0031-6997/89/4103-0259$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 41, No. 3
Printed in U.S.A.

Pharmacology of Cyclosporine (Sandimmune) IV. Pharmacological Properties in Vivo

JEAN F. BOREL

Preclinical Research, Sandoz Pharma Ltd., Basel, Switzerland

A. Antibody-Mediated Immunity

1. Summary of early work. Borel and his co-workers were responsible for all the initial in vivo testing of CS before it was evaluated in more sophisticated experimental models elsewhere (106, 107). It proved effective in a number of standard assays, notably the haemagglutinin inhibition assay. This in vivo assay represents a severe test for immunosuppression, in which a satisfactory suppressive index of < 0.75 is often not achieved with compounds clearly effective in the depression of plaque-forming cells (PFC). A short course of orally administered CS or azathioprine (AZ) to mice immunised with sheep erythrocytes led to a reproducible and dose-dependent inhibition of haemagglutinating antibody formation. The LD50 was attained at about 5×235 mg/kg for CS suspended in tragacanth and at 5×110 mg/kg for AZ (106). In view of the relative small dose-difference between efficacy and toxicity of most immunosuppressives, it is important to determine the dose-ratio between the toxic and pharmacological effect (therapeutic index). Since the oral LD50 (mortality within 3 weeks) was 5×660 mg/kg for CS and 5×200 mg/kg for AZ, the resulting index of 2.8 for CS is superior to that of 1.8 for AZ.

Moreover, a comparison of the in vivo results in mice treated with equiactive doses of CS and AZ demonstrated more clearly the difference in the therapeutic index between the two compounds. The counts and differentiation of peripheral blood leukocytes and thrombocytes indicated that CS had a more pronounced effect on lymphocytes than on granulocytes and thrombocytes, while AZ affected equally all blood cell types. Spleen weight in mice after repeated treatment with high doses of CS was evaluated at different time intervals and no significant reduction was observed (106, 107). These results markedly contrast with those from other immunosuppressives like AZ and cyclophosphamide (CP) used in equipotent doses, which are known to produce cellular depletion of spleen (107), and to cause severe depression of myeloid tissues (for review cf. 39). Myelotoxicity, in particular, was investigated by comparing the effects of CS and AZ on bone marrow cell counts and spleen cell proliferation in mice. The dose of CS corresponding to the ED50 in the haemagglutination assay did not significantly affect either parameter, while the equipotent dose of AZ diminished both by 77 to 99% of control values (107).

The pronounced suppressive effect of CS on haemagglutinin production against sheep erythrocytes was further shown in the rhesus monkey (100). Provided the

very hydrophobic CS was administered orally in a correct galenic form (i.e., which is well absorbed), complete inhibition of antibody production following a 5-day course was achieved (fig. 1). In contrast, other studies performed in cynomolgus monkeys treated with intramuscular CS, though attaining therapeutic drug blood levels, failed to demonstrate inhibition of antibody formation against murine monoclonal antibodies (596) or resulted in lowering only secreted IgA in tears to chlamydial antigens, but without affecting the other isotypes (1015). However, the same CS-treated animals became unresponsive to tetanus toxoid.

The rapid reversibility of the immunosuppressive effect of CS, once treatment is stopped, was demonstrated in the same stringent haemagglutination assay. Treatment received, up to one day before the immunising antigen, was ineffective in mice, although the dose given was reasonably high (>ED50) (107). This observation was further supported by data obtained in rats treated daily with 20 mg/kg per os for 19 weeks. At 13 weeks their haemagglutinating antibody titres (expressed in −log 2) were 2.9 compared to 6.2 in placebo-treated controls (suppressive index = 0.46). Another group of those rats was immunised 1 week after cessation of treatment, i.e., week 20. Nine days later the primary antibody response was fully recovered compared to the controls: titres expressed in $-\log_2$ were 5.7 and 6.3, respectively (J. F. Borel et al., unpublished results). Similar observations were also made in rats receiving 45 mg/kg/d in the feed, a dose level exerting toxic side effects. The production of PFC was suppressed by 99.6% compared to controls after 13 weeks of treatment (107).

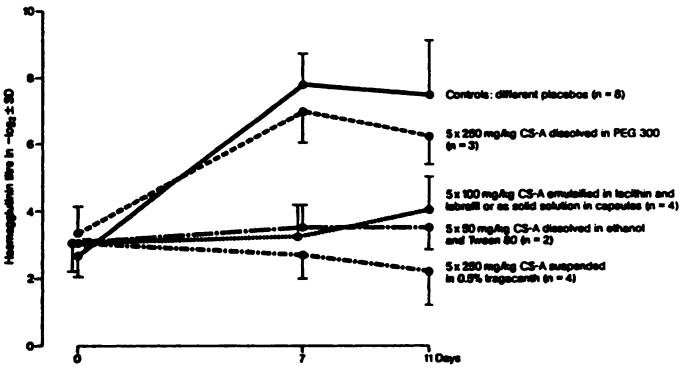

FIG. 1. Suppressive effect of cyclosporine A (CS-A) on haemagglutinin production against sheep erythrocytes in the rhesus monkey. Comparison of several galenic forms for oral administration. When CS-A is given dissolved in polyethylene glycol (PEG 300) it remains inactive: this is probably due to lack of absorption. The suspension in tragacanth is approx. 5 times less active than the solution in ethanol and Tween 80. Male rhesus monkeys of 3 to 9 kg body weight were immunized intravenously with 5 ml of a 10% washed sheep erythrocyte suspension on day 0. The animals were treated orally by gastric intubation on days 0 to 4 inclusive with either CS-A as indicated or the placebo equivalent. Blood samples were drawn before, and 7 and 11 days after immunization. Serum haemagglutinin titres were determined using Takatsy's microtechnique. (Reproduced with permission from ref. 100.)

Six weeks after discontinuation of the drug, the animals had fully recovered from toxicity and the number of direct PFC per spleen had returned to normal levels (J. F. Borel et al., unpublished results). The reversible suppression of alloantibody production by CS was further confirmed in skin allografted rats treated orally with 25 mg/kg/d. Withdrawal of CS treatment led to complete recovery of specific immune responsiveness and the time taken for recovery was independent of the duration of treatment (214).

The marked inhibition of haemolytic PFC by CS has been documented in mice (106, 107), rats (107, 413), rabbits, and squirrel monkeys (J. F. Borel, unpublished data). Furthermore, the effect of a single high dose of CS, AZA and CP on the kinetics of direct (IgM) PFC in mice was compared (106). CS and AZ showed a strong suppression (about 90%) on days 3 and 4, which was, however, less pronounced on day 5, while CP completely abrogated PFC on all days tested. Very similar suppressive effects were obtained on the kinetics of indirect (IgG_{2a}) PFC (106). When studying the dependence of the immunosuppressive activity on the time of drug administration with respect to immunisation in mice, CS was most effective when given on days −1, 0, or +1. AZ strongly depressed PFC on day 2, but less on day 3. CP was again equally suppressive any time between days −2 to +3. It was concluded that CS interfered with an early event in mitogenic triggering of lymphocytes and that its action could be clearly distinguished from the antimitotic activity of AZ or the unspecific cytostatic effect of CP (106).

The early studies also demonstrated that both short- and long-term treatment with CS were equally suppressing the primary direct PFC response, which suggested that long-term courses did not induce tolerance to the agent (107). It was also evident that the extent of PFC suppression by CS does not correlate with the concomitant reduction in spleen cellularity, since it was only significantly reduced after long-term, but not short-term treatment.

Moreover, CS significantly inhibited both IgM and IgG_{2a} PFC in a secondary response to sheep erythrocytes in mice which had not been drug treated during the primary exposure to the antigen. Optimal effects were again attained when CS was administered just before, at, and shortly after the immunising dose, i.e., on days −2, −1, 0, and +1. However, the secondary PFC were less suppressed than the PFC of the primary response, despite treatment with comparatively higher doses. The number of spleen cells was only marginally reduced, a finding that contrasted sharply with the severe depletion caused by CP (107).

Finally, the effect of CS on antibody formation to lipopolysaccharide antigens (LPS) was assessed in both normal and homozygote 'nude' mice (107). Doses of CS sufficient to strongly reduce haemagglutinin titres failed

to inhibit the formation of IgM antibodies against LPS in both types of mice. Since LPS is considered to be a thymus-independent antigen and the homozygote 'nude' mouse to be devoid of functional T cells, these results suggested that CS does not directly affect B lymphocytes. This assumption was further supported by the failure of CS to suppress in vitro proliferation of LPS-stimulated spleen cells derived from 'nude' mice (111). It was concluded that the inhibition of antibody formation by B cells was mediated primarily through the action of CS on T helper (T_H) cells.

2. Experimental thymus-dependent and thymus-independent antigens. The ability of CS to suppress in vivo the primary as well as the secondary antibody responses to the T-dependent (TD) antigen sheep erythrocyte has been reported in the preceding part. These results were partially confirmed when using the TD haptencarrier complex dinitrophenyl-keyhole limpet haemocyanin (DNP-KLH). CS ablated both IgM and IgG PFC in the primary response in mice (541). In contrast, the same dose of CS injected subcutaneously, a very effective route, did not inhibit the secondary response.

Similar results were obtained in a different system (590). CS totally suppressed the primary antibody formation to the soluble TD antigen human serum albumin (HSA) in the rabbit. However, the same dose failed to inhibit the secondary response as measured by an indirect haemagglutination method. The primary response was elicited using HSA without Freund's complete adjuvant (FCA); in the secondary response, however, the rabbits were both primed and challenged with HSA in FCA. It appears doubtful whether this difference in immunisation or a substantially higher drug dose would have profoundly influenced the antibody titres in the secondary response. In another experiment the TD antigen pneumococcus type 3 was used, which when given as a formalinised heat-killed vaccine, produces a brisk T-cell determined antibody response in the rabbit. Antipneumococcal antibody titres were measured on days 10, 18, and 30 and compared with preimmunisation values, which uniformly showed no antibody titre (293). The rabbits had been immunised on days 1 to 4 and boosted on days 15 to 18. All CS-treated rabbits (10 and 20 mg/kg/d or on alternate days intraperitoneally), when compared to saline-treated controls, had clearly depressed antibody titres on day 10. On day 18 titres were uniformly lower, reflecting antibody binding by booster antigen, while day 30 titres were depressed by CS in all drug-treated groups but less than during the primary response. It was inferred that in the rabbit CS inhibits the T-dependent limb of antibody production by B lymphocytes.

It follows from the results obtained in these three different systems that the secondary antibody response is not or is markedly less inhibited than the primary. The degree of suppression might speculatively be related to the type of antigen used: haptencarrier complex, particulate, or soluble antigen.

The importance of the nature of the antigen became evident when two classes of thymus-independent (TI) antigens were used: the TI-1 antigens such as DNP conjugated to LPS (DNP-LPS) or to *Brucella abortus* (DNP-BA) and the TI-2 antigens like DNP conjugates of Ficoll (DNP-FIC) or of dextran (DNP-DEX) (541). It was shown that, in the mouse, B cells responding to TI-1 antigens are resistant to CS (confirming the previous failure of inhibiting LPS responses; see 541), while those responsive to the TI-2 antigens are exquisitely sensitive. Thus, doses that completely abrogated responses to TI-2 antigens had a minimal effect on the DNP-BA response and even enhanced antibody formation to DNP-LPS (522, 416). These findings suggested that CS sensitivity represented a novel marker of functional B cell subsets in the mouse. A summary of the effects of CS on antibody responses in the mouse is shown in table 1 (522).

Nowak et al. (713) have examined the effect of CS administration on haemagglutinating antibody production to TD (sheep erythrocytes and human γ-globulins) and TI-1 (*B. abortus*) antigens in the chicken. The antigens were injected intravenously 3 times in 7-day intervals starting on the second day of intramuscular CS injection. Doses of 25 or 50 mg/kg/d were administered every 3 days for 21 days. The primary response was measured 7 days after the first immunisation and was chiefly of the IgM class. Despite the high degree of individual variation in agglutination titre, there was a significant titre difference to human γ-globulins and to *B. abortus*, but not to sheep erythrocytes. After the first antigenic boost (16 days) there was no significant difference in overall antibody titres to the TD antigens; however, the immune response to the TI-1 antigen was clearly enhanced. After the second antigenic boost (22 days) only the lower response to human γ-globulins at the higher dose was statistically significant, whereas there was again a trend to higher responses to *B. abortus* in the CS-treated groups. The secondary and tertiary responses were also predominantly of the IgM class. Therefore, the IgM response appeared normal or even elevated in treated chickens and only the IgG (2-ME treatment) response was significantly lower than normal. In conclusion, the failure to inhibit the primary haemagglutination response is likely due to insufficient doses of CS, although the same doses showed an inhibitory effect to human γ-globulins. The partial enhancing effect on IgM antibodies to the TI-1 antigen is in agreement with the above mentioned mouse data; this also holds true for the normal secondary and tertiary responses, except for the tertiary response to human γ-globulins with the high CS dose. In contrast the IgG antibody production to all three antigens was strongly suppressed.

Both class TI-1 (LPS) and TI-2 (Ficoll, dextran) can induce and reveal B memory cells and the induction of

TABLE 1

*Summary of effects of CyA on antibody responses in the mouse**

Antigen	Type	Response	Effects of CyA	Conclusion
DNP-LPS	TI-1	Primary	↑ (IgM)	CyA^R B cell
DNP-CPSK				
TNP-BA	TI-1	Primary	↓ (≈ 50%)	
DNP-Ficoll	TI-2	Primary	↓ (IgM, IgG)	CyA^s B cell
DNP-dextran				
DNP-KLH	TD	Primary	↓ (IgM, IgG)	CyA^s 1° T_H cell and/or 1° B cell
DNP-KLH	TD	Secondary	none or ↑ (IgG)	CyA^R 2° T_H cell and 2° B cell
DNP-KLH (in KLH-primed)	TD	Primary	non (IgG) ↑ (IgM)	CyA^s 1° T_H cell CyA^R 1° B cell

* CyA^R, CyA^S, CyA-resistant or -sensitive, respectively; 1°, 2°, virgin (1°) or memory (2°) cells; T_H, T-helper cell; ↑, ↓, enhancement or suppression, respectively. Abbreviations for antigens explained in text. (Reproduced with permission from ref. 522.)

these cells was shown to occur in spite of the presence of CS (854). At the level of B memory cell activation, it was found that those activated by TI-1 antigens are resistant to CS and thus share the same properties as the precursors of the B memory cells (antibody-forming cells or AFC). The B memory cell population activated by TI-2 antigen is inhibited by CS and is then similar to the AFC precursors responding to these antigens. A long lived hapten-specific IgM memory can be induced in the newt with a primary immunisation with TNP-LPS (TI-1). CS treatment from one day before until day 4 after reimmunisation with TNP-sheep erythrocytes (TD) affected the two responses differently. The primary anti-carrier response was reduced while the memory anti-hapten response was strongly enhanced compared to untreated controls (797). This confirms that primed B cells are resistant to CS and that CS potentiates the memory IgM response.

A puzzling finding is, that even though the progenitors of the B memory population are insensitive to CS, they give rise to two B memory subpopulations, one of which eventually acquires sensitivity to this drug (856). These results suggested two possible hypotheses. The first was that there are two broad compartments of B cells in mice. The one responds to TI-2 antigens and consists of $Lyb5^+$ CS-sensitive B cells, while the second responds to TI-1 antigens, with the aid of T cells to TD antigens, and consists of $Lyb5^-$ CS-resistant B cells. The second possibility (which is currently favoured) is that there is a single B-cell compartment, which can be activated by biochemically distinct pathways (519, 520; see also section V.A.).

The observation that the priming of B memory cells to the class of TD antigens could be suppressed by CS was attributed to the inhibition of T-helper function rather than to a direct effect on B cells (G. G. B. Klaus and A. Kunkl; unpublished data). Strong evidence for this was provided by immunosuppressing mice with CS at the time of a primary immunisation with sheep erythrocytes, but without drug treatment during the secondary immunisation 28 days later. The kinetics of IgM and IgG PFC formation indicated a nearly complete inhibition of all antibody responses. However, in vivo treatment of the CS-depressed mice at the time of antigen challenge with a semi-purified lymphokine (B-cell memory-forming factor derived from Jurkat cell supernatants) restored the secondary PFC response to the normal level of untreated controls (477). In contradistinction, another attempt to restore CS-suppressed primary responses to either TNP-KLH (TD) or TNP-Ficoll (TI-2) by injecting the mice with various lymphokines and LPS failed (997). Although some of these agents enhanced responses in CS-treated mice, none restored B-cell responses of CS-treated mice to near the level in mice treated with the agent alone.

As reported above, CS has been shown to suppress the primary TD responses in the mouse, whereas secondary responses were less or not inhibited. This appears to be due to the emergence of CS-resistant cells after priming with antigen. To distinguish between the possibilities whether primed TD B cells or primed T_H cells were resistant to CS, mice primed 4 weeks previously with KLH—which should have resistant T_H cells—were immunised with soluble DNP-KLH as shown in fig. 2. Since the 3 days' treatment with CS failed completely to depress both IgM and IgG PFC responses to DNP coupled to sheep erythrocytes (DNP-SE) on days 3 and 5, it was concluded that primary TD B cells and primed T_H cells are CS resistant, while primary T_H cells are CS sensitive.

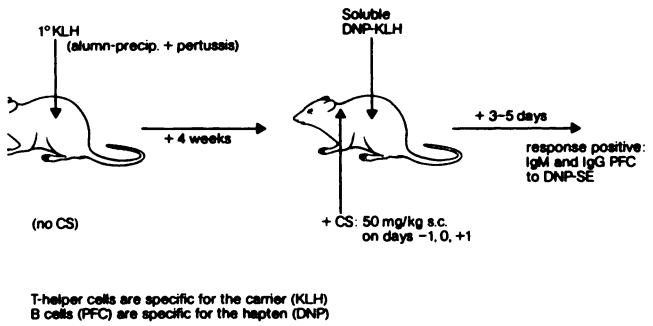

FIG. 2. Experimental design used by Klaus and coworkers to analyse which in vivo activation steps in the antibody-mediated immune response are sensitive or resistant to CS (see text under IV.A.2.).

In transfer experiments, irradiated mice were given DNP-primed B cells and either normal spleen cells or cells from mice immunised with KLH more than 1 month previously as sources of virgin or primed T_H cells, respectively. CS treatment of the recipients suppressed help given by the unprimed cells, but not that by the primed cells. Subsequently, mice were given KLH with or without CS daily, either day −1 to +1, or daily until day 6. Cell transfer on day 7 showed that donors treated with CS throughout had developed a normal level of help, while cells from those receiving a 3-day course of CS gave significantly enhanced help compared with controls, i.e., there was an 'overshoot' effect due to the emergence of supranormal numbers of T_H cells. It was further demonstrated that in this system there is a marked discrepancy between the dose of CS needed to suppress help during a primary response (ED50: 12.5 mg/kg) compared to that required to suppress helper-cell priming (150 to 300 mg/kg) (523).

Besides suppressing IgM and the subclasses of IgG antibodies, CS has also been noted to inhibit IgE production to ovalbumin (OVA) in rats as assessed by the passive cutaneous anaphylactic reaction (U. Martin, Sandoz, Basel; unpublished results; see also 922). Okudaira and coworkers (728) have further shown that persistent anti-OVA IgE antibody formation in the mouse was suppressed by oral administration of 50 mg/kg/d CS; however, the concomitant IgG antibody production was slightly, but insignificantly suppressed. Another group (153) administered high subcutaneous doses (30 mg/kg/d) of CS for 5 days following immunisation of BN (Brown-Norway) rats with DNP_{14}-OVA in alum and observed that the primary total and specific IgE response was strongly suppressed, whereas this treatment had no significant effect on the secondary IgE response. In contrast, rats receiving a single dose of CS (30 or 180 mg/kg) on the day of priming developed a normal primary IgE response, but the secondary IgE response was abolished independently of whether they did or did not receive a single CS dose at day 21 together with the antigen challenge. The inhibition of the secondary IgE response was attributed to the induction by a single dose of CS of nylon wool-adherent suppressor spleen cells as shown by passive transfer experiments (153). There is at least a second report in which the effect of a single dose of CS on the kinetics of the antibody response in the guinea pig was analysed (750). The effect of an intraperitoneal dose of 100 mg/kg CS, given as a single injection either before, at, or after immunisation with DNP_{50}-BGG (bovine γ-globulin), on antibody levels was investigated. Primary and secondary anaphylactic anti-DNP antibodies were only depressed when CS was injected 3 days after immunisation. The secondary anti-BGG IgG_1 and IgG_2 responses were also suppressed if CS was given 3 days after immunisation, but not otherwise. However, the way in which these responses are affected depends on the time of treatment in relation to the time of primary immunisation. The effect varies from depression to stimulation. The type of antibody affected varies both with the class and with the nature of the antigen as both primary and secondary responses may be altered. There are several reports describing that, under certain, rather artificial circumstances, CS can reproducibly and significantly enhance antibody production (501) and stimulate delayed hypersensitivity skin (925, 975) or localised graft-versus-host (GvH) reaction (806). Most of these findings, which are not limited to CS, might be explained by a rebound phenomenon triggered by inadequate suppression. In addition, they are confusing, of little practical relevance, and will not be discussed in this review.

3. Viral antigens. A few papers have been published on the in vivo effects of CS on the antibody response in virus-infected mice. The most straight-forward results were reported by Charan et al. (162) using mice injected intravenously with vesicular stomatitis virus (VSV). Both primary and secondary serum neutralisation antibody responses were assayed after intraperitoneal administration of 60 mg/kg/d CS for 8 to 12 days. The initial IgM response of mice to VSV is T-cell independent (TI-1) and CS had no effect, whereas the anti-VSV IgG antibodies, which are TD, were completely suppressed in the primary response. It therefore appears that the switch from IgM to IgG was prevented. However, once the switch to IgG in a primary response had occurred, the IgG response became refractory to CS. Moreover, the secondary IgG response was highly resistant to CS. This again is in support that priming occurs in spite of CS treatment and that memory T and B cells are quite resistant to the suppressive effect of CS.

In another study serum antibody titres of mice were measured at different times after intranasal influenza A virus infection and using two different techniques: haemagglutinin inhibition titre and total antiviral antibody determinations. Doses of about 100 mg/kg/d CS were given subcutaneously for 21 days. Using the former test, the appearance of antibody in CS-treated mice was both delayed and suppressed, but by 19 days after infection quite significant amounts of antibodies were formed in these mice. The latter technique revealed a prolonged production of IgM and decreased production of IgG in the CS treated mice compared to controls, suggesting that the main effect of CS was to interfere with the switch from IgM to IgG antibodies (831).

Other results are difficult to explain in view of widely differing experimental protocols. When mice were infected intraperitoneally with herpes simplex virus and given orally or intraperitoneally 40 mg/kg/d CS (4 to 5 times), it was found that CS did not affect the induction of B-cell immunity (25). Another study yielded similar results in mice infected intranasally with influenza A virus and treated orally with 200 mg/kd/d CS for the

first 10 days post infection. Although this treatment suppressed cytotoxic T cells, it did not inhibit the serum haemagglutinin inhibition antibody response (184).

4. Alloantibodies. The effect of CS on alloantibody production has been investigated in various rat models. The reversible suppression by CS of a primary alloantibody response to skin allografts has been described (214). It was shown that an oral dose of 25 mg/kg/d for different treatment periods was fully immunosuppressive and that the recovery of the humoral response to the grafts was independent of the duration of CS treatment. The rate of production and timing of appearance of the IgM and IgG_2 isotypes, with respect to graft rejection in animals recovering from CS-treatment, were identical with those found during the normal primary response. Since withdrawal of the drug led to complete recovery of specific immune responsiveness, these data do not support the suggestion that CS eliminates or permanently damages antigen-reactive T- or B-cell populations.

Another study deals with the influence of CS on alloantibody responses in F344 rats receiving weekly transfusions of DA rat whole blood (192). Whereas repeated transfusions resulted in a persistent alloantibody response in non-immunosuppressed controls, administration of daily oral doses of 15 mg/kg CS from days 0 to 7 resulted in prevention; from days 8 to 49 and 15 to 49 in the eventual suppression of alloantibody responses before the end of the experiment on day 49. This is clear evidence that CS, at least in this model, can abrogate an ongoing antibody-mediated response. The authors further suggest that the immunosuppressive action of CS may, in part, be due to the development of anti-idiotypic activity whose nature remains to be more fully characterised. Jones et al. (475) have also investigated the effect of CS on the alloantibody response to blood transfusion in inbred strains of rats by indirect haemagglutination and CELISA. Their data suggested that the sensitisation produced by blood transfusions (0.5 ml injected 9 times at weekly intervals) and the persistence or decline of the alloantibody response depended upon the responder status of the recipient. Blood transfusions given with CS (10 mg/kg/d orally for the first 7 days) were capable of inducing suppressor activity that was transferable in spleen homogenates. Subsequent alloantibody responses were influenced by the class I and II disparities of the donor and recipient rats. If these results were to be extrapolated to clinical practice, CS should be given with pretransplant blood transfusions to prevent sensitisation, and the transfusion donor should differ from the recipient at both class I and II loci. That CS therapy can provoke the disappearance of preformed lymphocytotoxic antibodies has also been documented in two retransplanted patients previously sensitised by a first cardiac allograft (712).

In a series of other experiments the effect of CS on the secondary antibody-mediated response in the rat was explored. Female rats were sensitised to paternal antigens in two semi-allogeneic pregnancies and later challenged with paternal or unrelated strain blood transfusions (476). The alloantibody responses were monitored by the indirect haemagglutination assay. Immunosuppression with CS was effective in preventing the primary humoral immune response produced by blood transfusion, but not in abrogating the established immune response to previous pregnancies.

In an attempt to create a rat model of the past-positive, current-negative lymphocyte crossmatch phenomenon, LEW rats were sensitised with ACI heart fragments or skin grafts (837). Subsequent ACI heart allograft survival in the presence of high titre alloantibodies in control animals was markedly shortened. In contrast, CS therapy significantly prolonged heart graft survival. Non-immunosuppressed, adoptively transferred LEW recipients of ACI hearts rejected their cardiac grafts in an accelerated fashion (MST (mean survival time): 4.5 ± 0.5 days as compared with 6.7 ± 0.5) and displayed an anamnestic antibody production. However, immunosuppression with CS prolonged graft survival over 30 days in all rats and prevented a secondary alloantibody response.

Similar results demonstrating the efficacy of CS to influence a secondary antibody response were obtained in a slightly modified model (474). LEW rats received weekly transfusions of DA rat whole blood for 8 weeks either with or without CS (15 mg/kg/d orally) after sensitisation with DA splenocytes. (AO × PVG) F_1 hybrid rats received only 10 mg/kg/d CS after similar sensitisation. Administration of CS did not affect the spontaneous decline of alloantibody titres against class I antigens, but prevented maintenance of high alloantibody titres against class I antigens in LEW rats transfused repeatedly after sensitisation. CS was also associated with a significantly reduced response to class II antigens at the end of the study at 56 days. IgG alloantibody subclass responses were altered by CS with significant reduction in titres of IgG_1, $_2$a and $_2$b, but not $_2$c, against class I antigens in rats transfused repeatedly. These results suggest that, contrary to those obtained with some other antigens, CS does affect secondary alloantibody responses.

Finally the effect of CS on the in vivo humoral response against the non-MHC (major histocompatibility complex) endothelial antigen that is present on the renal capillary and venous endothelium was tested in a rat model. The animals were treated during the sensitisation process with different doses of CS for different periods. CS suppressed the endothelial antibody response completely and irreversibly at a dose of 15 mg/kg/d injected intramuscularly with a minimum duration of therapy for two weeks. Furthermore, the results also showed the induction of a state of unresponsiveness with doses which were not effective in suppressing the initial antibody response (399).

5. *Autoantibodies.* The effects of CS on autoantibody production in several models of autoimmunity have been documented. Clearcut results were reported by Drachman et al. (229) in experimental autoimmune myasthenia gravis which is an antibody-mediated autoimmune disorder of acetylcholine receptors (AChR) at neuromuscular junctions. Intraperitoneal doses of 20 to 25 mg/kg/d given for 3 weeks, i.e., starting at the time of primary immunisation, suppressed the antibody responses to AChR virtually completely. Following 12 weeks of CS (1st week high dosed, then tapered to 10 mg/kg/d), the AChR-immunised rats responded like naive controls to a further challenge of AChR, but without a second course of CS treatment, thus suggesting that priming had not occurred under the previous longterm CS treatment. The secondary response to a challenge of AChR (without adjuvant) was prevented by CS treatment, but a large challenge dose in FCA partially overwhelmed the effect of CS. Interestingly, treatment of ongoing disease resulted in a reduction of the initial AChR antibody level by more than 50%. However, by 3 weeks after cessation of CS treatment, the titres had risen again to their original values. In this rat model CS exerts clear preventive as well as therapeutic effects as confirmed by others (365).

The in vivo effects of CS in the experimental allergic orchitis model in the guinea pig were studied by Hojo and Hiramine (427). Ten mg/kg/d CS injected by three systemic routes for the first 14 days prevented the antisperm antibody response in strain 13 guinea pigs, when examined 2 weeks after immunisation, and delayed its onset following cessation of the drug. However, when CS was started 2 weeks after immunisation, even higher doses did not anymore affect the antisperm antibody titres as compared to controls. This indicates that CS does not suppress already activated T_H and B cells (AFC). In cell transfer studies using lymph node cells from immunised and CS-treated guinea pigs, most recipients failed to develop orchitis at 2 weeks postchallenge, but the antisperm antibody response was unaffected. This shows that CS acts differently on different lymphocyte subpopulations. Lovett et al. (595) have further shown that pre-vasectomy treatment with CS (10 mg/kg/d intramuscularly for 7 days) significantly reduced or delayed the production of cytotoxic sperm antibodies in Lewis rats. Surprisingly, post-vasectomy treatment markedly advanced the onset of antibody formation.

The S-antigen, found in all mammalian retinas, is uveitogenic under experimental conditions and CS has been reported to prevent S-antigen induced uveitis in LEW rats (159). Although experimental allergic uveitis is predominantly mediated by T cells, CS therapy did alter the humoral immune response which resulted in lower peak and delayed production of circulating anti-S-antigen antibodies in a proportional relationship to the dose of CS.

Induction of experimental autoimmune thyroid disease by early thymectomy and subsequent sublethal whole body irradiation induces a reproducible model in the rat, which is characterised by progressive thyroid gland lymphocytic infiltration leading to follicular obliteration and associated with circulating antithyroglobulin antibodies. Eight weeks after their last irradiation the animals commenced treatment with CS (10 mg/kg/d p.o.) and were treated for varying time intervals thereafter. With commencement of CS there was a very significant fall in antibody levels lasting only for as long as CS was maintained (627).

The effect of CS was also tested in mercuric chloride-induced nephritis in the BN rat. The optimal regimen of 7 mg/kg/d for 2 months interfered with the polyclonal activation of B cells observed in mercuric chloride-induced autoimmune disease, i.e., the IgE antibody levels did not alter over the experimental period. The IgE levels started to rise after CS was discontinued or fell dramatically with the delayed introduction of CS (50). The drug exerted a striking preventive and curative effect in this model.

The New Zealand black × New Zealand White (NZB × NZW) F_1 hybrid mouse (NZB/W) model, in which the mice spontaneously develop an autoimmune disease resembling systemic lupus erythematosus (SLE) in man, has been used by some investigators to study the effects of CS on anti-DNA autoantibody production. Such mice were treated orally with 100 mg/kg/d CS 5 times a week from 16 to 60 weeks of age (472). This treatment resulted in a significant inhibition in the formation of PFC to TD sheep erythrocytes and the level of anti-dsDNA autoantibodies was slightly reduced when compared with female NZB/W controls. However, levels of other autoantibodies (anti-ssDNA, anti-IgG, anti-erythrocyte) and serum IgG were unchanged.

In another study the suppressive effect of CS on the development of autoantibodies in young (12 weeks old) as well as in old mice (36 weeks old) was examined (363). Both groups of mice were given orally 100 mg/kg/d CS for 12 weeks and had at the onset of treatment anti-DNA levels around 0.20 (optical density). This level dropped to 0.05 after 12 weeks of CS treatment, whereas it rose to 0.44 in the control mice. In the old mice, treatment started when they were already producing high levels (about 0.8) of anti-DNA autoantibodies, and at 48 weeks the level was progressively reaching less than 0.2. CS also reduced both the incidence and the titres of anti-sheep erythrocyte autoantibodies (Coombs positive). These results show that ongoing autoantibody responses can be suppressed in this murine lupus model. This observation is remarkable, since in vitro experiments have demonstrated that B-cell activation in certain forms of autoimmunity (e.g., the NZB strain) may be associated with decreased sensitivity to CS (759).

MRL/1 and BXSB mice develop around 4 to 6 months

of age an acute form of SLE. The major basic abnormality in MRL/1 mice seems to reside in the T_H circuit (Lyt 23^- phenotype), while in BXSB mice this seems to be the B-cell circuit. Mice from both strains were treated daily for 16 weeks with an oral dose of 25 mg/kg CS. This low dose, which was insufficient to inhibit a primary antibody response to BSA in normal mice, did suppress the cellular immune response, and did not alter significantly the anti-DNA and anti-IgG antibody levels in either strains compared to placebo-treated controls. However, the prominent effect of CS was an evident reduction in lymphoproliferation in the MRL/1 mouse, which is due to the presence of the recessive lpr gene (67). Similar experiments with MRL/1 mice performed in our laboratory, using a higher oral dose of 100 mg/kg/d, essentially confirmed the above results (364). It is finally noteworthy that in these last studies the levels of rheumatoid factors were unaffected (67) or even increased (472, 364) by CS treatment.

The effect of CS on the production of experimental anti-erythrocyte autoantibodies in mice has been investigated. These autoantibodies are induced by repeated injections of rat erythrocytes and thought to be regulated by auto-suppressive T cells. Daily treatment of mice with CS (50 mg/kg/d intraperitoneally) for up to 13 days during the course of injection of rat erythrocytes significantly inhibited autoantibody formation as well as antibodies against rat red cells. The CS-treated mice that did not form autoantibodies produced suppressor cells which inhibited autoantibody production in adoptive transfer experiment and which were destroyed by anti-Thy 1.2 serum and complement treatment (185). In another study mice were treated with CS (25 or 100 mg/kg/d orally) during the period of induction of autoantibodies. The high CS dose inhibited autoantibody production whereas the low dose temporarily potentiated it. Cell transfer and anti-Thy 1.2 treatment indicated the presence of T_S cells in the spleen of immunised mice. Their formation was inhibited by the high dose CS and potentiated by low dose CS. In addition, CS treatment after sensitisation had no effect on autoantibody titres (583).

Autoantibodies are thought to be implemental in the development of spontaneous autoimmune thyroiditis of obese strain chicken. CS has consistently failed to prevent or reduce antibody production in this model and also to influence the course of the disease (987).

The yet limited clinical studies undertaken in corresponding autoimmune diseases reveal contradicting effects of CS on autoantibody production also in patients. In agreement with experimental work there is a demonstrable suppressive effect of CS on anti-AChR antibody formation in patients suffering from myasthenia gravis. Preliminary results of a double-blind, placebo-controlled trial of CS in twenty myasthenia patients indicated a larger decline in AChR antibody titres in the CS-treated group than in the placebo group after 6 as well as 12 months (932). CS also reduced anti-thyroid autoantibodies present in a few positive patients suffering from endocrine ophthalmopathy (478). A substantial decrease in IgM-type anti-mitochondrial antibody levels, but not of IgG-type, was observed in four of five patients suffering from primary biliary cirrhosis (491). Decreased levels of autoantibodies against a series of autoantigens were detected in the sera of patients with unveitis receiving CS or CS plus bromocriptine following 3 months of treatment. In contrast to the decreased antibody titres the total immunoglobulin levels remained within the normal range (83 a).

In contrast, CS failed to alter anti-DNA titres in eight SLE patients treated for 6 months or more, although clinical improvement was observed in seven of those patients (268). In a similar study, positive clinical results were obtained in eight out of thirteen SLE patients treated for an average of 12 months which CS and steroids (269). However, antinuclear and anti-dsDNA antibody titres remained unchanged, but their serum binding capacity was slightly lower at the end of treatment (33 ± 7%) as compared to pretreatment value (42 ± 7%). Still another trial was performed with twenty patients suffering from SLE where CS was combined with fluocortolone (643). This therapy had a favourable effect in seventeen of these patients, but only half the patients had a marked drop in antibody activity against dsDNA. Concerning the antibodies to ssDNA, which appear to reflect the degree of non-specific B-cell activation, eleven out of sixteen had a demonstrable decrease and four had an increase, while the titre remained variable in one.

Moreover, no change in rheumatoid factor levels was reported from a small trial with eight rheumatoid patients completing a 24 week course on CS, although a marked improvement was noted in five of them (978). In a 4 months' double-blind, placebo-controlled study comprising fifty-two rheumatoid arthritis patients, the rheumatoid factor titres remained unchanged under CS treatment (226).

A recent important trial reports the effect of CS treatment on the production of autoantibody in diabetic (IDDM I) patients (89). Anti-islet cell and anti-insulin antibody production was studied over a 12-month period in eighty-two recently diagnosed diabetics. CS had a minimal effect on anti-islet cell antibodies whether directed to islet cytoplasmic (immunofluorescence) or membrane (cytotoxic assay) antigens even in patients undergoing remission. Conversely, CS completely suppressed the synthesis of antibodies elicited by exogenous insulin and decreased the autoantibodies to thyroid antigens, indicating that CS has variable effects on antibody production against various antigens.

6. Antibody response in humans. Transplant recipients on immunosuppressive therapy have an impaired antibody- and cell-mediated immune responsiveness. Con-

venient in vitro tests, such as enumeration of T and B cells and their subsets or the measurement of serum Ig, will not reliably detect a secondary immunodeficiency due to treatment with immunosuppressive agents. Since most vaccines and toxoids require helper T-lymphocyte involvement to achieve immunological memory, it is essential to assess in these immunocompromised patients either their established, preexisting responsiveness to a previous vaccine by an antigen challenge, or their residual immuno-competence to mount a primary immune response. Surprisingly few reports are available on this important issue.

Two clinical trials were performed in renal transplant recipients immunosuppressed with variable doses of CS combined with medium- to low-dose prednisone. Their antibody titres in response to a trivalent influenza primary vaccination were determined. The first study compared nineteen patients on CS + Pred (prednisone) to eight patients on (azathioprine) AZA + Pred and to twelve healthy volunteers (444). Nine patients (47%) in the CS group and five (63%) in the AZA group showed fourfold rises in titres to at least one virus strain compared with twelve (100%) in the control group. The second trial comprised twenty-one recipients on CS + Pred, thirty-eight recipients on AZA + Pred, twenty-eight patients on haemodialysis and twenty-nine healthy volunteers (962). CS-treated patients had a significantly lower antibody response than AZA-treated ones, whether mean antibody levels, fourfold titre rise, or seroconversion to protective titres were analysed. No significant differences in antibody responses were found between healthy controls and patients on AZA. The patients on haemodialysis showed an impaired response to vaccination but, in contrast to CS-treated recipients, booster immunisation proved valuable in this group.

Another rather extensive longitudinal study on the immune responsiveness was performed in thirty-two kidney transplant recipients receiving CS as the sole immunosuppressive drug (956). It was demonstrated that in the CS-group the primary IgG anti-KLH and IgG anti-tetanus toxoid responses were significantly decreased as compared to the control group consisting of healthy blood bank donors. CS did, however, not affect the secondary humoral or cellular response, as these responses were equal to that of the control group.

Two clinical studies report the in vivo effects of CS in bone marrow transplant patients. In the first trial eight children with severe combined immunodeficiency received mismatched haploidentical bone marrow transplantation and were treated with CS intravenously for 2 months (274). Five patients experienced full cellular and significant humoral immunological reconstitution within 4 to 6 months after bone marrow transplantation. This included the presence of IgG and IgM, and of antibodies to polio and influenza A viruses, tetanus and diphteria toxoids after the respective vaccination. The second study compared the immune responses to two neoantigens (TD antigen KLH and class TI-2 antigen DNP-Ficoll) in sixty-seven normal subjects to thirteen bone marrow recipients on CS as the sole form of immunosuppression (16). DNP-Ficoll induced similar antibody responses in control subjects and bone marrow recipients except that antibody levels declined more rapidly in patients. Both IgM and IgG classes of antibody showed similar thymus-independent behaviour upon first immunisation and re-immunisation. The TD response to KLH evoked an antibody formation in 88% of controls, but in only one bone marrow recipient on CS. Eight of the transplant patients were re-immunised with KLH 2 to 6 weeks after stopping CS and only one made a primary antibody response, arguing that CS inhibited priming.

Short-term CS treatment (24 mg/kg/d orally for 6 days) has recently been evaluated for its effect in suppressing anti-antibody production in cancer patients receiving repeated antitumour antibody therapy (573). Since, in this preliminary study, up to 4 times as many doses of antitumour antibody could be usefully given when CS was used, this protocol increases the potential for effective antibody targeted therapy of cancer.

Finally, eleven patients with chronic uveitis and treated with CS (starting dose of 10 mg/kg/d orally) were immunised with the TD antigens KLH and tetanus toxoid. Their responses were compared to that of both uveitis and normal controls (747). No significant differences in antibody responses to primary and secondary antigenic challenges were noted among the three groups, whereas delayed cutaneous hypersensitivity to both antigens was very significantly decreased in the CS-treated patients. These findings demonstrate clearly the selective effect of therapeutic doses of systemic CS on particular immune functions.

An interesting recent observation is the occurrence of so-called "autoantibody" production, mostly detected as anti-A or anti-B, following transplantation of 0 group allografts. Organ transplantation with a minor ABO incompatibility (presence of donor antibodies against the recipient's erythrocytes) is now frequently performed due to a shortage of suitable donors. A short-lived ABO haemolysis after transplantation of organs with minor mismatch (kidney, liver, lung, and especially bone marrow, but not heart and cornea) has been observed (see references in 450). Heart-lung allografts involve transplantation of a large amount of lymphoid tissue which creates a potential risk for GvH reaction in the case of an antigen mismatch. In contrast to nine fully ABO-matched heart-lung recipients, six out of nine ABO-mismatched patients suffered immune destruction of their erythrocytes (450). These findings clearly indicate that group 0 donor lymphoreticular tissue and circulating lymphocytes continue to produce anti-A and/or anti-B antibodies after transplantation and despite immuno-

suppression. The latency period between generation and onset of haemolysis is consistent with the time required for engraftment and generation of a secondary immune response, stimulated by the recipient's differing ABO antigens, from previously primed B lymphocytes. The predominantly IgG allotype of the ABO antibodies lends further evidence that they are of 0 group donor origin. Since CS has little effect on primed cells (table 1), the production of ABO antibodies by donor lymphocytes appears CS-resistant as testified by these cases. For yet unknown reasons the synthesis of ABO antibodies is self-limiting. It should be evident from the above, that these antibodies can not be designated as genuine autoimmune antibodies, because they arise only after cell transfer under mismatched conditions. In addition, CS does not suppress their production nor does it induce them, although it may enhance the phenomenon (see references in 450).

7. Conclusions. The relative paucity of studies on the effects of CS on antibody-mediated, as opposed to cell-mediated, immune responses as well as their heterogeneous and often contradictory results renders any attempt to search for a "unité de doctrine" illusionary. The early work clearly illustrates the potent blocking effect of this agent on primary antibody production against sheep erythrocytes in several species. Although the optimal doses needed to suppress antibody synthesis are roughly twice those needed for inhibiting cell-mediated immunity, the therapeutic index is significantly superior to that of other comparable immunosuppressants, e.g., azathioprine, cyclophosphamide, and methotrexate, and the inhibition is not mainly due to cellular depletion as observed with the above cytostatic drugs. Both IgM and IgG isotypes are suppressed in the primary response. Time-course studies show that CS acts on the early events of the immune response which precede mitosis. Especially noteworthy is the rapid and complete reversibility of the suppressive effect which is independent of the duration of CS treatment.

When comparing the effects of CS against various antigens, it is evident that the primary antibody formation against TD and TI-2 antigens is easily suppressed, but not against TI-1 antigens. It may well be that CS sensitivity is a novel marker of functional B-cell subsets in the mouse and possibly in other species (107). The hypothesis has been put forward that there is a single B-cell compartment which can be activated by biochemically distinct pathways, and which eventually splits into B-cell subsets sensitive or resistant to CS. Whether CS directly affects B cells or indirectly through suppressing T_H-cell function has been investigated using several experimental protocols. Some of the results of the effects of CS on antibody responses in the mouse are summarized in table 1. The emerging consensus indicates that primary TD and TI-1 B cells and primed T_H cells are CS resistant, whereas primary TI-2 B cells and primary T_H cells are CS sensitive. It was further demonstrated, when using viral antigens, that the TD switch from IgM to IgG isotype is suppressed by CS. However, the nature of the antigen, i.e., whether being soluble, particulate or a haptencarrier complex, seems to play no role concerning the sensitivity to CS.

CS effectively inhibits an ongoing humoral response as shown in models using repeated blood transfusions to elicit alloantibodies or in several autoimmune models with overt autoantibody production. This assertion should be restricted, since CS fails to affect the continuous autoantibody synthesis in a few other models. Clinical trials with patients suffering from various autoimmune diseases demonstrate both the success and failure of CS to influence the autoantibody titres.

The suppressive effect of CS on the secondary antibody response is particularly confusing. The secondary response to sheep erythrocytes was clearly inhibited by CS in mice which were not immunosuppressed during the primary immunisation. However, higher doses of the drug were necessary than of those needed to block the primary response. Using the same model, but treating the mice with CS only during the primary response, the booster immunisation failed to elicit a secondary response. Some experiments clearly suggest that CS inhibits priming in vivo; others do not. In another set of experiments, rats were presensitised with allogeneic spleen cells without receiving CS, and were subsequently challenged with donor-specific blood transfusions. CS treatment prevented the anamnestic alloantibody response. In experimental myasthenia gravis, the inhibition by CS of a secondary autoantibody response was dependent on the antigen dose used for challenge. In contrast, CS appears to affect the secondary antibody response very little in man, while it clearly suppresses a primary one. There is no doubt that, for whatever reasons, the secondary humoral response remains rather refractory to the immunosuppressive action of CS. However, there is evidence that significant reduction in secondary antibody titres may be obtained in certain experimental conditions, provided large CS doses and relatively low antigen amounts for challenge are being used. More systematic studies are needed to clarify why under identical conditions a secondary cell-mediated response is fully suppressed, while the antibody response is able to proceed unimpaired.

B. Cell-Mediated Immunity

The term cell-mediated immunity was originally coined to describe localized reactions to organisms, usually intracellular pathogens, mediated by lymphocytes and phagocytes rather than by antibody. In this chapter we are reviewing those responses in which B cells and antibodies play a subordinate role. Since CS primarily interferes with T-cell functions, it is, therefore, unlikely to affect T-independent sensitivity reactions such as anaphylactic sensitivity, antibody-dependent cytotoxic

hypersensitivity or immune complex-mediated hypersensitivity. However, it profoundly impairs both delayed-type hypersensitivity (DTH) reactions and the development of T_C cells in most experimental models. The effects of CS on alloreactivity are presented separately in the section on "Transplantation" (IV.C.) and on infections in the section on "Interaction of CS with defense mechanisms against infections" (IV.E.). The complex interactions of CS on the thymus and the developing immune system are being dealt with in section IV.C.2.f. Finally, the interference of CS with suppressor cells is discussed both under part IV.C.2.e. with respect to transplantation and under part IV.D.5. with respect to autoimmunity.

1. Delayed-type hypersensitivity reactions. DTH reactions can occur only in an individual previously sensitized (exposed) to a given antigen. In animals, sensitization is achieved by the injection of a given antigen emulsified in FCA into the skin. In both humans and animals, hypersensitivity is tested by the injection of the antigen dissolved in saline into the skin. Thickening of the antigen-injected site, occurring usually 24, 48, and 72 hours later, in comparison with the control site, is taken as evidence of DTH reaction. Important information about the mechanism of DTH can be gained by the transfer of the reaction from sensitized to normal individuals. This can be performed by harvesting lymph node, spleen, or thoracic duct cells from a sensitized donor and injecting them into an unsensitized recipient. Although the sensitization for DTH is always done in vivo, the actual testing of the reaction can be carried out either in vivo or in vitro (ex vivo) (for review see 524, 789, 560).

a. DELAYED-TYPE HYPERSENSITIVITY IN MICE. The skin hypersensitivity reaction to oxazolone in mice is done by sensitizing the animals on day 0 with oxazolone and by an antigenic challenge given 9 days later. The skin reaction is evaluated 24 hours later by measuring the increase in skin thickness (107, 97). CS was administered on days 0 to 4, inclusive, to test its capacity to inhibit the sensitization phase of this DTH reaction. It suppressed the primary skin reaction in a dose- and time-dependent fashion which showed a maximum inhibition (around 50%) at 70 mg/kg/d given orally immediately after sensitization. Increasing the dose resulted only in insignificant variations (107). The secondary response to oxazolone contact sensitivity, elicited about 7 weeks later, was suppressed to a similar extent as the primary reaction when the mice were treated on 4 consecutive days prior to the antigenic challenge (maximal effect again at 70 mg/kg/d orally), or with a high single dose just before the oxazolone booster. It should be noted that the secondary response was inhibited independently of whether the primary response had been partially inhibited or not (107). Similarly, when instead of oxazolone, alloantigen was given as an allogeneic spleen cell suspension to induce DTH, dose-response studies showed that DTH reactions were reduced to background levels when recipient mice were treated with CS (100 mg/kg/d intramuscularly) on days 0, 4, and 6 after primary alloantigenic challenge. The response to a second challenge given 14 days after primary sensitization was significantly decreased by CS treatment during primary and/or secondary exposure to alloantigen, and CS was as effective as ALS (antilymphocyte serum) in abrogating both primary and secondary DTH reactions (668).

Another modification of the treatment schedule consisted in treating the mice with a single high dose at the time of challenge of the primary response, i.e., on day 9 when sensitization had occurred. In this way an antiallergic rather than an immunosuppressive effect was measured (404). CS clearly depressed the DTH reaction but not as effectively as when administered during the early sensitization phase (97). Corticosteroids like dexamethasone are much more powerful inhibitors of this late reaction.

Several other natural CS analogues were also tested for their immunosuppressive activity in this DTH model (412, 413, 108, 104). The results are summarized in table 2. They indicate that a few other analogues are as potent immunosuppressants as CS in these models of cell-mediated immunity. Interestingly, (Val^2)dh-CS was also slightly more active than CS in the effector phase, i.e., when given 24 hours and 30 minutes before challenge with oxazolone on day 9. (ED30 for CS: 2 × 83 mg/kg orally, compared with 2 × 64 mg/kg orally for (Val^2)dh-CS). In the DTH skin reaction to Propionibacterium acnes in rats the derivative was half as effective as CS (108). In contrast to CS, the analogue (Val^2)dh-CS exerted no inhibitory effect in the tuberculin skin DTH model in the guinea pig (108; Table 3).

DTH can also be induced by the injection of antigen-specific, cloned T_H cells into the footpad of mice. The reaction is maximal at 24 hours, as assessed by local swelling (404). A single dose of CS (90 mg/kg/orally),

TABLE 2

Effect of some natural cyclosporins on two cell-mediated immune responses in vivo

Cyclosporins		DTH[a]	GvH[b]
		ED-30[c]	ED-50[c]
Cyclosporine A	CS-A (CS)	70	24
(Ala^2)-CS	CS-B	70	>50
(Thr^2)-CS	CS-C	ND[e]	25
(Val^2)-CS	CS-D	>70	25
(Val^2)dihydro-CS[d]	dihydro-CS-D	35	36
(desoxy-C_9^1)-CS	CS-F	>70	>50
(Nva^2)-CS	CS-G	35	35
($Nva^{2,5}$)-CS	CS-M	ND	≤50

[a] Oxazolone DTH skin test in mice.
[b] Local graft-versus-host reaction in female rats (Simonsen assay).
[c] Effective dose in mg/kg/d per os.
[d] Synthetic derivative of CS-D.
[e] ND, not done.

TABLE 3
Skin DTH reaction to tuberculin in the guinea pig. Effect of drugs given at the time of tuberculin (PPD) challenge

Drug	Dose mg/kg	Increase in skin thickness in % after:	
		24 h	48 h
1st experiment[a]			
Cyclosporine	2× 20 (i.p.)(8)*	−81 (0.0025)ξ	−83 (0.0025)
Azathioprine	2×100 (i.p.)(7)	−62 (<0.005)	−61 (<0.005)
Dexamethasone	2× 1 (p.o.)(8)	−43 (0.0025)	−20 NS
Indomethacin	2× 5 (p.o.)(8)	−17 NS	−16 NS
Phenylbutazone	2×100 (i.p.)(8)	−12 NS	−16 NS
2nd experiment[b]			
Cyclosporine	50 (i.p.)(6)	−86 (<0.0005)	−85 (<0.0005)
(Nva^2)-CS	50 (i.p.)(6)	−67 (<0.05)	−85 (<0.001)
(Val^2)dihydro-CS	50 (i.p.)(28)	−17 NS	−18 NS

[a] Methodology in ref. 97, 107 and 112. The animals were treated with drugs only around the time of challenge, i.e., 30 minutes before and 5 or 6 hours after by the route indicated in parentheses.

[b] Methodology in ref. 413. The drugs were injected intraperitoneally only once 30 minutes before challenge.

* Number of guinea pigs per group.

ξ p values; NS: means $p > 0.05$.

given up to 10 hours after sensitization with the cloned T cells, completely abrogated the DTH response, but had no effect when given after 15 hours. When the footpad swelling was induced by a lymphokine-containing supernatant, it already reached a peak within 2 hours. CS given 1 hour before the injection of the lymphokines did not depress the swelling; in contrast, a single oral dose of dexamethasone (0.5 mg/kg) totally blocked the swelling reaction (403). The results are consistent with the concept that CS inhibits the production of lymphokines but, unlike dexamethasone, does not alter their effects once they are released. A comparison of the efficacy of several drugs in the cloned T_H cell-mediated DTH model revealed that the oral ED50 in mg/kg was 31 for CS, 76 for (Val^2)dh-CS, and 0.22 for dexamethasone (402).

The effect of CS has been further analyzed in an immunological autoreactive experimental model in mice. The DTH reaction in this syngeneic DTH model was induced and elicited solely by the lymphoblasts' self-antigens (688). Surprisingly, CS (60 mg/kg/d intraperitoneally) injected close to the induction/sensitization phase was markedly less effective than when administered close to the effector phase at the time of challenge. Even a single dose of CS given 24 hours after challenge efficiently reduced the 48-hour reaction of the syngeneic DTH model (565). Adoptive transfer experiments revealed that T cells from immunized and CS-treated donors failed to efficiently transfer the syngeneic DTH response to naive recipients and, conversely, this DTH response was also blocked in recipient mice that were treated with CS (565). Further experiments disclosed that the effect of CS on the syngeneic DTH is associated with its ability to directly block the production of inflammatory lymphokines from CD5 cells, particularly since CD5 cells from CS-treated mice failed to transfer this DTH reaction, but nonspecific inflammatory responses were not affected (688).

CS was evaluated in a mouse model of allergic contact dermatitis to dinitrofluorobenzene (DNFB). It was found to significantly suppress the ear swelling reaction, similar to the oxazolone-induced DTH model, whether it was given during the early sensitization period or at the time of antigenic challenge to fully sensitized mice. This suppressive effect was, however, reversible when the animals were rechallenged with DNFB 96 hours after the first challenge. CS was not effective if given to sensitized mice as late as 6 hours after challenge (800). The suppression of the swelling reaction was closely paralleled in the histopathological sections (800). Using a very similar model, another group of investigators showed that simultaneous treatment of the mice with IL-2 preparations reversed the effect of CS on the induction of contact sensitivity as well as on the effector phase. However, neither phase of the response was restored by murine γIFN (997).

When CS (100 mg/kg/d intraperitoneally) was given to mice only at the time of priming with herpes simplex virus (pathogenic strain injected intradermally), it had little effect on the subsequent DTH reaction elicited 28 days later. However, the DTH response was substantially reduced when CS was injected repeatedly between the time of priming and challenge at 7 days (sensitization phase), or when previously primed mice received the drug shortly before challenge at 28 days (effector phase) (14). If mice were inoculated intranasally with influenza A virus and treated with CS (100 mg/kg/d subcutaneously) for 21 days, the DTH response to the virus could not be elicited at 6 and 12 days after infection (831). However specific effector T cells that mediate DTH to influenza virus (T_{DTH}) were found to be formed in vivo in these CS-treated mice, but the activity of these T_{DTH} cells could only be measured when they were transferred into untreated naive mice. When these T_{DTH} cells, which were MHC class II-restricted, were transferred into CS-treated recipients, no DTH activity could be detected (832). These results would imply that T_{DTH} cells are generated during CS treatment, but that the DTH reaction can only be manifested in the absence of the drug.

b. DELAYED-TYPE HYPERSENSITIVITY IN GUINEA PIGS. In the skin contact sensitivity model using DNCB (1-chloro-2,4-dinitrobenzene) in guinea pigs, CS treatment (20 or 50 mg/kg/d intraperitoneally) on days 0 to 4 inclusive completely suppressed the skin reaction on day 10 (challenge), since no redness or swelling were observed (107). In the DTH skin reaction to tuberculin in the guinea pig, several drugs were administered at the time of challenge only, to test their effects on the effector phase. Table 3 shows that CS and its congener (Nva^2)-CS were potent inhibitors of the effector phase; however, the other derivative, (Val^2)dh-CS, which was clearly active in other DTH models, failed to significantly suppress

the effector phase in this particular model (413, 108). Antiinflammatory agents are not inhibitory and corticosteroids weakly effective in suppressing this DTH skin response; the antimitotic agent AZA was effective only in toxic doses (97, 112).

The effect of CS on the effector phase of the allergic contact reaction to oxazolone in the guinea pig was investigated, and also compared with that of other immunomodulating agents. A single intraperitoneal or oral dose of CS (80 mg/kg) given with the oxazolone challenge markedly suppressed the appearance of erythema and oedema as well as all components of the dermal cellular infiltrate. This effect was still evident 72 hours after CS administration. In addition the same dose of CS had no effect on the toxic contact reaction to croton oil (19, 18). CS had by far the most marked capacity to suppress the allergic contact reaction to oxazolone when compared with CP, MTX (methotrexate) and AZA, thus indicating that it acts on the cell-mediated response rather than exerts mere nonspecific antiinflammatory effects (19). Using the DTH reaction to ovalbumin in the guinea pig, it was demonstrated that CS (25 mg/kg/d orally) given for 2 weeks following sensitization caused profound suppression of the DTH reaction elicited 14 days later. A very marked impairment of DTH was also observed when the drug was given at the time of skin testing, i.e., 24 hours before and 6 and 24 hours after antigenic challenge (925).

It is worth noting, that topical application of CS to the test site substantially inhibited the effector phase of contact sensitivity reactions to DNFB or DNCB in guinea pigs (77, 10, 684). Local elicitation was blocked in previously DNCB-sensitized animals that had received a single topical application of 15% CS in an Azone-containing vehicle just prior to challenge. However, elicitation was not blocked at a distant site, indicating a local effect of topical CS (77). Contact sensitivity skin reactions to DNFB were inhibited by twice daily topical application of CS (2% in ethanol: olive oil, 1:2) starting 24 hours before challenge and continuing for 3 days. Histological examination confirmed that this suppression was associated with marked inhibition of the characteristic mononuclear inflammatory cell infiltrate. Serum levels of CS remained below the sensitivity limit of the radioimmunoassay (10). The suppressive effect of topical CS to DNCB sensitivity was short-lived and reversible, since CS was not effective when given 6 hours or later after antigenic challenge. CS had no effect on the toxic contact reaction in normal animals either to croton oil or to DNCB in high concentration, which findings confirm its specific anti-T cell-mediated effects (684).

c. DELAYED-TYPE HYPERSENSITIVITY IN CLINICAL TESTS. Patients with chronic uveitis treated with CS were immunized with KLH and tetanus toxoid. Cutaneous DTH responses, mitogen-induced lymphocyte blastogenic responses, and antibody production were compared with those of similarly immunized control individuals. A significant decrease in DTH for both antigens was observed, but no significant differences in blastogenic or antibody responses were noted (747). These findings demonstrate that the majority of patients treated with CS had intact responses to T cell-dependent antigen as measured by both proliferative response and antibody production, but that other immune functions such as DTH are affected by therapeutic doses of systemic CS. The thymus-dependent primary immune response to KLH was measured in controls and in bone marrow transplant recipients treated with CS. DTH developed at the site of immunization in 68% of controls and in 88% upon subsequent challenge with KLH. None of the transplant recipients on CS developed DTH. When reimmunized only one out of eight patients made a primary DTH and antibody response, arguing that CS inhibited priming as well as any detectable response to KLH (16). The effect of topical CS on the elicitation/effector phase of allergic contact dermatitis to a variety of allergens was investigated in patients with known contact sensitivities to a variety of allergens. After a 48-hour pretreatment with 10% CS dissolved in Labrafil, skin sites were exposed to the appropriate contact allergen and 72 hours later the development of an eczematous patch test reaction was evaluated. Only one out of ten patients showed unequivocal signs of suppression of the skin hypersensitivity reaction (177).

d. CHRONIC HYPERSENSITIVITY PNEUMONITIS AND CHRONIC GRANULOMA. A rabbit model of chronic experimental hypersensitivity pneumonitis and desensitization was used to evaluate the effects of systemic CS. When administered (15 mg/kg subcutaneously) 12 to 18 hours before each inhalational challenge with aerosolized antigen (ovalbumin) and the adjuvant muramyl dipeptide, CS suppressed the development of disease as well as the anamnestic antibody response, particularly in bronchoalveolar lavage fluids. When administered at the time of sensitization only, CS (50 mg/kg by toepad injection) suppressed the primary antibody response, but not the anamnestic antibody response or the disease. Antigen- and mitogen-induced blastogenesis was inhibited by CS in vitro (5 ng/ml), but antigen-specific blastogenesis was not abrogated by CS previously administered in vivo. These results indicate that CS caused a profound transient suppression on T_H and T_{DTH} cell subsets (531).

The development of a state of cell-mediated hypersensitivity to bacterial products is probably responsible for the lesions associated with bacterial allergy such as granulomatous lesions. When the struggle between the replicating bacteria and the body's defenses fails to be resolved in favour of the host, persisting antigen provokes a chronic local DTH reaction. The effects of CS on granuloma formation caused by bacteria are discussed

in part IV.E.3.b. The numerous reports from the literature convey the message that the effects of CS on granulomatous reactions are far from clear and that the various models used are not directly comparable, since granuloma formation can be T cell-dependent or independent. This is shown in the following experiments with schistosomal eggs.

The modulating effect of CS was studied in the immune-based pulmonary granuloma response to *Schistosoma mansoni* eggs. In this mouse model, the inflammatory response is maximal 2 weeks after egg injection. Oral administration of CS (50 mg/kg/d) during the first or first two weeks of the response dramatically enhanced the levels of granuloma response in the lungs, but not when given from days 8 to 14. This enhancing effect was seen in high- and low-responder strains, but not in athymic nude mice, indicating that granuloma response and CS-induced enhancement were possibly dependent on functional T cells. While CS and CP augmented the inflammatory process, dexamethasone reduced the granuloma response compared with untreated, egg-injected controls (636). In contrast, granuloma initiation is considered as T-cell-independent, but T-cell participation is thought to enhance tissue reaction, in a model in which the grafting of hepatic schisotosomal egg granulomata from athymic mice elicits a granulomatous response in both naive athymic and euthymic mice. Euthymic mice treated with CS (150 mg/kg/d intramuscularly) for 2 weeks were injected with hepatic granulomata, isolated from mice with schistosomiasis, into their skin and CS treatment was continued for 3 additional weeks. Morphologically identical granulomata developed in both treated and untreated mice. Examination of T-cell functions strongly suggested that intact T-cell activity is not essential for the initiation of granuloma formation. In addition, granuloma grafts appeared to stimulate CS-resistant T-cell activation locally, which amplifies and organizes the granulomatous response (897).

e. MECHANISM OF ACTION OF CS IN DTH REACTIONS. Though the precise mechanism of the DTH response is not yet known, it is characterized by three main features: 1) the hard swelling of the injection site, caused by cellular infiltration; ii) the erythema, caused by the damage of underlying blood vessels and leakage into the tissues; and iii) the necrosis which is caused by enzymes and mediators released by activated monocytes and lymphocytes. The DTH reaction has thus two components: a minor specific or immunologic component consisting of few sensitized lymphocytes that, upon antigenic challenge, start the reaction, and a major nonspecific or inflammatory component, triggered by the products of the stimulated lymphocytes and responsible for most of the tissue damage. In addition, immediately after the second antigen exposure, a short-lived and transient state of desensitization occurs which may be due to the very few sensitized lymphocytes left to respond to a further antigenic challenge (524).

It is evident that CS inhibited both the induction and the effector phases of the DTH response in several species, including man. This suppressive effect was independent of the type of antigens used (chemicals, proteins, virus or spleen cells). Both the primary and the secondary DTH reactions were abrogated by appropriate treatment with CS and the secondary response was suppressed independently of whether the primary response had been modulated by the drug or not (107). If the DTH reaction was elicited by injection of activated, antigen-specific, cloned T_H cells, CS given up to 10 hours later was efficacious in preventing the reaction, but not when given after 15 hours (402). In contrast, when the challenge was induced with antigen, the effector phase was inhibited under the condition that CS was given before and/or simultaneously with antigen. It is worth noting that topical CS application at the site of antigen administration also prevented the effector phase of the DTH reaction (77, 10, 684).

To avoid confusion the point should be stressed that CS has to be administered during the sensitization period for a significant length of time and in significant amounts in order to exert its inhibitory effect. When treatment protocols are altered such as treating animals shortly before immunization (750, 975), or for very short periods after the immunizing dose (925, 8, 976), an augmentation of the DTH reaction was observed which could also be transferred with spleen cells (975, 925). This stimulating effect, which is often erroneously designated as a paradoxical effect of CS, is known to be expected as a rebound phenomenon in all those situations in which a threshold dosage is administered; this stimulation, which is not limited to CS, can also be observed with other immunosuppressants administered in improper conditions. When CS was administered over a wide dose range and by several routes before an otherwise tolerogenic dose of SE, but the CS blood levels ranged below detectable levels (<45 ng/ml) at the time of immunization or antigen challenge, an increase in the absolute numbers of splenic CD4 cells was observed, whereas no alteration in CD8 cells was recorded (976). Moreover, the suppression by CS is reversible and of short duration, since a secondary challenge performed 96 hours after the primary one did elicit a positive DTH response (800).

It is obvious that the target cells for the action of CS in the DTH response are those mediating the specific or immunologic component of the reaction, because CS is known to have no direct effects on the nonspecific, acute inflammatory component (106, 19, 684, 235). Various studies have demonstrated that the DTH reaction could be adoptively transferred by CD5 nonadherent T lymphocytes which are the cells affected by CS (31, 565, 688). These cells are also found to release lymphokines in vitro when cultured with the relevant antigen. CS

interferes with the DTH reaction by presumably suppressing the release of lymphokines and other mediators of inflammation. It has been shown that addition of rIL-2, but not of γIFN, reversed the inhibition by CS (997). Supernatants from activated, antigen-specific T_H clone cultures are also capable of overcoming the effects of CS, but not of dexamethasone (403, 402). Similarly, CS injected 8 hours before local injection of an active supernatant from activated and sensitized spleen cells containing chemotactic mediators did not interfere with phagocytic cell recruitment (855). Moreover, ex vivo and in vitro assays have clearly shown the efficacy of CS to impair antigen-specific induction of lymphokine release by sensitized lymphocytes, such as macrophage procoagulant activity and chemotactic mediators (926, 855, 653).

Of particular interest is the finding that CS did not interfere with the clonal expansion of antigen-specific DTH-mediating cells (653). This implies that DTH precursors are not sensitive to CS inhibition. The use of an in vitro system strongly supported this interpretation, since cells recovered from 6-day SE-stimulated spleen cell cultures were able to induce a DTH reaction to SE after transfer to naive mice even though CS was present during the culture period (857). The authors suggested that only the effector functions of the T_H and T_{DTH} lymphocytes were suppressed by CS, but that their proliferative capacity was resistant to the drug (940). This was clearly confirmed in other transfer experiments which showed that the induction of T_{DTH} effector cells was insensitive to CS, whereas the effector function of this T_{DTH} subpopulation was CS-sensitive (125). The results of Palestine et al. (747) in uveitis patients also seem to indicate that the peripheral blood lymphocytes retained a normal proliferative response (ex vivo) under CS therapy, while the DTH immune function was suppressed. In experiments on the effects of CS on antibody production in mice, Klaus and Kunkl (523) observed that in vivo CS could dissociate effector function (primary help) from T_H-cell proliferation (priming). Additional evidence supporting this contention was obtained in lung allotransplanted tolerant dogs. Unresponsiveness was a selective phenomenon in that donor alloantigens in mixed lymphocyte culture (MLC) induced a strong proliferative response of T cells from tolerant recipients ex vivo, but the donor-specific T_C activity was very low compared with that to third-party alloantigens (711).

There is some controversy concerning the effect of CS on suppressor cells in DTH reactions, possibly depending on the models used. When using the DTH response to alloantigens in the mouse two independent papers reported that induction of suppressor lymphocytes was not affected by concomitant CS treatment. Herrmann (402) had previously established, as a control, that CS did not inhibit the induction of T_{DTH} precursors. Mice were first sensitized intravenously with syngeneic (control) or allogeneic spleen cells and 7 days later immunized subcutaneously with allogeneic spleen cells. A challenge was given after 6 days by injecting again allogeneic cells into the footpad. The results demonstrated a markedly impaired DTH response in the mice sensitized twice with allogeneic lymphocytes compared with the fully developed reaction occurring in those mice receiving first syngeneic cells. When the animals were now treated with CS (range of 12 to 75 mg/kg/d orally) for 4 days starting on the day of primary sensitisation with allogeneic cells, the ensuing response remained depressed, indicating that the suppressor cells had been normally induced in spite of CS treatment. The derivative (Val^2)dh-CS also spared the suppressor cells, but treatment with 2′-deoxyguanosine (50 mg/kg/d for 8 days) strongly impaired their development (402). Mirisklavos et al. (655) used a similar model of DTH response to alloantigens in mice, but analyzed the effect of CS by transferring spleen cells from sensitized and CS-suppressed mice into irradiated naive recipients. Concomitant treatment of mice with alloantigen and CS (100 mg/kg/d intramuscularly on days 0, 4, and 6 after sensitization) suppressed the DTH response to alloantigens, and spleen cells transferred from those suppressed mice abrogated the response of sensitized cells co-transferred at the same time. The splenic T_S cells were MHC-restricted and antigen-specific with the surface markers Thy 1.2 and CD8 (655). Thus, it generally appears that in alloreactivity systems (cf., IV.C.2.e) CS spares or even promotes the induction of T_S cells.

In DTH models using chemical contact sensitizers, however, there is evidence, though in our opinion less convincing, that CS prevents the development of suppressor cells. It is claimed that intravenous overloading with the contact sensitizer 2, 4-dinitrobenzene sulphonic acid activates the T_S circuit which results in a decreased DTH response. Treatment of mice with a suboptimal, low dose of CS (12 mg/kg/d orally) during the sensitization period (days −1, 0, 1) with an antigen overload dose resulted in increased contact sensitivity. It was concluded that CS blocked the induction and functional expression of an afferent-acting T_S cell which was CP-sensitive, and was induced by a high dose contact sensitizer and assayed by transfer to naive recipients (125). When CS was administered during sensitization (on days 0 to 4 only) to guinea pigs, a reproducible enhancement of the DTH response to ovalbumin was obtained which could be adoptively transferred by systemic injection of pooled spleen and peritoneal cells. However, the expression of the augmented response was lost by additional transfer of cells from normally immunized donors at the time of skin testing. These data were interpreted as supporting the concept that augmentation of DTH by CS was presumably attributable to antigen-specific suppressor cell impairment (9). We would rather favour an alternative explanation, namely the enhanced DTH re-

action observed in both cases being due to a rebound phenomenon induced by a suboptimal CS dose (125) or by a very brief treatment (9) (see also table 1 in reference 806).

2. Local graft-versus-host and host-versus-graft reactions. The local graft-versus-host (GvH) reaction, which is also called local lymph node weight assay or Simonsen test, is an assay for measuring alloreactivity and is mainly performed in rats (284). Recipient F_1 hybrid rats are injected subcutaneously into one hind footpad with viable donor parental spleen cells (this combination avoids the host-versus-graft (HvG) reaction) to induce the GvH response. Seven days later both popliteal lymph nodes are removed and weighed. The weight difference between the ipsilateral (increase due to GvH reaction) and contralateral (normal control) lymph nodes is taken as the parameter for evaluating the reaction (806).

CS (50 mg/kg/d orally) given repeatedly on or after the day of sensitization strongly suppressed the local GvH response. In contrast, delayed treatment on days 4, 5, and 6 with the same dose did not significantly alter the ongoing immune reaction. A single dose of CS administered simultaneously with cell transfer profoundly augmented the response, in a manner reminiscent of the DTH reaction (9, 8, 976). Treatment with CS alone (no donor cells) exerted a negligible effect on lymph node weight, thus indicating that it was preventing proliferation without being lymphocytotoxic (806). As seen in table 2, other cyclosporins have also been shown to exert a potent suppressive effect in this assay for cell-mediated alloreactivity (413, 412).

In vitro incubation of parental donor spleen cells with CS (50 μg added to 25 $\times$ 10^6 cells/ml) prior to transfer into F_1 hybrid rats, markedly reduced the local GvH response. Inhibition of this response was reproducibly achieved with CS dissolved in two different solvents. However, when the compound was added in the form of finely dispersed crystals in Hank's solution, no suppression was observed. Surprisingly, if the concentration of undissolved CS was increased up to 500 μg, a highly significant enhancement of the local GvH reaction was measured, implying that this extremely high concentration was not cytotoxic, since only viable donor cells are able to elicit a response (806). Furthermore, when the donor cells were injected on day 0 into recipient rats which were in a steady state relative to CS levels, i.e., treated continuously from day -10 to 4, the ED50 values for oral administration could be lowered by a factor of at least 4 and for subcutaneous injection of about 10 (J. F. Borel; unpublished data).

Marwick et al. (616) have not only demonstrated the efficacy of CS to inhibit the GvH response, but also the HvG reaction, which was elicited by injecting F_1 hybrid spleen cells in rats of the parental strain. With the CS treatment protocol they were using, the popliteal lymph node enlargement was never completely abolished, and splenic lymphocytes from CS-treated recipients showed no significant reduction in their response to donor-strain lymphocytes in MLCs, suggesting that clonal deletion had not taken place (616).

The local HvG model is a modification of the local GvH model in that the allogeneic lymphoid cells are mitomycin C-treated before their transfer injection (225). This particular system confines the response to the single popliteal lymph node, and very few if any of the allogeneic stimulator cells reach the lymph node. Assessment of activation status of lymphocytes in the node by phenotype analysis or by ^{3}H-thymidine uptake can therefore by unambiguously attributed to the responding lymphocytes. The recipient rats were treated with CS (15 mg/kg/d intramuscularly) from day -2 until 4 when the lymph nodes were removed. The drug did not prevent the early increase in lymph node weight during the first 24 hours of the response. However, by day 4, when the increase is maximal in untreated recipients (225), the lymph node weight in CS-treated recipients was reduced by approximately 50% (166). In additional experiments, popliteal lymph node cell suspensions were subjected to a phenotype analysis designed to investigate changes in the surface glycoproteins expressed on T cells during activation: induction of class II MHC molecules and of IL-2R. Surprisingly, CS had no effect on the phenotypic changes seen in the lymphocyte populations found in the draining nodes. Within the first 24 hours a substantial proportion of the large lymphocytes in the nodes expressed class II molecules and this proportion remained high for the next 4 days. The early rise in blast cells expressing IL-2R in both control and CS-treated animals fell by day 4 to about 50% of maximal. The small lymphocytes within the same populations showed none of the changes described. This phenotypic analysis and the finding that activation proceeded as far as DNA synthesis (tritiated thymidine incorporation) suggested that CS had little or no inhibitory effect on the early stages of lymphocyte activation within the lymph node (166).

The increase in cell number in the involved lymph node derives from a combination of proliferation of the lymphocytes with specificity for the antigen involved, i.e., clonal expansion, and a nonspecific retention of lymphocytes from the recirculating lymphocyte pool that are arrested in their normal migration through the node, probably as a consequence of lymphokines released by the activated cells. The results obtained with this model suggest that CS had little effect on the activation and proliferation of responding T cells within the node, but did inhibit the local lymphokine release responsible for lymphocyte recruitment to the node. A comparison with the results obtained in a systemic GvH reaction (167, 166) and this local HvG response confirmed that CS appeared in neither in vivo systems to suppress lymphocyte activation as assessed directly by phenotype analysis

of and DNA synthesis by the responding lymphocytes. This confirms the dichotomy of the action of CS in vivo as discussed above (IV.B.1.e.), showing that proliferation (priming) is resistant but the effector function is sensitive to CS.

It is worth noting that in the systemic GvH reaction, CS (15 mg/kg/d intravenously starting 12 hours before cell transfer) treatment of the F_1 hybrid recipients did not prevent the selective sequestration of antigen-reactive cells into the spleen and lymph nodes and their activation as assessed by ^{3}H-thymidine incorporation. In the untreated F_1 hybrid, from 36 hours after injection, large number of dividing blast cells were released into the lymph. However, these cells did not appear in the lymph of recipients treated with CS (167). These data are in agreement with others, demonstrating that in unresponsive rats bearing cardiac or skin allografts T_C cells against donor targets are found residing in lymph nodes, but not among lymphocytes taken from the peripheral circulation (985). Kroczek et al. (537) have used a local GvH assay in the mouse that allows a quantitative assessment of several of the early events in the activation of lymphocytes in response to alloantigenic stimulation in vivo. Their results also showed that CS (range of 1.5 to 15 mg/kg/d intraperitoneally starting 36 hours before cell inoculation) administration in vivo had no effect on alloantigen-induced increases in cell size, percentage of cells expressing the IL-2R, the spontaneous or IL-2-driven proliferation of freshly explanted cells (ex vivo), or the induction of T_C lymphocyte activity (also ex vivo). Significant plasma levels of CS were achieved by the injection protocol used in this study (537). However, to the contrary of the local GvH model, CS (5 to 15 mg/kg/d injected intraperitoneally at 12-hour intervals beginning 36 hours before the injection of ConA) treatment of mice in the concanavalin A model was capable of inhibiting not only IL-2R induction but also proliferative responses with and without IL-2. Some reduction in cell size was also evident at the highest doses used (78). Butler et al. (136) have reported the effects of several immunosuppressants on the in vivo expression and regulation of murine IL-2R after sensitization with the contact sensitizer picryl chloride. Treatment of mice with CS (100 mg/kg/d orally for 3 days beginning on the day of sensitization) reduced both the proliferative responses as well as the number of IL-2R-positive cells. These findings taken together might possibly indicate that the mechanisms controlling the activation pathways in response to allogeneic cells, Con A and picryl chloride may differ markedly in vivo. (See also discussion on the mechanisms promoting unresponsiveness: IV.C.2.e.).

3. T cell-mediated cytotoxicity. In the first paper mentioning 'cyclosporin A', we analyzed in a comparative study its suppressive properties on T cell-mediated cytotoxicity in three different experimental protocols (96). Firstly, CS exerted a profound and dose-dependent ex vivo inhibition of allogeneic target cell lysis when administered repeatedly during the sensitization period of mice immunized with allogeneic spleen cells (in vivo oral treatment on days 0 to 7). The drug depressed both primary and secondary responses by preventing the development of effector T_C cells. Secondly, when a single, high dose of CS was given around 9 to 11 days post-immunization, i.e., 16 hours before sacrificing fully sensitized mice, CS did not affect the existing T_C cells (ex vivo). Thirdly, CS failed to suppress cell-mediated cytotoxicity after in vitro addition to sensitized mouse spleen cells. These results clearly indicated that CS was only suppressing the development of T_C cells, but completely failed to modify the cytolytic function of these cells both in vivo and in vitro.

Further studies, especially in allografted animals, confirmed and extended these early findings. Cells were recovered from allogeneic rat kidneys, transplanted into normal recipients or recipients given CS (10 mg/kg/d orally on days 0 to 4), and tested ex vivo for specific and nonspecific cytotoxicity. At this dose CS completely abrogated the rejection process, but did not substantially suppress the massive influx of mononuclear cells that infiltrate a rat renal allograft within 5 days of grafting; however, the number of CD8 cells was clearly reduced compared with that from rejecting animals. Moreover, cells harvested from kidneys undergoing unmodified rejection possessed both donor-specific and nonspecific cytotoxic effector populations, whereas those obtained from kidney allografts in CS-treated hosts, while largely retaining nonspecific cytotoxicity, were very deficient in donor-specific activity (617). These results may partly explain the antirejection effect of CS, since it was demonstrated that specific T_C cells, rather than nonspecific responses, play an essential role in allograft rejection in the rat (124). These observations were substantiated in bone marrow as well as in lung transplanted dog experiments. Indeed, it was shown that DLA (dog leukocyte antigens) haploidentical littermate chimeras, receiving long-term treatment with decreasing doses of CS (MTX had been added on days 1, 3, 6, and 11), failed to produce T_C cells directed against host targets or unrelated targets from dogs homozygous for one of the host's DLA haplotypes. However, the chimera lymphocytes exerted rigorous cytotoxicity against targets from third-party donors. In contrast, lymphocytes from DLA nonidentical chimeras were unable to generate T_C cells both against host and histoincompatible third-party targets (200). In the lung allograft dog experiments, a mitogen-dependent cell-mediated cytotoxicity assay was used to examine the development of the total intragraft and peripheral blood T_C-lymphocyte activity during CS dose tapering. It was demonstrated that intragraft cell-mediated cytotoxicity remained low during the periods of unresponsiveness and increased upon onset of rejection. In contrast, peripheral blood cell-mediated cytolysis did not correlate well with

evidence of rejection, because it was usually absent (710). Using the MLC assay, it was found that lymphocytes from unresponsive lung allograft recipients had a diminished ability to generate donor-specific T_C cells, but retained normal cytolytic responses to third-party allogeneic stimulator cells, thus demonstrating specificity of the tolerant state. In contrast, high levels of specific T_C cell activity were detected in cell preparations from lung allografts undergoing rejection (711). Rejection phenomena observed after termination of CS therapy were reversed by resumption of CS treatment, which caused also a decrease in T_C-lymphocyte activity (710, 708).

The effect of CS on T_C cells was also investigated in several infectious models. Oral doses of CS (200 mg/kg/d) on 5 consecutive days, the first being given 2 hours before influenza virus inoculation of mice, strongly suppressed the induction of primary T_C cells (24). Complete suppression of T_C-cell responses against both vaccinia virus and lymphocytic choriomeningitis virus (LCMV) could be observed only when CS treatment was started immediately before immunization. If treatment was started later than 48 hours after the initiation of infection, T_C-cell activity to vaccinia virus reached normal levels or was reduced to 20% of that of control mice to LCMV; whereas beginning 96 hours after infection resulted in a normal generation of T_C cell activity to LCMV (449). In contrast, CS (50 mg/kg/d intraperitoneally from day 0 to 9) treatment of mice infected with murine cytomegalovirus (CMV) (day 0) did not appear to influence the development of MHC-restricted virus-specific T_C cells (129).

Hodgkin et al. (423) have demonstrated that lymphocytes recognizing class I alloantigens were able to lyse appropriate target cells and to release lymphokines in vitro. However, the relative contribution of these activities to in vivo functions of these T_C cells is unclear, but it should be possible to discriminate between them using CS, since the drug inhibits lymphokine release from class I-specific T_C cells but has no effect on their cytotoxic activity. The in vivo function of class I-specific T_C cells was analyzed in two models; the local GvH reaction induced by transfer of sensitized T_C cells to the footpad in mice and the islet allograft rejection model in the mouse induced by the passive transfer of sensitized T_C cells. CS (75 mg/kg/d subcutaneously) inhibited the in vivo functions of the transferred sensitized T_C cells in both systems; hence, these functions appeared to be lymphokine-dependent (423). Similar findings were also obtained in a murine influenza virus model. CS inhibited the function for transferred, influenza-specific, class I-restricted T_C cells which normally led to clearance of virus in the lungs of infected mice. The results strongly suggested that the in vivo clearance of influenza virus by class I-restricted T_C cells involved a lymphokine mechanism (830). In conclusion, these data demonstrate the ambivalence of this T-cell subset in relation to biological function; the cells express direct cytotoxic activity and produce lymphokines. The cytotoxic activity of the class I-restricted T_C cells appears dependent upon the latter activity.

This generalized statement may not always apply, because there are models showing that allograft rejection is not dependent on lymphokine release. Noble and Steinmuller (706) treated specifically sensitized T_C cells in vitro only, not host mice, with CS and washed these cells prior to injection. They used a murine skin allograft model in which T_C lymphocytes generated against epidermal alloantigen-1 (Epa-1), a tissue-restricted, non-H-2 alloantigen that is a target-cell determinant of both skin allograft rejection and cutaneous GvH reaction, directly produce full-thickness ulcerative skin lesions in Epa-1^+ mice. Anti-Epa-1 T_C cells also indirectly cause an 'innocent bystander reaction' (when injected admixed with Epa-1^+ target cells into the skin of Epa-1^- hosts), which unlike the direct destruction of host tissue by T_C cells in immune lymphocyte transfer reactions, is initiated by the release of lymphokines that recruit host inflammatory cells to the injection site. Treatment of T_C cells with CS in vitro abrogated IL-2 production, but did not affect cell-mediated cytolysis. Moreover, this in vitro CS treatment did not impair the ability of T_C lymphocytes to evoke immune transfer reactions, nor did it significantly affect their ability to mediate the bystander reaction. Therefore, when T_C cells were treated in vitro with CS in such a way that they lost their capacity to produce IL-2, their cytotoxic activity in vitro as well as their ability to directly and indirectly mediate tissue destruction in vivo were left intact. It was concluded that the ability of T_C lymphocytes to mediate allograft rejection may not be dependent on their ability to produce IL-2 and that cell-mediated cytolysis may play a direct role in the rejection process. However, these studies differ from the previous ones (423, 706) in the antigen differences (H-2 vs non H-2) and target tissues (cultured islets lacking class II-bearing leukocytes vs normal skin). Since the suppressive effects of CS are reversible, it can not be excluded that the in vitro CS-treated T_C cells might regain their ability of producing lymphokines once transferred into the host environment. In contrast to the direct tissue destruction seen in the above immune transfer reaction, the nonspecific tissue destruction in the bystander reaction appears to result indirectly from the antigen-specific interaction of T_C cells and admixed target cells. This reaction may possibly be triggered by host cells producing the relevant mediators in response to the cytolytic process, since injection of allogeneic epidermal cells without admixed T_C cells did not induce the bystander reaction (706). This example proves once more the difficulty in bridging in vitro and in vivo results and also in analyzing in a stringent way in vivo events.

Orosz and coworkers (736, 735, 734) have further investigated the effect of CS on T_C-cell modulation by

attempting to correlate results from both in vitro and in vivo models. Under limiting-dilution culture conditions, CS was shown to block terminal T_C cell clonal expansion. These results would suggest that the signaling systems from proliferation and cytolytic function in T_C cells are separable and differentially sensitive to CS. This hypothesis would also imply that CS has an inhibitory effect on T_C lymphocytes, which is independent of T_H-cell dysfunction (735, 734). Studying the effect of CS on the alloantigen and/or lymphokine-driven proliferation of murine T_C cells clones, it was observed that lymphokine-driven proliferation of T_C cell clones continues in the presence of CS, but that alloantigen does not synergistically increase lymphokine-driven proliferation if CS is present (735, 734; see also 743). The sponge matrix model of allograft rejection has been used to study in vivo immunologic events associated with allograft rejection. Sponge matrix allografts accumulate both donor-reactive T_C lymphocytes in an active cytolytic state and donor-reactive alloantibodies within several days of allograft implantation (736). The fluids from sponge matrix allografts did also allow measuring the concentration of drug. CS interfered with the accumulation of donor alloantigen-reactive T_C cells in sponge matrix allografts by markedly reducing their number in a dose-dependent manner as determined by limiting dilution analysis. However, CS little affected the number of donor-reactive T_C lymphocytes in the peripheral blood. Consequently, it appeared that the donor-reactive T_C cells were available, but were unable to enter the graft site under the influence of CS. Optimal impairment of T_C cell accumulation required the daily delivery of 30 mg/kg/directly to the graft site or, when CS was injected into a distant subcutaneous site, about 50 to 70 mg/kg/d were necessary to achieve similar levels of immunosuppression (735). Further experiments demonstrated that CS could differentially impair the accumulation of donor-reactive T_C cells, but not donor-specific alloantibodies in sponge matrix allografts (736). Acute rejection is primarily a T-cell-mediated phenomenon, whereas hyperacute and possibly chronic rejections are mediated by alloantibody. The above results would suggest that CS therapy would be effective only against acute rejection, and that antibody-mediated forms of rejection would be rather unresponsive to CS treatment. Clinical observations appear to support this suggestion.

4. Natural killer cells and tumour cells. a. EFFECT ON NATURAL KILLER CELLS IN ANIMAL MODELS. Natural killer cells (NK) appear closely related to killer cells mediating antibody-dependent cell-mediated cytotoxicity and are part of the natural or innate immune system. They are members of the T-cell lineage, but they are thymus independent and do not discriminate self from non-self components. They mediate cytolysis through direct cell contact without prior sensitization. The activity of NK cells is not MHC-restricted and their repertoire of separate specificities recognized is considerably smaller than that of T_C cells. Their heterogeneity has been suggested to reflect different stages of differentiation of the same cell type. NK cells lack most T-cell markers, but bear receptors for the Fc portion of IgG (FcγR), and are CP- and cortisone-sensitive; they are activated by γIFN and their morphology is that of large granular lymphocytes. They need IL-2 to grow in culture conditions but do not require additional stimulation by lectins or antigens which delineates them from the requirements of T_C lymphocytes. The functional role of NK cells in vivo may be severalfold: in vivo resistance to growth of NK-susceptible tumour cell lines, resistance against metastatic spread of tumours and against growth of primary tumours, natural resistance against primary infections, natural resistance against bone marrow transplants, some role in GvH disease, and possible regulation of differentiation of normal haematopoietic or other cells (for review see 400).

There is some controversy concerning the in vivo effect of CS on NK cells. A 5-day treatment of mice with CS (70 mg/kg/d orally) caused a short-lived decrease in splenic NK activity when the test was performed 2 hours after the last CS dose. Total recovery of the NK activity was, however, observed when the ex vivo assay was performed two days later (5). Mice treated with a single intraperitoneal dose of CS (50 mg/kg) exhibited a marked but not total inhibition of natural killing by spleen NK cells. CS added in vitro also significantly depressed NK activity and its action seemed to be caused by direct interaction with NK cells (1001). CS-treated mice (50, 100 or 150 mg/kg/d intraperitoneally on days 0 to 3) were inoculated with replicating murine cytomegalovirus and/or nonreplicating Newcastle disease virus and sacrificed on day 3 to assess ex vivo their spleen cell NK activity. CS inhibited in a dose-dependent manner the ability of these two viral IFN inducers to enhance NK activity, although it did not significantly suppress IFN induction by Newcastle disease virus. In vitro it had no effect on NK activity per se, but it markedly inhibited the ability of IFN to enhance NK activity. NK cells are ordinarily activated by the IFN induced by these two viruses, and if that action is blocked (CS exerted a clear IFN-blocking effect), then NK activity is not stimulated (358). Early NK activity after herpes simplex virus 2 infection is an important cell function which correlates with resistance in most mouse strains. CS (50 mg/kg/d intraperitoneally on 4 days, beginning 3 days before infection) treatment totally inhibited or drastically reduced NK activity. This inhibitory effect was transient. In contrast, oral administration of CS did not affect intraperitoneal NK responses, whereas it induced a complete suppression of T_C-cell responses against influenza A virus. It was again observed, that CS therapy did not diminish the enhanced IFN levels induced by herpes virus (26).

In vivo treatment of mice with most immunosuppressive agents dramatically reduced in vitro cytotoxicity against NK-sensitive targets by direct reduction in either percentage specific lysis or lytic units per spleen. In most cases in vitro addition of rIL-2 enhanced NK activity of treatment groups to a normal value which, however, remained below the rIL-2-enhanced range of nontreated animals. In vivo treatment with CS (100 mg/kg as a single intraperitoneal dose 24 hours before sacrifice) did not affect NK activity and addition of rIL-2 augmented natural cytotoxic activity in a similar fashion to the profile of naive cells (3). The systemic NK activity was studied both in untreated rats which acutely reject allogeneic heterotopic heart grafts and in CS-treated (15 mg/kg/d intramuscularly on days 0 to 6) rats which tolerate their transplants. The trend and magnitude of changes in NK activity were similar at all time points for the two animal groups. Compared to naive rats, peak NK activity was noted 7 to 8 days after engraftment in untreated rats and 7 to 12 days after engraftment in CS-treated hosts. In both groups, NK activity returned to normal levels by 3 weeks. In addition, no evidence could be found for inactivation of NK cells or their precursors in vivo in ungrafted rats undergoing CS treatment alone (947).

b. EFFECT ON NATURAL KILLER CELLS IN MAN. The effect of CS on NK cells in immunosuppressed patients seems minor or nonexisting; in some studies it is blurred by the co-medication of other potent immunosuppressants. Landegren et al. (564) have used human peripheral blood lymphocytes which, when activated in MLC, express cytolytic activity against a range of target cell types. CS at 1 μg/ml efficiently prevented the development of T_C cells directed against target cells expressing the phenotype of the stimulator cells while not preventing an increase in NK activity measured against tumour targets. The drug was further shown not to influence the short-term induction of increased NK activity by IFN. Since already established T_C cells were not sensitive to CS, the drug, therefore, seemed to be selectively involved in the induction of immune responses requiring T-cell proliferation. The only study reporting a significantly decreased mean NK activity and ability of IFN to enhance in vitro this NK activity in the first 18 week posttransplant period was performed with cells derived from renal recipients treated with high-dose CS and prednisone (359). However, another group reported that the impaired NK function in renal transplant patients receiving CS as their sole immunosuppressant was partially restored by IFN stimulation (312). In contrast, in a prospective, longitudinal study of sixteen CS-treated renal allograft recipients, NK activity was not significantly altered during the first 6 months following transplantation from pretransplant values (574). Gaeta et al. (303) did not observe in high-dose CS-treated transplant recipients any adverse effect of CS on NK function; in the cases of viral infection the NK response was higher than in patients without seroconversion. McGinnes et al. (625) reported that the low NK cell activity found in cardiac transplant recipients pretransplant was not due to low numbers of NK cells but rather to decreased lytic activity. During the first 10 days posttransplant, the number of NK cells was depressed and NK activity was low, although NK cell function and numbers did not correlate. This early posttransplant depression of NK cell numbers was probably the result of ATG therapy. Following the cessation of ATG, NK cell numbers and activity returned to within the control range by 3 months posttransplant (625). Moreover, in a follow-up study of recent-onset type I diabetes mellitus under CS treatment, Müller et al. (675) reached the conclusion, that NK activity did not seem to be severely impaired in these patients, while ADCC (antibody-dependent cell-mediated cytotoxicity) experienced a significant decrease. CS therapy neither decreased the normal NK levels nor impaired the normalization of ADCC under insulin treatment. Finally, a recent study was conducted by Versluis et al. (963) in eleven recipients of cadaveric renal allograft with stable function before and after being converted from CS to AZA therapy one year after transplantation. This prospective study was undertaken to assess the basal NK cell activity of peripheral mononuclear cells in renal transplant patients after long-term CS therapy and also investigated the capacity for stimulating NK cell activity in vitro by γIFN as well as for γIFN production after induction with concanavalin A. In stable renal transplant recipients NK activity after one year of continuous CS therapy was significantly better than 3 months after conversion to AZA and was nearly equal to that of healthy volunteers. A significant increase in NK cell activity in response to in vitro γIFN was observed in these CS-treated patients, whereas this γIFN-stimulated NK activity was profoundly decreased after conversion to AZA, suggesting a more profound effect of AZA on pre-NK cells. When monitoring NK subsets with MAbs (monoclonal antibodies), a significantly lower number of NK cells expressing the Leu-11a antigen was found after drug conversion. (Anti-Leu-11a detects an antigen associated with the FcγR present on NK cells.) No significant effect was observed in Leu-7$^+$ cells (a marker for large granular lymphocytes). The capacity to produce γIFN after in vitro mitogen stimulation of unprimed lymphocytes was increased 3 months after switching therapy from CS to AZA, suggesting a more profoundly inhibited but not deficient γIFN production under CS therapy (963).

In conclusion, there seems to be more stringent evidence for CS not influencing NK cell activity than the opposite. The results derived from mouse models are highly controversial, although the inhibitory effect of CS on NK cells was usually partial and transient (358). In a detailed study in transplanted rats, CS was reported to have no influence on NK activity (947). Concerning the

situation in humans all studies except one, in which patients were treated with high-dose CS and prednisone (359), demonstrated that CS did not negatively affect NK activity found in peripheral blood mononuclear leukocytes. On the contrary, CS was considered useful for discriminating between allogeneic T_C cell and NK cell cytotoxicity (564).

c. EFFECT ON TUMOUR CELL GROWTH IN VITRO AND IN VIVO. The cytostatic effect of CS (10 μg/ml) on murine mastocytoma cells (P-815X2) appeared marginal and may well coincide with the cytotoxic level of the drug. The difference between the IC-50 of CS for lymphocyte and for mastocytoma cell proliferation is about 2000-fold, while cytostatic drugs like cytosine arabinoside and colchicine inhibit both cell types at very similar concentrations (P. Hiestand and J. F. Borel, Sandoz, Basel; unpublished data). Two oncogenic murine lymphoid tumour lines (BW, a T-cell-like line and NSI, a non-Ig-secreting B-cell-like line) were cultured in the presence of increasing concentrations of CS and their growth rate (^{3}H-thymidine incorporation) measured. CS inhibited the growth of the BW T-cell line (≤100 ng/ml) at doses that failed to affect the NSI B-cell line (<100 μg/ml) (984). The in vitro effect of CS on tumour cell lines was further investigated in a joint but unpublished study by P. Alexander (Southampton, UK) and R. E. Handschumacher (New Haven, USA; personal communication). Several non-lymphoid cell lines and even murine leukaemias, generally classified as null or B-cell lines, were insensitive to as much as 10 μg/ml. However, certain T-cell lines (e.g., BW 5147 and S-49) bearing Thy 1 surface antigens were clearly inhibited by doses between 2 to 10 μg/ml. Moreover, the sensitivity of eighteen permanent haematopoietic cell lines to CS has been investigated by W. Knapp (Vienna, Austria; personal communication). Two human T-cell lines (MOLT4 and CEM) were significantly inhibited at a CS concentration of 0.5 μg/ml. All the other non-T-cell lines, including three human B-cell lines (4413a, Daudi, Raji), were not affected at all or inhibited only at 10 to 20 times higher concentrations. Thus, CS may selectively recognize a series of differentiated functions in lymphoid cells and their malignant counterparts, though in the latter at concentrations very much higher than those needed to inhibit mitogen-induced T-cell proliferation.

Clinical immunological studies have also assessed the antileukaemic effect of CS on the cells of a number of human haematopoietic biopsies. CS killed the tumour cells, but not the non-malignant control cells, from three of four patients with T-lymphocytic leukaemia/lymphoma at concentrations comparable with those recommended in clinical immunosuppressive therapy. The leukaemic cells from patients with B-type chronic lymphocytic leukaemia were resistant to CS. The drug was cytostatic and cytolytic to three of five T-lymphoblastic leukaemia cell lines tested (936). CS has finally been reported to inhibit T cell growth of peripheral blood mononuclear leukocytes from patients with Sézary's syndrome indirectly as a consequence of suppression of IL-2 production. CS did not interfere with intracellular events leading to the expression and the biologic function of the IL-2R both in Sézary and normal T lymphocytes (877).

The oncostatic activity of CS was evaluated in vivo in actively immunosuppressed mice inoculated with two lymphatic leukaemias (L 1210 and P 388). CS treatment did not influence the survival time in these mice, but dramatically reduced their spleen size compared with control mice. This yet unexplained effect occurred solely in leukaemic animals whose increased spleen weight is mainly due to leukaemia cell infiltration (H. Stähelin, Sandoz, Basel; unpublished results). CS was further screened in eleven murine transplantable neoplasms and revealed a significant increase in lifespan with some long-term survivors after intraperitoneal injections of drug with three ascites tumours (Taper liver, sarcoma 180 J, and Ehrlich ascites) (536). However, the effective doses were in most instances close to tolerable toxicity. A detailed study was performed with the HRL leukaemia which arose spontaneously in a Hooded rat and resembles human T-cell acute lymphoblastic leukaemia. Even when CS was given at the maximum doses which the rats would tolerate, it exerted no significant antileukaemic effect (13). Also in the rat, the transplantable Roser T-cell leukaemia exhibits features of human acute T lymphoblastic leukaemia and, in particular, the blast cells have been shown to have a T_H phenotype. In leukaemic animals, all organs examined, apart from the testes, showed infiltration by malignant lymphoblasts. In CS-treated rats (25 mg/kg/d orally from days 0 to 14), there was no reduction in the extent of lymph node involvement and other organs, but the spleen showed areas of residual white pulp. However, by day 17 there was a 12-fold reduction in the mean number of peripheral lymphoblasts. Withholding CS treatment until day 14 resulted in a significant reduction of leukaemic blasts on day 19 (870). This antileukaemic effect, however, was achieved at a dose of 25 mg/kg/d, which is at the lower end of the nephrotoxic range in this species. Moreover, three T-cell lymphomata in mice were not affected in their growth rate by CS-treatment. However, the drug-treated animals died more rapidly than controls because of enhanced metastatic dissemination (13). Making a number of simplistic assumptions leads to the conclusion that approximately 10 times the immunosuppressive dose or more would be required to reach a threshold antitumour action, i.e., halving in growth rate. These high CS concentrations can not be maintained in vivo because of excessive toxicity.

It is evident that for certain tumours, which are demonstrably immunogenic, T-cell function is required not only for the initiation of an immune response but also

for its maintenance, even after primary tumour resection. Therefore, when CS, given orally or parenterally in repeated doses, was investigated for its effect on a variety of syngeneic mouse and rat tumours, including sarcomas, carcinomas, and a T-cell lymphoma, they showed a marked increase in their propensity to grow metastases, in some cases even when administration of the drug was delayed until after excision of "primary" tumour implants. In contrast, no effect of CS on metastasis was observed in rats bearing poorly immunogenic mammary or squamous-cell carcinoma. The metastases developing in CS-treated animals, when transplanted into normal syngeneic animals, showed no evidence of enhanced metastatic potential compared with their "parent" tumours (245, 13). Tumour growth, although at a normal rate, persisted unhibited in CS immunosuppressed mice injected intradermally with the highly malignant P-815 mastocytoma. As expected, the CS-treated animals did not develop in vitro T_C cells neither in the spleen or in the draining lymph nodes (68). In another experiment, several spontaneously occurring solid canine tumours were transplanted into a group of normal adult dogs suppressed with CS therapy. Five out of six osteogenic sarcomas and the single thyroid adenocarcinoma proliferated into macroscopic tumours. When CS was stopped all but one tumour were rejected. Biopsies obtained during drug administration showed minimal necrosis and inflammation in the tumour; however, after cessation of CS, dense lymphocytic infiltrates and tumour necrosis were observed. Reactivity of lymphocytes from tumour transplant recipients in MLC was normal when carried out in normal serum, but was suppressed when serum from CS-treated dogs was added (198). These results demonstrate that CS allowed for engraftment of allogeneic tumours in normal dogs by interfering with host T-lymphocyte function. (See also last part of IV.C.1.d. for xenogeneic tumour transplantation.)

The influence of host immunity on the hepatic colonization of intrasplenically injected B16F10 melanoma cells in syngeneic mice was studied. CS (80 mg/kg/d subcutaneously) was used to treat tumour-bearing animals either from the time of tumour cell injection (days 1 to 5) or once micrometastases had appeared (days 7 to 12). CS treatment during the early neoplastic period enhanced the implantation in the liver of B16F10 melanoma metastases coincident with the lowering of both T lymphocyte and macrophage cytotoxic activity, although the drug did not seem to affect significantly the tumoricidal action of NK cells. When CS treatment was begun once the micrometastases had appeared in the liver, a significant doubling time enlargement of metastatic volume in liver was observed as reflected by reduction of tumour growth. In contrast to the latter finding, when CS was given until day 5, the number of micrometastatic foci was doubled and growth rate was comparable to that of untreated mice, in spite of the previous immunosuppression. In addition, CS (≥1 μg/ml) produced a direct antiproliferative effect on the B16F10 melanoma cells in vitro (52). The effect of CS-induced immunosuppression was assessed in a rat model of progressive and regressive colonic tumours. CS (20 mg/kg/d subcutaneously) injected for 30 days after tumour cell inoculation, drastically enhanced the local growth of the progressive tumour and increased the number of metastases. It also increased the local growth and prevented the regression of the regressive tumour which persisted long after discontinuation of CS treatment and occasionally yielded metastases. CS prevented the accumulation of inflammatory T-lymphoid cells at the periphery of both tumour types, but without altering the tumour infiltration by macrophages and NK cells. CS did not modify the natural cytotoxicity of peripheral blood mononuclear cells against tumour target cells (861).

Using the classical models of two-stage induction of experimental tumours (862), it was demonstrated that CS (diet containing 0.015% for 25 to 35 weeks beginning 7 to 10 days post-sensitization) enhanced the induction of thymic lymphomata in Swiss Webster mice initiated with a single subcarcinogenic dose of N-methyl-N-nitrosourea. The serum CS level in these mice receiving CS diet for 4 weeks averaged 453 ng/ml. No mice treated with CS alone or receiving basal diet developed lymphomata (862, 863). Another similar experimental model revealed that a single dose of whole body radiation (350 rad) as an initiator followed by CS treatment (same diet as above) led to the development of lymphoid tumours in half of the CS-treated mice. The Swiss Webster mice produced lymphomata of B-cell lineage in mesenteric lymph nodes and spleen, whereas C57BL/6 mice developed thymic tumours of T-cell lineage; none of the CS alone treated or basal diet fed mice developed tumours (391). Using the two-stage induction model of urethane (ethyl carbamate)-induced pulmonary adenomata in mice, the same CS diet for 22 weeks caused a 3-fold increase in average size of the adenomata, but there was no significant difference in the number of pulmonary tumours developed in mice receiving basal or CS diet (863). In conclusion, three experimental models demonstrate that CS can act as a classical tumour promoter in the two-stage induction of lympomata of both T-cell and B-cell lineages in mice, although CS alone was not oncogenic. The above results clearly stress the importance of intact host immunity for resisting the invasion and growth of transplanted tumours as well as the oncogenic potential of combined tumour-promoting agents.

d. MUTAGENIC, ONCOGENIC, AND TERATOGENIC POTENTIAL OF CS THERAPY. Although the concept of immune surveillance is not universally accepted, there is overwhelming evidence that immunodeficiency may result in an increased incidence in developing malignancy. It is also well known that many cytostatic agents, including radiation, possess a direct mutagenic potential. It

was, therefore, an important task to carefully assess the mutagenic, oncogenic, and teratogenic potential of CS in both in vitro and in vivo toxicological studies. The previous results (IV.B.4.c.), however, do not indicate that CS administered in immunosuppressive doses to healthy animals exerted an oncogenic action. The experimental toxicological studies with CS are in full agreement with these results in so far as there is no convincing evidence for a mutagenic, oncogenic, or teratogenic effect of the drug given to several species for very prolonged periods in several doses. Summaries of the toxicological results have been published (803, 804, 1024, 620). Moreover, the effect of immunosuppressive doses of CS (60 mg/kg/d orally) on epidermal cell mitotic activity and the proliferative response of epidermis following ultraviolet radiation was not different from that of control hairless mice (498). Long-term toxicological studies in mice revealed an increase of benign osteomas containing abundant viral particles in control and CS-treated mice, both groups being equally affected. All other tumour types also occurred with the same frequency in control and treated groups. In particular, no increase in lymphoreticular neoplasms was observed (see tables 10 and 11 in reference 803). These findings contrast with those observed by Hattori et al. (391a) who reported an accelerated spontaneous development of thymic lymphomata in AKR mice treated with CS. The explanation could be that the potent T-cell-specific suppressive effect of CS may off-set the labile immunological balance in particularly tumour-prone mouse strains, as for instance, the AKR strain.

The problem of the oncogenic potential of this drug is by no means an academic one, and it is of enormous concern for the clinical situation. The early report by Calne and colleagues (146) stating that three lymphomata occurred among thirty-four transplant recipients caused a great surprise and seriously endangered the future of CS. Fortunately, Calne and his coworkers discovered that they had heavily overimmunosuppressed their patients (due to nonexisting clinical experience with this drug), and by dose reduction the situation rapidly improved. The enhanced risk of lymphoma is inevitable with an effective general immunosuppressive regime and a balance must be struck between effective immunosuppression against allograft rejection and incidence of lymphoma. The postulated mechanism of lymphoproliferation, as advanced by Bird (75), is that the direct consequence of reduced T-lymphocyte surveillance of latently Epstein-Barr virus-infected B cells results in lymphoblastoid cell line outgrowth and subsequent progression via selection and mutation towards far more aggressive B-cell clones. It was very encouraging to note that newer CS protocols using much lower drug doses immediately led to an acceptable incidence of cancers (752, 405). This incidence following the use of CS is no higher than with conventional immunosuppressive therapy (172). However, the types of malignancies and their clinical behaviour show significant differences between the two forms of therapy. In essence, CS-treated patients (usually with prednisone and often additional drugs) appear more likely to develop potentially life-threatening tumours of various internal organs rather than low-grade skin cancers and in situ cervical carcinomata as in conventionally immunosuppressed patients (752).

e. REVERSAL OF MULTIDRUG RESISTANCE. The role of the cyclosporins in cancer chemotherapy must be viewed with both interest and caution (936, 877). Recent work has revealed that CS potentiates the effect of some cytostatic drugs both in vitro and in vivo in both tumour and normal cells (738, 823). In some systems, however, a clear potentiation of cytostatic drugs is seen in cells with acquired and pleiotropic drug resistance, or MDR, compared with their sensitive counterparts (868, 869, 635, 946, 944). Hence CS and its derivatives may have a role as a resistance modifier in MDR tumours. Since a number of weakly or non-immunosuppressive CS analogues also act as resistance modifiers in vitro, the structure-activity requirements for immunosuppression and resistance modification appear to differ (946, 944, 945). Unfortunately, very little is known about the mechanistic basis at the molecular and cellular drug handling levels. Over-accumulation of 100-fold or more of the cell surface P-glycoprotein (170 kD), which occurs in normal liver, kidney, colon, jejunum, and adrenal gland and which functions as a transport system for cytotoxic compounds, is observed in MDR cells. MDR is thought to result from an increased rate of efflux of anticancer drugs, suggesting that the P-glycoproteins may pump many small hydrophobic molecules out of cells (883, 323, 190). Although several CS derivatives have been found to act as highly effective resistance modifiers compared with CS (944; 309a), the suggested explanations for this phenomenon are contradictory and must await further investigations for full evaluation of the therapeutic potential (738, 823, 635, 139, 193, 317). Since MDR tumour sublines have decreased membrane potentials compared with their corresponding drug-sensitive parental tumours, it was suggested that CS and verapamil may correct this alteration by restoring the membrane potentials to normal levels (960).

It is of interest to add in this context that the mechanism of resistance in *Plasmodium falciparum* to chloroquine may be similar or the same as in multi-drug resistant mammalian cancer cells. The most striking difference between susceptible and resistant parasites is the rapid release of chloroquine by resistant parasites (538). The ability of verapamil, a calcium channel blocker, to inhibit the efflux of chloroquine from the resistant parasite is remarkably similar to its ability to inhibit the release of anticancer agents from cancer cells with MDR phenotype (538, 828). Since there is no cross-resistance between CS and chloroquine (696), and since some CS

derivatives are known either to reverse MDR or to possess antimalarial potency, it is tempting to speculate on the therapeutic potential of a new CS analogue expressing both properties, when given to chloriquine-resistant malaria patients (see also IV.E.5.).

5. Mechanism of action in vivo: a unifying hypothesis. Most laboratories have investigated the mode of action of CS in vitro by studying its effects on lymphocyte stimulation by mitogens or antigens, usually in conjunction with lymphokines (see recent reviews: 405, 853, 939, 233, 290, 187). The resultant fairly coherent, though still incomplete, working model essentially proposes that CS causes immunosuppression by preventing the activation of resting lymphocytes at an early stage of the cell cycle, thus inhibiting primarily the production and release of IL-2 and other lymphokines by T_H cells (see chapters V. and VI. of this review). While evidence for this concept is compelling, various phenomena in vivo are not readily explained by this model. This contradiction first led Klaus and Chisholm (521) to ask the seminal question: Does CS act in vivo as it does in vitro? More recent work has clearly underlined the importance of this unresolved problem (e.g., 299, 78, 522, 587).

We have placed much emphasis on the in vivo results and their interpretation throughout this section. It became evident that several findings obtained in vitro under well defined conditions are not directly applicable to the extremely complex situation encountered in an animal. Although necessary and often helpful, ex vivo results may be misleading, because the experimental reactions are occurring outside the organism, in which CS may often still be present. We are fully aware of these major, almost insurmountable difficulties, but we have nevertheless attempted to present a very preliminary hypothesis which tries to unify a number of results that have been discussed here. This scheme (fig. 3) should be considered only as food for further thought and in no way as a conclusive statement. Moreover, only the data obtained from experiments relevant to long-term immunosuppression, as for instance those used for studying allograft protection, have been included. Findings of purely academic interest were, for the sake of simplicity, not integrated in this concept which, however, does not imply that they are irrelevant to the mode of action of CS in vivo.

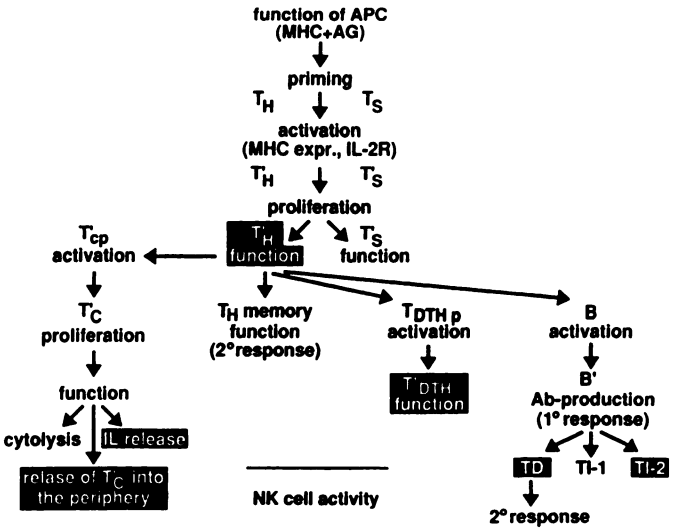

FIG. 3. Mechanism of action of cyclosporine in vivo. Attempt to present a unifying hypothesis. APC, antigen-presenting cell; MHC, major histocompatibility complex; AG, antigen; T_H, T helper cell; T_S, T suppressor cell; IL-2R, interleukin-2 receptor; T′,B′, activated cells; T_{Cp}, cytotoxic T precursor; T_C, cytotoxic T cell; T_{DTHp}, precursor of T_{DTH} cell; T_{DTH}, T cell mediating delayed type hypersensitivity; Ab, antibody; TD, thymus-dependent antigens; TI, thymus-independent antigens; IL, interleukin; NK, natural killer cell. *Black boxes* indicate CS-sensitive steps; all other steps are presumably CS-resistant.

C. Transplantation

1. Experimental organ and tissue grafting. Experimental transplantation is an ideal playground for testing immunosuppressants. The very first transplantation experiments of Borel et al. (106) demonstrated significant prolongation of fitted pinch skin allograft survival in the model using DBA/2 donors and BALB/c mice which differ at the MLS-1 locus and which were treated orally with CS for 10 days. Innumerable papers have since appeared which report the effects of CS in both experimental and clinical transplantation. Excellent reviews have been written by Green (343, 344) and Morris (666) for experimental transplantation and by Beveridge (72) for clinical transplantation. As announced in the introduction, we shall limit ourselves to experimental transplantation.

A summary of the different organs and tissues transplanted in various species and whose survival, with the exception of xenografts, have been successfully prolonged with CS is presented in table 4. The list of references is representative but not exhaustive, especially in the classical renal and cardiac allograft models in rats and dogs. The experimental grafts listed in the table have been assigned arbitrarily into five categories: classical allografts, new allografts which had seldom or not been attempted before the use of CS, composite grafts comprising more than a single organ, xenografts where the rejection process may qualitatively differ from that causing an allograft rejection, and finally bone marrow graft whereby the whole immune system is being replaced and which causes graft-versus-host (GvH) reaction. The novel immunosuppressive properties of CS have prompted intensive experimentation and resulted in major advances in both basic and applied transplantation. These results will be grouped in clusters and discussed below.

a. CLASSICAL ALLOGRAFTS. The early transplantation experiments were aimed at demonstrating the efficacy of CS to prolong allograft survival and to compare its potency with that of other available immunosuppressive agents and means. Among the so called classical models used, the skin allograft model in the mouse (576, 106), in the rabbit (332), and in the dog (204, 110) showed the

TABLE 4

Summary of experimental allogeneic and xenogeneic organ transplantation performed using cyclosporine

Species	Organ	Selected references
1. Classical allografts		
Chicken	Skin	987
Mouse	Skin	106; 576; 215; 622; 320; 577
	Bone marrow	954; 106
	Heart	35; 874
Rat	Cornea	989
	Bone marrow	670; 943; 954; 213; 212
	Pancreas	931; 223; 123
	Skin	214; 937; 758; 360; 561; 81
	Heart	770; 186; 260; 682; 654; 838; 377; 23; 532; 432; 732; 742
	Liver	1023; 457; 251
	Kidney	51; 1013; 430; 330; 881; 1011; 562; 413; 124
Rabbit	Cornea	851; 451; 990; 283; 816; 417; 60
	Bone marrow	333; 73; 331; 463
	Skin	333; 335; 294; 513; 872
	Kidney	238; 237; 347
Cat	Bone marrow	308; 137; 389
Sheep	Liver	278
Goat	Heart	42
Pig	Bone marrow	950
	Heart	151
	Kidney	280; 439
	Skin	280; 281
	Liver	280; 281; 93; 570
	Pancreas	327; 534
Dog	Bone marrow	204; 652; 206; 203; 889; 21
	Skin	204; 110
	Kidney	775; 434; 179; 244; 468; 4
	Liver	138; 992
	Pancreas	244; 554; 534; 878
	Heart	253; 780; 899; 633; 744
Primates	Skin	919
	Kidney	114; 113; 871; 370; 917; 918
	Heart	370; 753; 393; 726
	Pancreas	240; 241; 243
2. New allografts		
Mouse	Islet cells	867; 909; 488; 496
	Muscle cells	974; 571
Rat	Hepatocytes	600
	Nerve cells	1017; 1018; 1019; 826; 34; 43; 44
	Retina	22
	Muscle	361
	Small bowel	610; 387; 598; 840; 848; 211; 860
	Islet cells	341; 568; 216; 485; 965; 881
	Lung	770; 515; 769
	Parathyroid	84
	Aorta	834
	Adrenal cortex	781
	Vascularized bone	773; 378
Rabbit	Islet cells	471; 247
	Nerve	209
	Uterus	181
	Vein	258
Pig	Small bowel	782; 28; 768; 329
Dog	Tumour	198
	Islet cells	881; 534; 528; 11; 993
	Small bowel	221; 188; 707; 772; 297; 298; 180; 991; 49
	Lung	961; 709; 822; 516; 711; 864
	Vein	47
	Vascularized bone	773
Primates	Islet cells	242
	Lung	751

TABLE 4—Continued

Species	Organ	Selected references
3. Composite grafts		
Rat	Heart + lung	898; 771; 551
	Limb	409; 79; 765
	Kidney + islets	776
	Neuromuscular portion	78
Rabbit	Forelimb	222; 300; 295
	Craniofacial parts	300; 295
	Knee joint	866
	Ovary + adnexa	348
Dog	Kidney + segmental pancreas	279
	Pancreaticoduodenal graft	220
Primates	Heart + lung	394; 386
	Limb	194; 884; 817; 689
4. Xenograft		
Mouse (*mus caroli*)	Embryo	191
(rat)	Heart	894
(rat)	Islet cells	385; 911
(human)	Tumour	272; 62; 740
Rat (rabbit)	Skin	886
(human)	Skin	76; 311; 318
(hamster)	Liver	952; 662
(guinea pig)	Heart	2; 958
(hamster)	Heart	436; 527
(rabbit)	Heart	526
(human)	Tumour	438; 704; 319
(mouse)	Neurons	131
(mouse)	Retina	22
(mouse)	Brain	458; 459; 441; 273
(hamster)	Islet cells	685; 841
Rabbit (hare)	Kidney	219
Cat (rabbit)	Kidney	349; 499
Goat (lamb)	Heart	41
Dog (fox)	Heart	780
(wolf)	Heart	252
(wolf)	Kidney	540; 88
Baboon (macaque)	Heart	641; 547; 640; 639; 548; 810; 777
(pig)	Heart	582

ability of CS to prevent graft rejection for as long as treatment lasted; but once the drug was withdrawn rejection invariably ensued. Similar results were obtained in heterotopic cardiac allograft in rats treated with CS for days 0, 2, 4, and 6 only (532) and in orthotopic heart grafts in pigs treated on days 0, 2, and 4 (151). The reversibility of the CS inhibition suggests that clonal suppression rather than deletion is the likely mechanism of immunosuppression. However, using orthotopic renal allografts in the rat (432) and the rabbit (239, 345), it was unanimously reported that short-term treatment with CS led to sustained allograft survival. This was also confirmed in allogeneic bone marrow transplantation in the rat (942). There followed a debate on the nature of this CS-induced unresponsiveness: Was it graft-specific (345, 432, 346), or was this functional tolerance non-specific (237, 239, 236)? In view of the conflicting results of long-term graft acceptance obtained from several models, it was speculated that CS elicits both specific and non-specific unresponsiveness and that the preponderance of one type over the other may vary with time (682). It was claimed that CS induces a non-specific state of unresponsiveness in T_H cells actively maintained by the presence of non-specific suppressor cells and that subpopulations of specific suppressor cells may remain active for longer than the non-specific cells (for review see references 342, 343, 344, 666). It is known today that this operational tolerance is not achieved through a CS-induced specific deletion of T-cell clones responding to the donor's histocompatibility antigens (578). Long-term or indefinite acceptance was not found in rats receiving allogeneic islets of Langerhans (965), in kidney allografted dogs (147), or in heart transplanted primates (464).

All the extensive work (see reference in table 4) which followed these early experiments confirmed that this state of unresponsiveness occurs rather as an exception in some species and is further restricted to few organs. CS has to be administered as a rule for very long-lasting periods or indefinitely to protect an allograft from being rejected, although maintenance doses can be substantially reduced compared with the starting doses necessary

to prevent sensitisation and acute rejection (see also section IV.C.2.). It is, however, worth noting that in certain allograft models, e.g., heart or liver in pigs(983, 280, 93), lung in dogs (711), and kidney in monkeys (114), some animals will not reject their allografts when the drug is discontinued after several months of treatment. Similar scattered cases have also been observed in patients with renal allografts who for various reasons stopped taking medication (761; and personal communications from several investigators). Unfortunately, detailed clinical reports are not available and the cause for long-term graft acceptance in some individuals as opposed to others remains obscure. Anomalous results of this kind can be irritating, but they may turn out to be important.

Several early studies addressed essentially practical questions; for example, to find the most effective vehicle for CS. The hydrophobic nature of the CS molecule seriously endangered its development: First at the very beginning of its discovery and later before its clinical assessment (101). Long-term efforts were required to resolve this basic problem which initially assumed considerable importance (see figure 1). As high a dose as 250 mg/kg/d dissolved in an inadequate solvent may remain without effect, whereas a much lower dose of 50 mg/kg/d in a suitable solvent is able to completely abrogate antibody formation in monkeys (106). Mice are also very sensitive to the galenic formulation of the drug and, depending on the vehicle used, the dosage may have to be increased 10-fold to achieve equipotent effects (J. F. Borel, unpublished data). In contrast, bioavailability in rats is much less dependent on the galenic preparation (idem).

CS was shown to clearly inhibit both antibody- and cell-mediated immunity when administered by several routes, though differences are observed. Intravenous injection leads to the most rapid effect, but subcutaneous and intraperitoneal injections are also very effective. Intramuscular treatment results in a delayed and slow increase in drug blood levels and long-term administration is known to cause inflammation at the injection site. In contrast to the systemic routes, oral administration may require higher doses, but it has the advantage of mimicking best the clinical situation. A meticulous pharmacokinetic study performed in baboons (547) clearly underlines how much care must be exercised in interpreting CS dosages in experimental protocols, since the inhibitory effects depend not only on the route of administration and the species investigated, but also on the vehicle in which the CS is dissolved (128, 156).

Experiments performed in renal allografted rats by Morris and coworkers (667) showed very clearly that there is not only an optimal dosage of CS that will, in the rodent, produce indefinite graft survival, but of greater note, also showed that the time period over which CS exerts its immunosuppressive effect is very narrow. Neither pretreatment nor treatment commencing 4 days after transplantation produced any prolongation of graft survival. In other words, the drug must be given during the initial exposure of the recipient animal to donor histocompatibility antigens to suppress the induction phase of the immune response to the allograft.

In view of this finding, it is surprising that CS can effectively reverse a rejection crisis; however, the effectiveness of CS largely depends on the background of the secondary response. The situation is reminiscent of that discussed for antibody-mediated immunity in section IV.A. When the ability of the drug to influence second-set rejection was investigated, it was found that it was much less effective in prolonging kidney survival in a recipient rat previously sensitised by donor skin grafting (431). In a different experiment with the same rat model, CS was effective not only in preventing, but also in inhibiting chronic kidney allograft rejection depending on the preceding treatments (611). Low-maintenance doses of CS are effective in supporting long-term survival of skin in rats, but when the drug is discontinued, acute rejection of the graft occurs. However, this rejection episode can be reversed by a short pulse of daily CS followed by tapering the dose back to maintenance levels (937). Where donor-specific blood transfusion was used to prevent acute rejection, CS prevented the development of obliterative arteritis and chronic rejection in allografted kidneys, but it had no effect when the initial treatment was a single dose of CS alone. In contrast to the kidney model, CS was very effective in reversing rejection of a secondary heart allograft in rats (838). Lewis rats sensitised to LBN skin grafts or subcutaneous heart fragments rejected heterotopic vascularised grafts within 5 ± 0.4 days, but a short-course of CS (10 mg/kg/d intraperitoneally on days -2 to 7) prolonged survival to 12.3 ± 2.5 days. In contrast, a maintenance regimen of CS, given in a tapering dosage for at least 60 days, markedly extended survival from 77 to 100+ days. However, rejection occurred following withdrawal of the drug which was not the case in non-sensitised rats in this strain combination.

Delay in the administration of CS to recipient dogs of renal allografts until day 4, at which time sensitisation and rejection should have begun, did prolong survival in four out of six animals (in contrast to the rat), suggesting that acute renal rejection might be suppressed by CS at an early stage of the response (429). Another study was performed to evaluate the effectiveness of CS in reversing ongoing kidney rejection and in preventing pancreatic rejection in a simultaneous kidney-pancreas allotransplant model in DLA-identical littermate beagles (554). CS alone proved more effective than the AZA-steroid combination in reversing ongoing acute rejection. Moreover, liver-allografted dogs, experiencing slow rejection when CS therapy had been stopped after 60 days, had their acute rejection reversed if CS was administered at

a dose of 20 mg/kg/d for 6 to 20 days (992). Also in dogs, CS given as a 21-day course protected skin grafts from DLA-non-identical, unrelated donors but signs of rejection then developed on days 27 to 33. These were reversed in most dogs by a second course of treatment and, in addition, second set graft survival was also prolonged by CS (204). Somewhat comparable results have been reported from the clinic. The efficacy of CS in patients undergoing repeated renal transplants (identified as immunologically high risk individuals) has been documented in many trials (e.g., 793, 6, 957, 618). The available data would indicate that the prognosis of kidney retransplants under CS therapy is now approaching that which is achievable in primary transplants (for review see references 563 and 850). However, CS used as antirejection therapy in steroid-resistant, acute and chronic renal allograft rejection episodes, although successful in a number of patients, appears significantly less effective than when used prophylactically (607, 766).

Of particular interest is the work concerned with corneal allografts, because topical application of CS appears to protect the cornea from rejection. In the rabbit model, in which the corneal allograft provokes local revascularisation accompanied by first-set rejection, it proved possible to prevent rejection with topical CS applied 5 times daily for several weeks (451, 60, 282), but the grafts were rejected when administration was terminated (487). It has since proved possible to reverse ongoing rejection with CS, but only in those rabbits which had previously been treated with the drug (795). General experience gathered from the literature and from our own unpublished work strongly indicates that an ongoing first-set rejection (resulting from discontinuation of drug treatment) can only be reversed or a second-set rejection can much more easily be prevented with CS, if the individual has previously been immunosuppressed. It also appears that the previous treatment is not necessarily limited to CS but may comprise other immunosuppressants.

It is noteworthy that, with the addition of CS, corneas have been successfully transplanted in many high-risk patients, in whom rejection would have previously occurred. Successful engraftment of high-risk corneal allografts has been reported in twelve out of fifteen patients after short-term immunosuppression with systemic CS (5 mg/kg/d orally for 12 weeks) combined with topical application of corticosteroids (649). Another group of twenty-five patients presenting a high corneal rejection risk were treated with a combination of topical CS (2% in castor oil) and dexamethasone (eyedrops 1%), the dosages being slowly reduced and then discontinued after a few months. Long-term graft survival was recorded in twenty-two cases (315).

The work with classical allografts has also shown that there exist clear organ and species differences. As an example, CS doses of 20 mg/kg/d are appropriate to prevent renal allograft rejection in the dog, while this dose needs to be doubled in order to obtain similar results with a pancreatic transplant in the same species (244). Similar differences were observed in animals receiving transplants of two organs. Thus, it was found that kidney and pancreas allograft rejection responded differently to CS immunosuppression. Following cessation of CS in rats bearing both kidney and pancreas from identical donor-recipient combination, it was found that pancreatic allografts were regularly rejected while renal allografts survived for very prolonged periods of time (525). Using the same rat strain combination, a similar phenomenon was seen; heart allografts survived permanently after short-term CS, while all pancreatic grafts were rejected following cessation of CS (525). Opposite results were reported in rabbits grafted with both ear-skin and pancreatic islets transplanted to the liver via the portal vein. Tapering doses of CS were injected intramuscularly for 60 days. After cessation of drug treatment the skin allografts were rejected. The most intriguing observation was the continued survival and functioning of the islets after the rejection of skin grafts (471). Discrepancies in the rejection of either kidney or pancreas without the other organ have also been documented in patients receiving two allografts from a same donor (143). The same holds true in patients transplanted with heart and lung en bloc (628). On the other hand, there is good evidence from a series of clinical cases that a liver graft can protect a kidney from rejection provided both allografts stem from the same donor (608).

Many examples for species' differences in susceptibility to immunosuppression can be documented. For instance, orthotopic kidney allografts are accepted indefinitely in rats after a short-term treatment of 7 to 14 days with CS (432), but dogs will inevitably reject a renal allograft following cessation of even long-term treatment with CS (434). Orthotopic heart allografts in the pig were maintained for long periods of time after only a few days of CS treatment (151), while, in contrast, long-term CS therapy, even in combination with other immunosuppressants, seldom prolonged cardiac allotransplants in the monkey beyond a few months (753).

In summary, the profound immunosuppressive effect of CS is common to a range of species and to a variety of tissues as shown in table 4. Before CS became available, many of these tissues had defied attempts to transplant them. In each case, CS was more potent and had fewer side effects than other immunosuppressive agents and means in clinical use. In vivo experiments support the in vitro observations that CS is most effective when given early with antigen presentation. In general, CS is rather less effective at protecting second grafts in sensitised recipients than primary allografts of the same tissue. These experiments demonstrate in which situations CS works, but they tell us little about how or why it works (233). Some aspects of these major questions will be discussed under IV.C.2.

It is, however, worthy to note that many additional findings were obtained. Experiments with cardiac allografts during neonatal life in goats suggest that newborns subjected to orthotopic cardiac transplantation have potential for reaching full maturity, size, and adult exercise capacity without need for interval operative revisions (42). Since CS is used routinely in the immunosuppressive regimen after liver transplantation and its side-effects include hepatotoxicity, it seemed important to study its effect on hepatic regeneration. Rats pretreated with 25 mg/kg/d of CS orally for 7 days were submitted to partial hepatectomy and sacrificed at 6, 24, and 48 h later (481). The data demonstrated that CS did not adversely affect hepatic regeneration, but actually augmented the regenerative response. Others have confirmed the stimulatory effect of CS (10 mg/kg/d orally on days −1, 0, 1, and 2) upon liver cell proliferation after partial hepatectomy in the rat (510). In contrast, AZA and methylprednisolone treatment substantially suppressed hepatocyte mitosis when compared with the control group (511). An experimental massive third-degree burn model was developed in the LEW rat (82). The results obtained indicate that this model follows the clinical burn wound course, and treatment of massive burns with primary excision, skin allografts, and low doses of CS (8 mg/kg/d) could provide immediate and complete functional repair of the burn wound. Last, but not least, the high success rate obtained with CS alone or in combination with other drugs has inspired many investigators to attempt the transplantation of new and composite allografts.

b. NEW ALLOGRAFTS. All organs and tissues listed as 'new allografts' in table 4 are considered in the clinic as experimental procedures. The results obtained in animals immunosuppressed with CS have been consistently and markedly superior to those using standard chemical suppression (AZA, MTX, steroids) or TLI and bone marrow transplantation or combinations thereof. The particular problems associated with each of these allografts will be briefly reviewed.

The difficulty with lung transplantation is the poor healing at the bronchial anastomosis with conventional immunosuppression. In contrast, CS was shown not to interfere with healing of bronchial anastomosis in autografted dogs since the breaking strain of bronchi and the skin strength were similar to that in untreated controls (822, 317). It was also found that lungs were not rejected in the majority of dogs upon termination of long-term treatment with CS (961, 709) and, since third-party skin grafts were promptly rejected, this suggested that specific unresponsiveness had been generated (709). Ultrastructural analysis on acute rejection of canine allograft using CS (864) and several studies relating to the role of cytolytic and suppressor cells were preformed (709, 516, 711). There was evidence in long-term survivors for diminished donor-specific cytotoxicity relative to donor-specific proliferative response, i.e., the mixed lymphocyte reaction remained normal (709, 711). A study of cellular events associated with lung allograft rejection in the rat showed that broncho-alveolar lavage-derived cells from CS-treated rats blocked the formation of specific cytotoxic effector cells and the development of responsiveness to IL-2 (515). The importance of timing of CS treatment was demonstrated in lung-allografted rats. It was found, surprisingly, that acute lung rejection could be prevented as well as that most of the grafts would survive long-term, if a single dose of CS was given only on day 3 after transplantation (769). Delay to day 4 resulted in most allografts rejecting briskly. Such studies (769) are clearly more of theoretical than of practical interest (751).

Small bowel transplantation has been studied in rats, pigs, and dogs, but the currently available immunosuppressive regimens do not appear adequate to prevent rejection in patients, since the longest survivor died at 6 months (325; for review see 767). The small bowel is unusual among solid organs considered for transplantation because of the large quantity of lymphoid tissue present in Peyer's patches and in the lymph nodes of the accompanying mesentery. This lymph serves as a potent antigenic-stimulus, resulting in a vigorous rejection response. Therefore, the major problems are the control of rejection, anastomotic healing, and the graft-versus-host (GvH) reaction. Due to poor absorption from the gut, it is crucial that CS be given parenterally until the graft has become established (175). Studies in the rat have demonstrated that CS does not interfere with anastomotic healing and effectively prevents both allograft rejection and GvH disease (211, 387, 598, 840, 848, 610, 860). One study indicated a relationship between CS doses and sizes of intestinal segment: Lower drug doses were necessary for rats that received shorter intestinal allografts (512). However, CS has proven much less effective in large animal models; despite significant prolongation of graft survival in most series of small bowel transplantation, only a minority of the overall number of attempts became long-term survivors. Addition of steroids or other adjunctive therapies have not improved on results obtained with CS alone in the dog model. The combination with MAb and ex vivo irradiation of the graft (991) might possibly be more promising in the future (see reference in table 4 and review 767).

The introduction of CS, which allowed immunosuppression of recipients without the necessity for continuous maintenance steroids, renewed interest in pancreatic islet cell grafting. In marked contrast to most other classical organs, it has not proved feasible to obtain long-term survival of isolated islets of Langerhans following a short course of CS therapy, except when these were implanted under the renal capsule of syngeneic diabetic rats and then allografted together with the kidneys into allogeneic diabetic hosts (776). It appears that

isolated islets are not necessarily particularly immunogenic (322), but are very sensitive to the effector mechanisms of rejection (341, 525). It seems that macrophages are largely responsible for the rapid rejection of islet allografts, since the combination of the anti-macrophage agent silica and CS or ALS results in synergism (690). Moreover, in rat fetal pancreatic allografts, exocrine tissue does not develop while endocrine tissue reaches full maturity provided CS is given to the recipients (645). Interestingly, the immunogenicity of pancreatic allografts can be reduced by certain organ culture procedures, which achieve destruction of endothelial cells expressing MHC class II antigens and which may act as antigen-presenting cells (APC) (557, 123, 568, 485).

It is still not possible to state whether CS exerts a beneficial effect or not in islet cell transplantation, because so many reports are contradictory. The purity of the islet tissue has to be considered in such experiments, since it is known that all contaminating tissues markedly increase immunogenicity of the graft. In contrast, carefully purified islets express only MHC class I antigens and appear weakly immunogenic (for review see 339). It has repeatedly been experimentally demonstrated in vivo that CS has a negative effect on glucose metabolism and is toxic for islet cells both in rats (397, 371, 1000, 372, 383) and in dogs (53, 306, 54). However, the opposite has also been documented for mice (496), rats (341, 216, 485), dogs (528, 11), and humans (288). Müller-Schweinitzer (Sandoz Basel; manuscript in preparation) has tested the glucose tolerance in conscious male beagle dogs treated with single oral doses of CS (10 and 30 mg/kg). CS significantly impaired both intravenous as well as oral glucose tolerance. Determination of plasma insulin levels after CS revealed, in addition to reduced basal plasma insulin levels, a marked suppression of insulin peak levels during both intravenous and oral glucose tolerance tests, suggesting that the impaired glucose clearance following drug administration was due to reduced insulin secretion. It is worth mentioning that neither basal plasma insulin nor glucose levels 2 hours after drug administration, but before glucose challenge, were significantly different from those of placebo-treated control dogs. In addition, a recent placebo-controlled clinical study performed in patients with multiple sclerosis treated with conventional doses of CS for as long as 2 years did not indicate any evidence for impaired glucose homeostasis (785). Furthermore, the reversibility of the diabetogenic effect of CS in toxic doses (50 mg/kg/d intramuscularly for 2 weeks) in normal rats has been demonstrated (371). Three weeks after drug withdrawal, pancreatic tissue concentrations were still at 13.4 μg/g, i.e., 100 times higher than in serum, but glucose tolerance had already improved.

It is most difficult to reconcile the following three experiments in dogs. One study with established islet autografts (transplanted into the trabecular venous system of the spleen) suggests that CS begun the day before autografting gravely compromises graft success, whereas CS treatment starting after the graft is well established does not adversely affect islet cell function (53). In contradistinction, treatment of diabetic dogs with CS (20 to 40 mg/kg) beginning 3 to 5 days before islet allograft transplantation into the liver and adjusted to maintain drug through levels between 400 and 600 μg/ml prevented rejection in all but one out of seventeen animals in which the initial level exceeded 400 μg/ml (11). After stopping CS between 30 and 99 days of treatment, eight allograft recipients had sustained fasting euglycemia for 7 months, two for 8 months, and the remainder for at least 2 months. In the third study pancreatecomised dogs received intrasplenic allotransplants of unpurified islets (528). High levels of CS allowed prolonged (over 100 days) graft function in five out of twelve animals, with a median duration of 85.5. days. Non-immunosuppressed controls and AZA plus steroid-treated dogs had a median survival of 4 and 3 days, respectively. It was concluded that CS at whole-blood HPLC levels of 600 to 1000 μg/ml can achieve prolonged normoglycemia in these dogs. Therefore, we remain confronted with both positive (528, 12, 11) and negative (53, 993, 881, 534) results concerning the beneficial effect of CS in islet allografts in the dog.

The possibility of bridging large defects in peripheral nerves by neural allografts has been explored experimentally in rats and rabbits. A short course of CS does not induce unresponsiveness to nerve allografts in either species, but long-term treatment appears necessary to prevent rejection (1019, 209). Continuous CS treatment was also effective in preventing a second nerve allograft in sensitised rats and in allowing the regeneration of numerous host axons (1018). Using the peripheral nerve allograft model in the rat, Bain et al. (43, 44) have investigated the dose-response curve and assessed the regeneration across nerve allografts in animals immunosuppressed with CS. CS was, furthermore, effective in prolonging the survival of cryopreserved nerve allografts in the rat. Since discontinuation of treatment resulted in graft rejection, it seems that cryopreservation did not alter their antigenicity (34). These positive results have encouraged the attempt to transplant composite limbs, and assessment of peripheral nerve graft function indicated at least partial recovery in rats (765, 79) and in primates (194, 884, 817, 689).

Postnatal day-21 rat retinas, which are related to neural allografts, were grafted into adult rat retinal lesion sites. Treatment of the recipients over a 6-day time span with CS could to some extent rescue these retinal grafts (22).

Suspensions of mononucleate cells allografted into regions of regenerating host muscle survived for 65 days (termination of experiment) in mice treated with CS for 42 days, suggesting development of long-term graft acceptance (974). Furthermore, the demonstration that CS

administration permits cloned normal myoblasts to survive and develop in histoincompatible dystrophic mice indicates that clonal cell lines of superior myoblasts can be established, selected against tumorigenicity, and stored in cell banks ready for injection (571). Whole-muscle allografts in rats have also been protected from rejection by CS but only so long as therapy was maintained (361).

CS did not apparently improve healing of nonvascularised bone allografts in mice, even though it clearly depressed the alloantibody response (378). However, in vascularised limb grafts in rats, CS markedly prolonged graft survival across a strong MHC difference, but graft viability declined once the drug had been withdrawn (378). Further experiments on the survival of vascularised knee allografts in rats, showed that the use of CS inhibited the rejection response across a major MHC difference for as long as treatment lasted, whereas a short course was sufficient across a minor MHC difference (773). CS treatments resulted in survival of cortical osteons in a vascularised posterior rib graft model in outbred dogs (773).

The potency of long microvenous allografts in rabbits treated with CS was investigated (258). Intramuscular CS doses of 15 mg/kg/d over 8 or 22 days significantly increased the patency rate of veno-venous allografts to 100% and maintained allografts of normal vein morphology to 3 weeks. Another study explored the ability of CS to prevent arterial allograft rejection and failure across a major histocompatibility barrier in rats (834). Subcutaneous doses of 5 to 10 mg/kg/d of CS reduced or prevented aneurysmal dilation and delayed cellular infiltration and intimal thickening in the graft. It was also shown that a low maintenance dose (continuous 5 mg/kg q.o.d.) provided effective graft function.

c. COMPOSITE GRAFTS. The encouraging results obtained with CS in allografting classical and new types of organs and tissues (as just described) have led the experimental basis for transplantation of composite grafts. Heart and lung en bloc have been first transplanted in rats and primates and are now performed clinically on a limited scale in specialised centres (627). Of prospective importance are the integumentary/musculoskeletal allografts for posttrauma tissue replacement. Such allografts may have lifesaving capabilities in addition to their utilisation in functional and aesthetic surgical reconstruction. CS was tested in a newly developed experimental massive third-degree burn model in the rat (82). Doses of 8 mg/kg/d for the first 20 days, then 3 times a week thereafter, protected extensive skin allografts (75% body surface area) from being rejected and the long-term surviving grafts appeared healthy and had nearly perfect hair growth (for review see 81). Several composite tissue allografts have already been discussed (see IV.C.1.a, and b). Table 4 clearly shows the reawakened interest in transplantation of composite tissue allografts of skin, muscle, bone, vessel, and nerve in rats, rabbits, and primates. Indefinite survival of limb allografts has been obtained in the rat with low-dose CS (80). As previously mentioned, the return of nerve function in limb transplantation is essential and has been shown to occur in the rat (765). Primate allografted skin and composite tissue can only be maintained long-term with the use of high level CS and low-dose steroids (817). Although many of the target structures in composite allografted tissues do become innervated by host axons and regain some of the anatomic relationships seen in normal receptors, the quality of innervation is questionable in the case of axons serving rejected allografted skin. This combined immunosuppressive therapy (high levels of CS and low levels methylprednisolone) may be conducive to the production of morphologic abnormalities at the axon/Schwann cell level in primate tissues (817). Of rather anecdotal interest may be the feasibility of pregnancy after allografting en bloc vascularised ovaries with adnexa in rabbits (348, 350); however, this example demonstrates once more the immunosuppressive potency of CS while allowing the composite allograft to function normally.

D. XENOGRAFTS. Improved results with allogeneic transplantation, a worsening organ shortage, and extended criteria for recipient selection are now stimulating a resurgence of interest in xenografts (790). Although the distinction between concordant and discordant species is imperfect, these terms coined by Calne indicate whether organs transplanted between members of two species will suffer hyperacute rejection (142). Thus, species combinations are defined as concordant or discordant depending on the absence or presence of preformed antibody, respectively. Concordant means the xenogeneic equivalent of crossmatch-negative, ABO compatible in human allogeneic transplantation (for an exhaustive review see reference 32). Many experimental studies performed with xenogeneic transplants between discordant species demonstrate the predominant role of preexisting species antibodies and the subsequent role of complement components, platelets and vasoactive substances in mediating the hyperactute xenograft rejection. However, if the relationship between the donor and recipient species are of a minor evolutionary divergence, i.e., concordant combination, immediate graft rejection due to complication of an early antibody-mediated response is unlikely to occur and the 'normal' rejection may be the consequence of a cell-mediated response similar to that observed with allografts.

Results obtained from many experiments using CS in several concordant xenograft models, where rejection in untreated controls usually takes place in 2 to 4 days, demonstrate its effectiveness in protecting the graft (see reference in table 4 section 4). However, in discordant xenograft models, where microcoagulopathy occurs within minutes of reperfusion with blood, the drug does

not prevent hyperacute rejection (2, 349, 499, 582). Therefore, it is obvious that the problem of eliminating the preexisting species antibodies (e.g., by plasma exchange or absorption) must be solved before using CS to control the cell-mediated xenogeneic responses (958, 582). When rats underwent plasma exchange one day prior to receiving a heterotopic guinea pig heart and were treated on days -3, -2, and -1 with CS (30 mg/kg/d orally), xenograft survival was modestly prolonged to a mean of 418 min compared with 16 min in untreated animals. The natural cytotoxic antibodies remained low for the next 24 h, whereas in plasma-exchanged, but not immunosuppressed rats, the titres returned to their initial levels (958).

Discordant skin xenografts represent a special situation, because skin is not immediately vascularised and hence not rejected in a hyperacute fashion, thus allowing time for CS to exercise its immunosuppressive effects (344, 756a, 32). Prolonged viability of human split-thickness skin xenografts in rats using CS as the sole immunosuppressant has been reported. The first series of experiments showed excessive CS toxicity due to an unsuitable vehicle (76). This was later corrected and oral administration beginning at doses of 20 to 40 mg/kg/d for 10 days and later decreased to 3 times weekly produced prolonged survival (up to several months) of human skin xenografts with gross and microscopic morphology, labelling index, and karyotype similar to human skin in situ (318). Another discordant skin xenotransplantation model consisting in grafting full-thickness skin from rabbits to the tail of rats compared several immunosuppressive treatments (866). CS alone resulted in slightly prolonged graft survival, but in combination with CP and methylprednisolone a synergistic effect was observed: Doubling median graft survival as compared to controls.

Xenogeneic liver transplants may also be resistant to hyperacute antibody-mediated rejection. For instance, guinea pig livers in rats have a survival of 17 h compared with 26 min survival of guinea pig hearts in rats (847) and pig-to-baboon liver xenografts can be tolerated for a few days, while those of other organs undergo hyperacute rejection in an hour (150; for review see 32). It was further shown in the hamster-to-rat model that orthotopic liver xenografts survived for 7 days (662), while heart xenografts were rejected in an accelerated fashion in 3 to 4 days (662) or in 2 days (436). Oral doses of CS (20 to 40 mg/kg/d) were completely ineffective in prolonging liver xenograft survival. However, the same group has demonstrated that prolonged survival of hamster-to-rat liver xenograft could be achieved if CS administration was combined with splenectomy (952). In contrast to renal or cardiac xenografts there is an additional problem with liver xenotransplantation. One of the most important functions of the liver is protein synthesis and these proteins are genetically coded by the origin of the liver donor. Therefore, successful liver xenotransplantation would depend upon whether or not a recipient animal can survive with xenogeneic proteins (662).

Even in models of cell-mediated rejection (concordant species), prolonged xenograft survival is generally more difficult to obtain than allograft survival. It has been demonstrated that long-term acceptance of cardiac allografts in newborn goats can be achieved with continuous administration of tapered maintenance doses of CS (42). However, in the case of lamb heart xenografts transplanted orthotopically into newborn goats, massive immunosuppression using high CS doses combined with AZA and methylprednisolone resulted in an average survival of 72 days. Most of the ten recipients showed mild-to-moderate subacute and chronic graft rejection at autopsy (41). Since in the model of heterotopic intrathroacic heart transplantation rejections of the graft are not lethal for the recipient animal, the immunologic rejection mechanism can be observed to the final stage. The outcome of allogeneic dog and xenogeneic fox hearts under immunosuppression therapy, including CS and steroids, was observed by cytoimmunologic monitoring and endomyocardial biopsies. The transplanted allogeneic hearts survived for 53.2 ± 14.8 days (controls 6.8 ± 0.8) and the xenogeneic fox hearts for only 20.2 ± 4.1 days (controls 8.4 ± 1.9). The allografts were rejected in an acute cell-mediated fashion, whereas xenografts showed humoral and cellular rejection mechanisms (780). Based on limited observations it has also been suggested that alternative mechanisms exist for xenografts that do not play a role in allograft destruction. It has been speculated that xenograft rejection might be additionally mediated by natural killer (NK) cells or by antibody-dependent cell-mediated cytotoxicity (ADCC). A further possibility for late xenograft rejection might be a chronic, antibody-mediated process typified by a histopathological picture comprising scant cellular infiltration and particular vascular changes in the graft. Unfortunately, clear in vitro data indicating either a significant quantitative or qualitative difference in the cell-mediated response to xeno- as compared with alloantigens are lacking (for review see reference 32).

It is well-known that the nature and extent of xenograft rejection is strongly determined by the genetic disparity between species. Cardiac xenografts were performed in two concordant species; combinations using TLI (total lymphoid irradiation) and CS (526). In the more distantly related combination of rabbit-to-rat transplant (average survival in controls: 3.2 days) treatment with TLI alone or TLI plus CS doubled the survival time of the heart xenografts. However, in the more closely related hamster-to-rat combination TLI alone had only a marginal effect, but TLI combined with long-term intramuscular doses of CS (5 to 10 mg/kg/d) resulted in markedly prolonged survival (>100 days). It is worth mentioning that the rat anti-hamster cytotoxic

antibody titre remained below detectable levels on postoperative day 100 (526) and that the cellular immunity, as measured by one-way mixed lymphocyte reaction, was profoundly suppressed at postoperative day 46 (527) in recipients of beating xenografts. Earlier work had already demonstrated the effectiveness of CS alone in the latter combination, but high doses (35 mg/kg/d for 14 days) were required to attain significant prolongation of heterotopic cardiac xenografts with a median survival of 21 days compared with 2 days in controls (436).

Comparable intra- and cross-species primate cardiac transplantation was performed in the neck and the recipients were treated with 15 mg/kg/d CS intramuscularly from day 1 onward combined with steroids. The results of xenograft vs. allograft survival indicate a slight but statistically insignificant tendency toward more prompt rejection in the xenograft vs. allograft controls. However, there was no significant difference in survival between xenografts and allografts in immunosuppressed animals. This macaque-to-baboon cardiac transplantation model, in which readily available animals with a humanlike anatomy and physiology are used, has been proposed as the model of choice and most relevant to study xenotransplantation in humans (641). It is evident that successful xenogeneic transplantation of any organ requires that it is able to support life by living and functioning adequately in the environment of a different species, e.g., in spite of species variation of enzymes and hormones.

The ability of CS as the sole immunosuppressant to prolong survival of heterotopic cardiac xenografts has been tested in the rat-to-mouse model as well as in the reverse combination of mouse-to-rat (894). The results show an apparent differential effect of CS on xenograft survival that depends upon the recipient species. Rat cardiac xenografts in mouse recipients receiving intramuscular doses of 20 mg/kg/d CS for 14 days showed prolonged survival of rat hearts: 14.4 ± 3.2 days compared with 5 days in controls. Five out of seven mice rejected their heart grafts while receiving CS and the remaining two mice rejected after CS treatment was stopped. However, in the reverse combination no significant prolongation of graft survival (twenty-one of twenty four rats from all groups rejecting at day 3) was obtained; this is somewhat paradoxical since it is known (and documented by a large body of literature) that CS is generally a rather more effective immunosuppressant in rats than mice.

The surgical procedure to transplant islet cells into the liver via the portal vein is uncomplicated. Provided unresponsiveness to the graft could be achieved, ideally by a short-term and safe immunosuppressive therapy, and since porcine or bovine insulin is effective in diabetic patients, and since the sources for human islets are very limited, it is worthwhile studying the possibility of curing diabetes with islet xenotransplantation. Based on the results obtained with islet allograft (see IV.C.1.b.) CS alone is unlikely to promote long-term graft acceptance. Additional procedures, such as modification of the graft before transplantation or using particular organ sites for the injection of isolated islet cells, have been assessed to improve islet xenograft survival in both the mouse and the rat. Thus, long-term graft acceptance was induced in the mouse by prior ultraviolet irradiation of the rat donor tissue and a short course of peritransplant CS (385). Culture of rat donor islets at 24°C for 7 days combined with a 3-day course of CS therapy (50 mg/kg/d injected subcutaneously on days 0, +1, and +2) produced a marked prolongation of rat islet xenograft survival in diabetic mice (911). The mean graft survival reached 56 ± 10 days including three long-term survivors > 100 days. On the other hand, injecting isolated islets from hamsters into the cryptorchid testes of spontaneously diabetic BB/W rats, which received, immediately postoperatively, an intravenous injection of 25 mg/kg CS, followed by daily intramuscular injections of the same dose times 6, and then daily subcutaneous injections of 7 mg/kg for another 10 days, produced a limited cure of the diabetic process. These CS-treated normoglycemic rats with abdominal intratesticular islet xenografts continued to have functional grafts for periods ranging between 12 and 22 days when they were completely off immunosuppression (841). All these combinations clearly resulted in synergism and they should encourage further research in the field (see also IV.C.2.c).

Animal models of Parkinson's disease, Alzheimer's disease, Huntington's chorea, and other pathological conditions have shown functional improvement following transplantation of embryonic brain allografts (441). Because of the ethical and logistic problems associated with the use of human fetal tissues, cross-species transplants are an attractive alternative. The survival and function of cross-species mouse-to-rat grafts of fetal mesencephalic dopamine neurons, implanted as a cell suspension in the striatum of rats with lesions of the mesostriatal dopamine system, were studied in animals with and without treatment with CS (131). CS doses of 11 mg/kg/d were given intraperitoneally first 18 h prior to transplantation and continued for 6 weeks. The results demonstrated that in the presence of immunosuppression with CS the survival rate, the number of dopamine neurons surviving, the extent of dopamine fiber outgrowth, and the magnitude of the functional effects of the mouse xenografts were all comparable to the results obtained with syngeneic mesencephalic grafts. Another study has investigated the effect of CS on the survival of newborn mouse cerebral cortex into the third ventricle of adult rats (459, 458). CS treatment consisted in intramuscular doses of 10 mg/kg/d starting immediately after operation and lasting for 14 days. Histological and immunohistochemical examination revealed that the mouse brain tissue was rejected by 4 weeks in the control group. In the CS-treated group, however, both neuronal and

glial elements in the grafted tissue could survive and mature in the third ventricle of the recipients. Similarly, the effect of CS on the survival and differentiation of solid grafts of fetal mouse hippocampi (from 16 to 17 day old embryos) transplanted to the brain of adult rats was examined. Treatment of recipients with subcutaneous doses of 15 to 20 mg/kg/d CS started the day before grafting and lasted for 5 to 8 weeks. Eleven out of seventeen xenografts (65%) were recovered after 5 weeks and nine out of twenty-one (43%) after 8 weeks in the treated group compared with 36% and 18%, respectively, in the controls. The surviving mouse xenografts were organotypically organised, including the exchange of normal nerve fiber connections with rat host brain (273). An experimental model of brain xenotransplantation has been developed to evaluate cell survival in untreated and CS-treated animals. Cholinergic ventral neurons from embryonic mice were transplanted into the frontal lobes of rats using a cell suspension technique (441). The drug-treated rats were injected intramuscularly with 10 mg/kg/d CS for 13 days; the control group was not treated. The animals were sacrificed at 12 weeks and histochemistry studies performed. Although xenografts survived well in the untreated group (cell counts: 91 ± 19 per 2 cu mm volume), the CS-treated animals showed a statistically significant increase in transplant cell survival (119 ± 15). All these available data thus support the view that, although the brain is immunologically privileged relative to other sites in the body, the protection against immunological rejection is only partial and that treatment with CS can effectively prolong survival of xenografted brain tissue.

In recent years there has been extensive use of human tumour xenografts in animal models for the study of the biology and therapy of human cancer. These animal models are based on either genetically immune deficient hosts (nude mice and rats) or immune deprived hosts (thymectomy, irradiation, chemotherapy) (438). In contrast to other immunosuppressants, which have antineoplastic as well as antilymphocytic properties, CS could be particularly useful in improving existing models. This has been confirmed in several models of tumour xenografts.

The morphologic and growth characteristics of xenogeneic transplants of human bladder- and renal-cell carcinoma into CS-treated NMRI mice were studied and compared with those of the same tumours grafted into NMRI nu/nu mice. Tumour growth was best, in fact almost similar to that observed in the nude mice, in the normal mice receiving daily oral doses of 150 mg/kg CS. An important point was the demonstration of 80% acceptance rate of xenogeneic tumour grafts in the CS-treated mice compared with 100% in the nude mice. The observed far-reaching identity of primary and transplanted tumours, and the well-preserved correlation of growth rates of four different tumours with the clinical course of the corresponding patients, indicate that this tumour model is also reliable for obtaining data related to therapy of the corresponding patients (740). The possible advantage of CS-treated normal mice over the athymic nude mice was also investigated using a human malignant melanoma maintained in athymic nude mice which was successfully implanted and grown in CS-suppressed rats. Solid tumour sections were implanted subcutaneously in rats receiving subcutaneous doses of 25 mg/kg/d CS for 1 week and 3 times per week thereafter. 85% of the implanted tumour sections resulted in tumour growth and the tumours retained pretransplant gross and microscopic morphology, karyotype, and labelling index (319). Yet another study reports that the total growth of a transplanted human breast cancer or a rodent tumour (MTB-2) in the subrenal capsule of mice was much improved by treatment with CS (subcutaneous dose of 80 mg/kg/d for 2 to 8 days after implantation). CS treatment of CD-1 mice allowed allogeneic tumour growth for 12 days that was not significantly different from tumour growth in syngeneic C3H mice. These results are similar to results with human breast cancer xenografts in CS-treated normal mice versus athymic nude mice. In contrast, immunologic regression accompanied by prominent T-cell infiltration occurred after 6 days in the absence of CS (272). Finally, a preliminary paper relates the successful transplantation of pieces of a human midgut carcinoid tumour to the anterior chamber of the eye in rats subjected to treatment with CS at subcutaneous doses of 20 mg/kg/d throughout the observation period of 18 days. The xenografts consisted of growing tumour cells which upon pharmacological stimulation released large amounts of active tumour products such as 5-HT (705).

In conclusion, CS can be regarded as a major advance in xenotransplantation although, used as the sole drug, it does not resolve the major problems encountered in prevention of early and late xenograft rejection. Xenografts between discordant species are rejected in a hyperacute fashion which is thought to be mediated by the presence of preexisting species antibodies and which does not respond to CS therapy. In concordant species, in the absence of hyperacute rejection, most xenografts are believed to be rejected by cellular mechanisms which are sensitive to CS treatment. Xenogeneic grafts, even between closely related species, are generally more difficult to prolong than allografts, a possible explanation being that rejection is complicated by other, poorly understood mechanisms which may be absent in allograft rejection. Chronic antibody-mediated processes with concomitant cell-mediated rejection might in theory be controlled by CS, but numerous examples show that CS alone, even in high doses, fails to induce long-term survival. Rejections occur in spite of the ability of CS to prevent the formation of species antibodies. The most promising attempts were those in which CS was used in combination with

TLI, splenectomy, or pretreatment of the organ graft. Another favourable combination with CS is to implant concordant xenografts into immunologically privileged sites, such as cryptorchid testis, brain and the anterior chamber of the eye. Moreover, immunosuppression with CS of recipient animals receiving human tumour xenografts has allowed the development of useful experimental models for studying the biology and therapy of human cancers. Xenotransplantation still remains a difficult field, but it will no doubt increase its importance at the same pace as basic immunology progresses.

e. BONE MARROW GRAFTS. Bone marrow transplantation differs in its immunobiology from transplantation of other organs. To eradicate the basic disease (e.g., leukaemia) patients are given a conditioning regimen which kills their bone marrow cells and concomitantly wipes out their own immune system. This treatment will also prevent rejection of the subsequently transplanted bone marrow. The latter has first to engraft before it expands and substitutes for the deleted haemopoietic tissue and immune system of the recipient. In contrast to solid organ transplantation, where the main objective of immunosuppression is to prevent the host from rejecting the graft (host-versus-graft (HvG) reaction), the major problem in bone marrow transplantation is to prevent the ensuing rejection reaction of the transplanted immunocompetent bone-marrow-derived cells against the host (graft-versus-host (GvH) reaction). The immunosuppressive properties of CS aim at both the prevention of rejection (HvG) and of GvH reaction; they are not specific for either one. Besides the classical cell-mediated response, GvH is likely to be complicated by additional effector cells such as B lymphocytes, natural killer (NK) cells and macrophages, all reacting against the host's antigens. It is thought that multiple clones of different effector cells must exist in GvH disease and that, at the nontoxic blood level concentrations obtained in bone marrow recipients, CS acts probably only on T cells with de novo antigen recognition, while having no effect on presensitised effector cells which are potentially present in the large pool of involved clones (for review see 818, 891).

The early, fundamental work in experimental bone marrow transplantation performed by various groups in mice, rats, rabbits, and dogs treated with CS has been reviewed with competence by Deeg and Storb (202) and by Gratwohl and Speck (336). We shall focus on three major aspects which are: the effects of CS on engraftment, prevention of GvH reaction, and treatment of GvH disease.

The role of CS on engraftment of bone marrow transplants has been tested in rats (943), rabbits (331), and dogs (204, 203). Subcutaneous doses of CS (25 mg/kg/d) administered from day −8 to +18 clearly enhanced engraftment of allogeneic bone marrow in busulfan-treated rats and was more effective than CP or heterologous anti-lymphocyte antiserum (943). A similar beneficial effect of CS on engraftment of fresh and especially of cryopreserved allogeneic bone marrow was seen in the rabbit (333, 331). Results in dogs differ from those seen in rodents (206, 202). All recipient dogs were given 9 Gy TBI followed by haemopoietic grafts consisting of bone marrow and buffy coat cells. Among recipients of DLA-identical littermate grafts all had sustained engraftment, which is not different from MTX-treated dogs or controls without immunosuppression, and which indicates that CS has no direct marrow toxicity. Among recipients of DLA non-identical unrelated grafts 65% given CS alone failed to show sustained engraftment which is similar to the incidence seen when marrow is transplanted without buffy coat cells. Since the addition of MTX to CS resulted in engraftment in thirty out of thirty one dogs, it appears that MTX inactivated a host cell population that was involved in mediating resistance to histoincompatible marrow grafts. Therefore, the presence of MTX eliminated the need for a functionally intact lymphocyte population of donor origin, i.e., the buffy coat cells, and the inactivation of this population by CS had no bearing on engraftment. The reason for this detrimental effect of CS in dogs which contrasts with the results in rats and rabbits might be related to species differences. Indeed, such interference is also not seen in human bone marrow transplantation (336, 943). Another discrepancy between rats and dogs was observed when the effect of CS on engraftment in animals alloimmunised before transplantation was investigated. CS was not able to abrogate transfusion-induced sensitisation and graft rejection across major histocompatibility barriers in rats (943). Similar experiments carried out in the canine model showed that nine out of nine transfused DLA-identical dogs receiving CS (20 mg/kg/d intramuscularly on days −5 to 0) had sustained engraftment, whereas nineteen transfused recipients not given CS rejected their grafts (889). These combined data suggest that CS might abrogate transfusion-induced sensitisation to minor but not to major histocompatibility antigens.

Experimental results on prevention of GvH disease are difficult to compare, because of the many variables between individual studies such as different conditioning regimens (CP, busulfan, TBI), various transplant models (bone marrow cells, spleen cells, lymphoid cells), different species and strains with variable degrees of histoincompatibility, and various treatment schedules with CS. Early attempts at delaying rather than preventing GvH disease in mice were successful with short-term intermittent CS treatment (106, 954). The drug did suppress the GvH reaction in mice injected with subcutaneous doses of 15 to 60 mg/kg/d and for as long as treatment lasted (954).

More extensive studies performed in the rat model are summarised in table 1 of reference 202. CS given in subcutaneous doses of 10 to 30 mg/kg/d or q.o.d. pro-

moted more rapid and complete repopulation of lymphoid tissue as compared with appropriate controls and effectively prevented GvH disease for prolonged periods (954, 943). In addition, CS therapy was able to reverse a delayed or an established GvH reaction (954, 106). It was further shown in a rat study that CS therapy (10 to 20 mg/kg/d given orally from day 13 to 38) reversed and suppressed acute GvH disease, but upon cessation of treatment most animals eventually relapsed into a chronic GvH reaction (99, 169). Tutschka et al. (943) claimed that transplantation tolerance in the recipient chimeras was due to the presence of T_s cells that were able to suppress MLC and were radiation sensitive. The development of the T_s was dependent upon the presence of a functioning thymus. However, Denham et al. (212) showed that all haematopoietically reconstituted (fully allogeneic) rats, which were CS-treated for 6 to 26 weeks as prophylaxis for GvH disease, developed GvH reaction following termination of CS treatment. The potential GvH reactivity of normal donor strain cells was specifically suppressed in the chimeras and this suppression could be transferred via chimeric spleen cells to secondary irradiated recipients, but attempts to demonstrate a role for T_s in the maintenance of the chimeric state yielded inconclusive results.

The ability of CS to prevent GvH disease was also assessed in the rabbit, but the results are somewhat contradictory. In an early study, conditioned rabbits with 12 Gy TBI and grafted with allogeneic bone marrow cells were treated with intramuscular doses of 10 mg/kg/d CS on days 1 to 28. Median survival was 40 days and 33% survived 100 days. Only 25% of CS-treated animals developed GvH disease but several died with perforated gastric ulcers or infections (463). Stable, long-term complete chimerism (>200 days) was achieved in adult RLA-matched unrelated rabbits prepared by 825 cGy TBI and treated with CS (10 mg/kg/d subcutaneously from day −1 through day 14, then oral intake of 25 mg/kg/d through day 40) for prophylaxis of GvH disease. Untreated controls died on days 14 and 26 (73). In contrast, Gratwohl et al. (333) were not able to affect acute GvH disease nor to increase survival of rabbit conditioned with 12 Gy TBI, then reconstituted with either fresh or cryopreserved allogeneic bone marrow and treated with CS at 15 mg/kg/d subcutaneously for 60 days. The same CS dose, however, allowed allogeneic histoincompatible skin graft survival in all treated rabbits as long as the drug was given (333). They suggested that part of the early GvH reaction as well as early graft rejection was mediated by a subclass of cells which is resistant to CS and were later able to experimentally demonstrate the presence of such a subpopulation of T effector cells (sensitive to steroids, radiation and cryopreservation, but not to CS) in rabbit skin allografts (335). The same group has shown that CS enhances engraftment of T cell-depleted bone marrow without preventing GvH disease (333, 336). From the results of another experiment, they conclude that the combination of irradiated donor buffy coat cells, irradiated autologous bone marrow, and CS effectively restores engraftment of T-cell depleted mismatched bone marrow in rabbits without losing the benefit of reduced GvH reaction (331). It should be stressed that several investigators working with CS in rabbits have complained about a species-specific toxicity which has been described by Gratwohl et al. (334) and which is dose-dependent and characterised by wasting and anorexia. Ultimately the animals die within 60 days of treatment with a distended stomach and intestines full of dry, undigested feed. This highlights the difficulty in a number of studies in obtaining a therapeutic dosage level in rabbits on long-term CS therapy (e.g., 463, 333).

Most of the pioneering studies done by Deeg et al. in the canine model are summarised in table 2 of reference 202. All dogs were conditioned with 9 Gy TBI. Twelve out of sixteen recipients (75%) of marrow grafts from DLA identical littermates treated with CS were long-term survivors which is significantly better than 46% long-term survivors in dogs given no immunosuppression after grafting, but somewhat inferior to the 94% survival in seventeen dogs treated with MTX. In dogs given transplants from DLA nonidentical unrelated donors, tolerance could not be induced after discontinuing CS on day 25. Although early GvH disease could be prevented by CS, overall survival was not different from that in concurrent controls given MTX. Similar results were obtained by Miller et al. (646) who found that CS facilitates engraftment of a bone marrow stem cell graft and its acceptance in DLA-identical dogs; however, CS was ineffective across a DLA one-haplotype barrier. Deeg et al. (206) showed that when MTX was given in addition to CS in the DLA nonidentical unrelated combination, all but one dog showed sustained engraftment, and survival (median 100 days) was longer than in concurrent groups. Acute GvH reactivity was prevented completely but some dogs developed chronic GvH disease after immunosuppression was discontinued. These results indicate that CS can induce graft to host tolerance in DLA-identical littermates but not in recipients of DLA nonidentical, unrelated dogs. The latter results are comparable with those seen in canine (204, 110) and rabbit (333, 335) skin grafts models where prolonged graft acceptance is dependent upon continued CS administration. When a combination of MTX and CS is used after grafting, however, half of the animals become long-term survivors. Interestingly, these authors were able to isolate from the peripheral blood of these chimeras treated with both drugs a population of nylon wool-adherent lymphocytes capable of suppressing the reactivity of donor cells stimulated by host cells (202). Marrow grafts across major histocompatibility barriers in the dog provide a severe test for any immunosuppressive regimen. Without postgrafting treatment all dogs given haemo-

poietic grafts from DLA-nonidentical littermates died with GvH disease. In a further experiment ten dogs transplanted with marrow grafts from DLA-haploidentical littermates had sustained engraftment when treated with a combined regimen of intermittent MTX (intravenously on days 1, 3, 6, and 11) and tapered doses of CS for 100 days. The seven long-term survivors (>210 days) are all complete chimeras, but two of them developed chronic GvH disease on days 145 and 149, i.e., after CS had been stopped (203).

There is clear evidence that CS can be effective in the treatment of established GvH disease. This effect has been demonstrated in experiments with rats in which a short initial treatment period with CS preventing GvH reaction would regularly be followed by a later onset of GvH reaction which would respond favourably to reinstitution of the drug (106, 99). However, it was also shown that a delay of CS treatment until day 3 after grafting in rats (943) or until day 5 or 11 in dogs (202) could not prevent the development of GvH disease even during continued CS administration. The situation here seems reminiscent of that seen with some other organs like skin in dogs (204), cornea in rabbits (795), or kidney in rats (431, 433), where a rejection episode becomes reversible only if it results from a previous withdrawal of CS but does not respond to delayed introduction of CS administration.

CS was introduced to clinical bone marrow transplantation in the late 1970s and is now widely used for a variety of different disorders such as acute leukaemia, chronic myeloid leukaemia, pre-leukaemia, aplastic anemia, thalassemia major, severe combined immune deficiency, and inborn errors of disease (for review see 336 and 880). A large survey of bone marrow transplantation in Europe shows that the procedure has changed during the past decade from being an experimental form of therapy at a few centres to an established one all over western Europe (995). Clinical results clearly demonstrate that CS prevents marrow graft rejection in aplastic anemia (442, 30) and also facilitates engraftment (205, 784).

There has been controversy with regard to both beneficial and adverse effects of CS and MTX in clinical practice. In a large, retrospective European multicentre analysis it was shown that CS, alone or in any combination, increased leukaemia-free survival and reduced transplant-related mortality (995). However, the results obtained in three long-term, controlled prospective trials in Seattle led to the conclusion that CS and MTX were comparable in their ability to prevent chronic GvH and probably acute GvH disease (890, 1003). These findings are supported by additional independent prospective trials (784, 74, 285). The best evidence that CS is active in the prevention of GvH disease comes from the observation that this phenomenon occurs 4 to 8 weeks after withdrawal in about two-thirds of the patients who received CS therapy for 1 year or more (336). This late onset of an acute form of GvH reaction, which has not been described as occurring with MTX, regularly responds to resumption of CS therapy. This provides clear evidence that CS can be effective in the treatment of established GvH disease in man (see also references 199, 500, 134).

Although the role of the MHC in marrow transplantation is well defined in laboratory animals, there are few clinical data available to assess the allowable limits for HLA incompatibility in human bone marrow transplantation. A major progress in this field, which has been limited to HLA-identical siblings, would consist of expanding the number of potential donors by using related and unrelated volunteers only partially compatible for HLA. Powles et al. (764) have obtained rather discouraging results in an attempt to use mismatched family donors for bone marrow transplantation as treatment for acute leukaemia. Patients treated with CS alone had prompt haematological reconstitution and were chimeric. However, they developed a vascular endothelial syndrome best described as adult respiratory distress syndrome. Combination of an early course of MTX to CS treatment, in order to alleviate this latter syndrome, resulted in complete failure of engraftment in most patients. Beatty et al. (57), in a different important study using MTX alone to prevent GvH disease, clearly demonstrated the relevance of HLA to clinical marrow transplantation. Delayed engraftment in some patients, an increased risk of rejection, and an increased incidence and earlier onset of GvH disease were found to be associated with HLA incompatiblity. In spite of these complications the projected survival of study patients given transplants during remission, particularly with marrow that was incompatible with one HLA locus only, was not significantly different from the survival of controls (HLA-identical sibling donors) (see also 442, 40, 443). It is obvious that this problem needs to be pursued further. Careful timing and varying drug regimens including CS might yield some promise: First in the dog model and perhaps later in the clinic.

Of particular interest, in our view, is the possibility of correcting inherited enzyme deficiencies by allogeneic bone marrow transplantation. This is documented by the case of a 2-year-old male Siamese cat which received histocompatible bone marrow from a sibling female cat to cure a state of mucopolysaccharidosis VI (308). Following haematological reconstitution the recipient was treated with oral doses of CS (15 mg/kg/d) from day 19 to 104. Karyotypic analysis on day 183 posttransplantation revealed a stable chimera having 73% donor-origin cells. The significant clinical improvement of this cat suggests that allogeneic bone marrow transplantation may provide curative therapy for human enzyme deficiencies. A clinical case of bone marrow transplantation for glycogen storage disease type II (Pompé's disease)

has been reported (973), but the outcome is inconclusive since the child died 33 days later of sepsis after successful engraftment with CS. Recently the case of a 9-year-old girl with juvenile Gaucher's disease, which is an inherited lipid storage disease caused by a lysosomal enzyme deficiency, and who underwent splenectomy and allogeneic bone marrow transplantation (donor: HLA-identical brother) was reported. The girl is described as active and healthy 5 years after her bone marrow transplant (785). There is another report of seven patients with Gaucher's disease treated by bone marrow transplantation and CS (446). Six children had successful engraftment followed by progressive clearing of Gaucher cells.

The cure of experimental osteopetrosis by allogeneic bone marrow injection has been obtained in "op" mutant rats treated with CS (50 mg/kg/d subcutaneously). This high dose treatment was able to prevent rejection of the transplanted marrow without impairing the property of these cells to restore bone resorption in this severe osteopetrotic model (670). Two infants with malignant osteopetrosis were treated by allogeneic bone marrow transplantation and CS and engraftment without GvH disease occurred in both. Osteopetrosis was completely resolved in one at 30 months and was improving in the other 11 months after transplantation (865).

CS-induced syngeneic or autologous GvH disease has been described in lethally irradiated rats and mice following syngeneic or autologous bone marrow reconstitution. This special type of GvH reaction, first reported by Glazier et al. (314), occurred in rats treated for 40 days within a dose range of 7.5 to 25 mg/kg/d CS injected subcutaneously following irradiation and transplantation. Clinical symptoms as well as histologic lesions of GvH disease were observed 12 to 40 days after discontinuation of CS. This phenomenon has been confirmed in rats by Sorokin et al. (879) and by Bos et al. (116) using the same protocol. Cheney and Sprent (165) reported experiments in the mouse, in which lethally irradiated animals reconstituted with syngeneic bone marrow cells were treated intraperitoneally with 10 mg/kg/d of CS for 5 to 6 weeks. A high proportion of mice developed histological signs of GvH disease 2 to 4 weeks after cessation of drug treatment. Furthermore, when normal rather than irradiated mice were given a similar course of CS, lymphoid cells from these animals caused a severe, fatal GvH reaction upon adoptive transfer into irradiated syngeneic hosts. However, successive attempts by Chow et al. (169), Kosugi et al. (533), and by G. J. Prud'homme (Montréal, personal communication) to reproduce the CS-induced syngeneic GvH in the mouse model failed. Bryson et al. (132a) have just recently demonstrated that syngeneic GvH disease could be induced only in 3- to 4-week-old DBA/2 and C3H/HEN mice but not in several other strains. Marcos et al. (605) were able to reproduce an autologous GvH reaction in the immunodeficient (xid) CBA/N mouse, but not in other strains. Their mice were irradiated (8 Gy TBI) after shielding of a leg and injected intraperitoneally with 15 mg/kg/day of CS continuously from the day of irradiation. Syngeneic or autologous GvH disease has also been observed in man, but treatment with CS is not a prerequisite (see references in 169 and 818). Induction of this phenomenon in animals is also not restricted to CS, since it has been induced with 4-hydroperoxy-cyclophosphamide, the in vitro active derivative of CP, and with thalidomide (818). The incidence of the syngeneic GvH reaction could be accentuated by supplying nylon wool non-adherent spleen cells and/or sensitisation with DNCB as an antigen trigger after marrow grafting (818).

Syngeneic or autologous GvH disorders strongly resemble chronic GvH disease seen after allogeneic bone marrow transplantation in animals and patients. The two conditions share clinical autoimmune features (794), have potentially common exogenous triggers, possibly viruses, and may not represent histoincompatible rejection. There is also evidence that these GvH reactions may be directed against "altered self." Therefore, it was proposed that syngeneic GvH, rather than being a distinct entity, represents chronic GvH disease in the syngeneic setting—just like the latter occurring allogeneically (169). However, this interpretation has been recently challenged with valid arguments (606) and the phenomenon of syngeneic GvH will be discussed at length in part IV.C.2.f.

In summary, bone marrow transplantation is used to treat haematologic cancers and certain inherited enzyme deficiencies. It firstly consists in eradicating the recipient's immune system and, subsequently, in reconstituting him by engraftment of a foreign (allogeneic) system. This results in a GvH reaction which is the major problem encountered in marrow transplantation. CS can be effective in favouring engraftment, preventing graft rejection and only partially GvH reaction, and treating established GvH disease. This has been conclusively demonstrated in several animal species as well as in clinical trials. Since CS alone does not significantly contribute to controlling a GvH reaction across a MHC barrier, the number of potential donors, which is still limited to HLA identical siblings, remains very small. This is a crucial issue to be addressed in the future. Finally, CS and a few other drugs have been found to induce an autoimmune-like GvH disease after syngeneic or autologous marrow transplantation.

2. Induction of Unresponsiveness. a. IMMUNOLOGICAL BASIS OF ALLOREACTIVITY. Contemporary experimental work has firmly established the immunological basis of graft recognition and rejection (557). Tissue grafts from one genetically different individual to another require suppression of the immune response if the graft is to survive. Histocompatibility antigens are those cellular determinants specific for each individual of a species that are responsible for immune rejection when attempts

are made to transfer or transplant cellular material from one individual of the same species to another (allograft). These histocompatibility antigens, comprising numerous specificities, are controlled by the major histocompatibility complex (MHC). In addition, numerous minor histocompatibility antigens have been identified that are not encoded by the MHC. It is the incompatibilities of MHC antigens between donor and recipient that induce acute rejection responses and constitute the major barrier to successful clinical transplantation.

The MHC consists of a series of linked genes coding for cell surface glycoproteins anchored in the cell membrane. Class I region genes control tissue antigens responsible for graft rejection; class II genes code for the lymphoid cell surface markers that are required for self recognition between cells and cooperation in the induction of the immune response, i.e., they determine the ability of T lymphocytes of a given individual to recognise and cooperate with antigen-presenting cells (macrophages, dendritic cells, etc.) of the same class II type in the response to foreign antigen (843, 789). Therefore, both MHC class I and II molecules can induce graft rejection.

During a rejection response (alloreactivity) T cells are stimulated by foreign MHC antigens (alloantigens) on antigen-presenting cells (APC) (see fig. 4). The helper/inducer cells recognise class II MHC and the cytotoxic/suppressor series recognise class I MHC markers. The distinction between the two MHC classes is facilitated by two coreceptors referred to as CD4 for class II and CD8 for class I. CD4 is expressed usually on the helper/inducer T cells and CD8 on the cytotoxic/suppressor T cells. It is the critical role of the T_H cells to stimulate clonal expansion of specific clones after selection by antigen, i.e., the precursor T and B cells which are already committed to a single specificity by rearrangement of V region genes of the respective cell receptor for antigen. The activated T_H cells function through release of lymphokines like IL-2 targeted by direct cell-to-cell contact. This cell contact is established by recognition of MHC and a foreign antigen presented on the partner cell (MHC restriction). The T_H cells are capable of helping in the expansion of T_C precursors and their subsequent maturation into effector cytotoxic (T_C) cells, and resting B precursors into antibody-forming cells, or mediate themselves effector functions such as DTH (T_{DTH}) (843, 555).

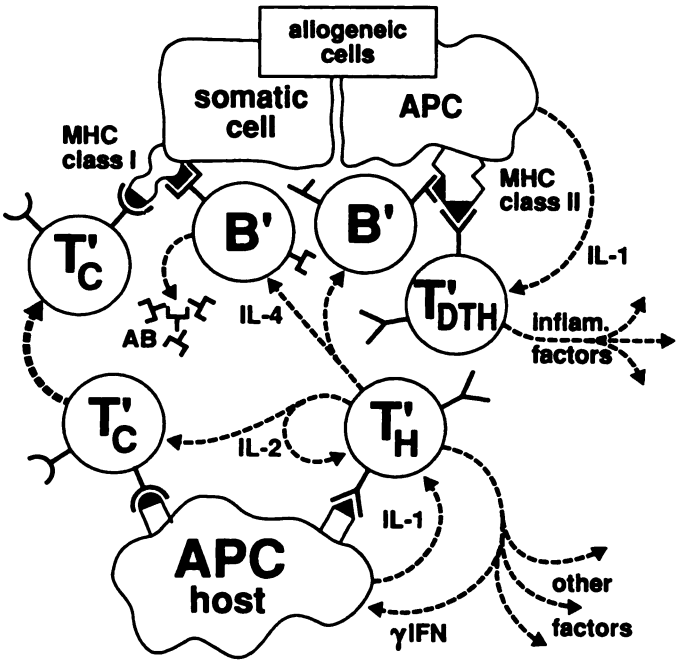

FIG. 4. Allograft reactivity represented in a simplified scheme based on a composite of data from different systems (see text for comments: IV.C.2.a.). APC, antigen-presenting cell; T′/B′, activated lymphocytes; T_H, helper T cell; T_C, cytotoxic T cell; T_{DTH}, T cell mediating delayed type hypersensitivity; IL, interleukin; IFN, gamma-interferon; AB, antibodies; MHC, major histocompatibility complex.

In order to appreciate the mechanism by which CS interferes with sensitisation and allograft rejection, it is essential to understand the above basic events governing allogeneic reactivity. Furthermore, recognition that lymphoid dendritic cells (DC) can cluster with T cells in a unique manner, are also present in most non-lymphoid tissues, and have the potency for triggering immune responses in vivo, raised the possibility that these so-called interstitial DC could be important passenger leukocytes in transplantation (456, 559, 261). It is believed that the DC within the graft are soon replaced by those of the host and that this event contributes to reduce alloreactivity despite the continued expression of alloantigens in the allografted organ itself (572). Various pretreatments of an allograft, which result in depletion of DC, i.e., passenger leukocytes, lead to acceptance of a functioning graft (for review see reference 557). Several studies indicate that donor DC within an allograft may be able to trigger rejection by presenting alloantigens to host T cells which will become activated. However, there may be another route for sensitisation, in which recipient DC might be able to present graft antigens to host T cells (852, 557). The relative roles of these two pathways in different systems remain to be clarified.

b. BASIC CONSIDERATIONS FOR ESTABLISHING IMMUNOSUPPRESSIVE PROTOCOLS WITH CS. With present-day knowledge, the full potential of CS is slowly emerging, as illustrated in fig. 5. It is crucial that the immune system should be strongly immunosuppressed in the early days immediately following transplantation. In the early times, when there was little knowledge about dosage and side effects in patients, it was thought that the use of CS alone in high doses would be preferable to its combination with other drugs and sufficiently effective in preventing allograft rejection (149). Over the years the alarming results, mainly concerning nephrotoxicity (cf. section VII.), have led to a fundamental reappraisal of the treatment protocol for using this drug. Massive reduction of the initial doses coupled with a gradual intro-

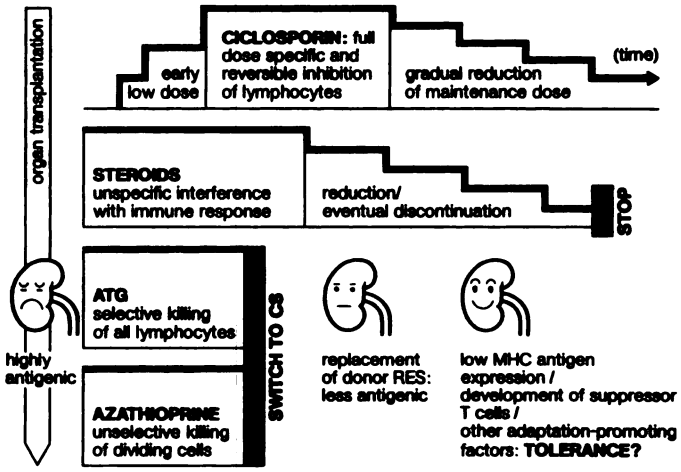

FIG. 5. Schematic representation of new strategies for immunosuppression in organ transplantation. (From Transplant. Proc. 18 (suppl. 5): 271–272, 1986.)

duction of CS have substantially contributed to avoid nephrotoxicity, mainly progressive kidney fibrosis, and lymphoma induction. However, the resulting early post-transplantation immunosuppressive deficit has to be compensated by the addition of other agents, e.g., AZA, ATG, MAbs and TLI. To avoid oversuppression and consequent development of malignancies, one should switch early to CS plus steroids, or if necessary to triple therapy, the procedure as chosen by some centres. With time, both CS and steroids can be lowered and, as is done very successfully in several places, the steroids stopped altogether. The low maintenance dose of CS has proven to be effective and very well tolerated.

Another aspect that supports this approach is the alterations in antigenicity that occur in the graft with time. In a first phase the reticuloendothelial cells, the passenger leukocytes bearing MHC class II antigens, are replaced by similar host-derived cells, thus rendering the graft much less foreign. Because CS inhibits the production of all kinds of lymphokines, including γ-interferon, which is instrumental in the enhanced expression of MHC antigens, the host's antigen-presenting cells become less effective and the cells of the graft are less well recognized as targets.

In addition, it has been repeatedly reported that CS allows the occurrence of suppressor cells in animal allograft models, as has been demonstrated by cell transfer experiments. There may be even more factors leading to a relative, though in no way absolute, state of tolerance. This could explain the extremely low doses of CS needed to ensure toleration of a graft for years.

CS does not yet, and alone may never, achieve antigen-specific tolerance, which is the ultimate goal of transplantation. Better understanding of the mechanism of action is likely to improve the prospects of further refining clinical protocols in both transplantation and in the newly developing indications for autoimmune disorders. In the following we shall review the numerous experimental attempts on how to achieve optimal immunosuppression or to induce unresponsiveness with CS alone or in combination therapy.

c. IMMUNOSUPPRESSION WITH CS ALONE OR IN COMBINATION REGIMENS. The ultimate goal in clinical transplantation is to achieve antigen-specific tolerance following a short course of immunosuppression. Since this is not yet feasible, a relative unresponsiveness to the allograft with minimal long-term immunosuppression is attempted as an intermediate goal. Therefore, animal experiments using CS alone or in many different combinations with other immunosuppressive measures were undertaken in view of supporting new clinical approaches. We shall summarise but a few interesting preclinical data, because basic considerations have been presented in IV. C.2.b. and several results were previously discussed in part IV.C.1.

CS mono-drug therapy. Several animal experiments were performed using CS as the sole drug; for instance, extensive survival of large size skin allografts was obtained with low-level long-term administration of CS in a rat burn model (407). The treatment protocol consisted of initial daily, subcutaneous injections of 8 mg/kg CS for 20 days and thereafter the same dose was given orally in the feed every other day for an indefinite period. In spite of a small, slightly erythematous lesion appearing in five out of eight rats at day 34 ± 11, no rejection occurred in these animals for as long as treatment lasted (>50 days). Hewitt et al. have also claimed that following systemic short-term CS therapy in various allograft models, biologically significant residual drug levels were found in recipients with indefinitely surviving grafts. This may possibly be due to accumulation of CS in body storage sites which would result in protracted CS release in the body fluid; however, whether residual CS may actively contribute to long-term allograft acceptance is uncertain and under investigation (410). The immunosuppressive effect of CS on skin graft survival and inhibition of antipneumococcal antibody titre was compared in rabbits treated with the drug on a daily or on an alternate-day basis. The latter treatment did not allow for dose reduction, since the dose had to be doubled to obtain similar effects as when given in daily doses (293). The use of CS alone as maintenance drug in a very low-dose (oral dose of 2 mg/kg/d) following an "inductive phase" regimen without CS was shown to be effective in a rat cardiac allograft model (903). It should be remembered here that Salaman (815) has used CS mono-drug therapy and compared it with combination therapy in two sequential controlled clinical trials. CS monotherapy was at least as good as combination therapy.

The feasibility of applying CS topically might be of practical importance in some clinical indications. Therefore, the effect of various dosages of topical CS, prepared in olive oil and dimethyl sulfoxide, was studied in skin allografts from Buffalo to LEW rats. Untreated allografts

were rejected in 7 days but survived for 19, 29, or 41 days after 10, 20, or 28 days of topical CS (10 mg/rat/d) application, respectively. Long-term graft survival (>100 days) was seen with continuous CS treatment at 10 mg/rat/d, 10 mg/rat q.o.d., and 5 mg/rat/d. Although the therapeutic blood level of CS ranged from 250–500 ng/ml, direct application of CS onto the allograft resulted in longer survival compared with that of recipients given topical CS on the normal skin 6 cm distal from the allograft (561). Another study demonstrated T cell-mediated site specific immune unresponsiveness by locally applied CS in a dual rat skin allograft model (LBN to LEW rats) (83). Immediately after grafting LBN skin onto LEW recipients, the latter received subcutaneous injections of systemic CS (8 mg/kg/d for 10 days) prior to transdermal application (5 mg/kg/d; vehicle not disclosed) which lasted until day 40. Prolonged skin allograft survival was observed both grossly and histopathologically in the presence of topically applied CS, while contralateral vehicle-treated control grafts were rejected. The results also show that towards the end of treatment systemic T-cell-mediated immunity appeared unaffected, while CS levels were low systemically, but showed relative site-specificity in terms of tissue concentration.

While CS can be used clinically as the sole drug, as initially recommended by Calne and his group (145, 148), Starzl and coworkers suggested combining CS with steroid therapy from the onset (885). Today, CS is seldom used as mono-drug therapy (815); it is most often combined with steroids or with steroids plus azathioprine (563).

CS in combination with other chemical immunosuppressants. Experimentally, a clear synergistic effect was observed when combining very low-dose CS (1.25 mg/kg/d intraperitoneally) with prednisolone (6 mg/kg/d) in a rat cardiac allograft model (809). The synergistic effect of oral low-dose CS (2.5 or 5 mg/kg/d) and topical corticosteroid (fluocinolone acetonide) was highly significant on the survival of rat allogeneic skin grafts (1022). However, using a dog renal allograft model, a suboptimal dose of CS (10 mg/kg/d orally indefinitely) combined with a subtherapeutic dose of prednisolone (1 mg/kg/d) did not result in an additional effect (435). Combined immunosuppressive therapy with CS and AZA resulted in a synergistic effect in three of four experimental models. The best results with the least toxicity were obtained with a combination of 1.25 mg/kg/d CS injected intraperitoneally plus 15 mg/kg/d AZA for islet allotransplantation in rats across a minor MHC barrier and for heterotopic heart allotransplantation in rats across a major MHC barrier (881). It was also found that low-dose AZA (5 mg/kg/d) is synergistic with low-dose CS (10 mg/kg/d intraperitoneally) in prolonging rabbit skin allograft survival (294).

Interesting results were obtained with alternating-day regimens using CS and AZA. For example, 2.5 mg/kg/q.o.d. of CS combined with 1.5 mg/kg/d AZA resulted in over 100 day graft survival in a heterotropic cardiac allograft model in rats (1004). Collier et al. (179, 178) discovered that alternate-day CS (25 mg/kg/q.o.d.) and AZA (5 mg/kg/q.o.d.) were clearly superior to a half-dose daily of each drug for prolonging experimental renal allograft survival in the dog. Median survival with alternate-day treatment was 187 days compared with 68 days with daily half-dose therapy. In the first group five out of twelve dogs survived over 420 days compared with one out of fourteen dogs in the second group. These results have been confirmed in a preliminary clinical trial (144). Finally, very recent clinical data are indicating that alternating-day CS and prednisone have acceptable toxicity and appear to improve survival in patients with high-risk chronic GvH disease (895).

CS combined with MTX has produced very positive results in canine bone marrow transplantation as discussed previously (see IV.C.1.e.). The combination prevented failure of engraftment of histoincompatible marrow (206), produced superior results in GvH disease prophylaxis to MTX alone in dogs given marrow grafts from DLA-haploidentical littermates (203), and induced specific tolerance and immunocompetence in haploidentical, but not in completely allogeneic canine chimeras (201).

Combined synergistic effects of CS and sodium salicylate upon survival of rat heart allografts have been documented (849). A combination of CS (5 mg/kg/d orally), prednisone (5 mg/kg/d orally) and FK 506 (Fujisawa Pharmaceutical Co. Ltd., Japan) (0.5 mg/kg/d orally) was used in a canine renal allograft model and prevented rejection in five out of six dogs (935). Omission of the prednisone reduced the survival rate to three out of six dogs. A synergistic effect of CS with FK 506 has also been reported in heterotopic cardiac transplantation in the rat (678) as well as in the MLC in vitro (1020). In contrast, we have observed a moderate potentiating effect of the two drugs in two rat models (plaque-forming cell assay and experimental allergic encephalomyelitis), but no such effect was found in two other rodent models (localised GvH reaction or lymphnode weight assay in the rat and DTH induced by antigenprimed T_H cells in mice). The subtherapeutic doses of CS were given orally and of FK 506 subcutaneously (J. F. Borel and P. Hiestand, Basel; unpublished results). *Bordetella pertussis* vaccine potentiated the effect of CS (20 mg/kg/d subcutaneously for 14 days) in prolonging skin graft survival in rats. The synergistic effect of the vaccine occurred only when it was given before grafting (758). Finally, an interesting attempt to induce tolerance with CS and CP after renal transplantation in swine was undertaken (439). In the kidney recipients, immune response to the renal alloantigens takes place immediately after grafting. CP needs to be administered during clonal expansion, because lymphocytes are most susceptible to the toxic

action of CP when they are rapidly dividing. After clonal deletion or reduction by CP, low-dose CS (10 mg/kg/d orally) was given to sustain the inhibition of the residual allograft response and to allow generation of T_s cells. Although significantly prolonged survival was observed in one group, induction of specific unresponsiveness was not conclusively achieved with this combined protocol (439).

A clear synergistic effect of subtherapeutic doses of CS (2 mg/kg/d orally until rejection) and subtherapeutic doses of mizoribine (Bredinin) on the survival of heterotopic heart and partial-lung allografts en bloc was reported in the rat (898). This synergism was also confirmed with low-dose CS (10 mg/kg/d orally until rejection) in combination with subtherapeutic doses of mizoribine in dog renal allografts (15). An additive effect was observed in canine renal allograft recipients administered a combination drug therapy consisting of CS (5 mg/kg/d orally) and mizoribine (3 mg/kg/d orally). It was concluded that low-dose mizoribine enhanced the immunosuppressive effect of low-dose CS without complications due to hepatotoxicity, myelosuppression or infection (352). This combination therapy suggests the possibility of realising steroid-free immunosuppression.

CS in combination with ALS/ATG or MAbs. Very promising interactions have been observed in experimental models between CS and enhancing serum (containing antibodies against donor alloantigens), antilymphocyte serum (ALS/ATG), or various monoclonal antibodies (MAbs). A first study showed a positive interaction between low-dose and short-term CS and ALS, but not CS and enhancing serum, for suppressing renal allograft rejection in the rat (430). A second study used a different enhancement protocol, namely by immunising actively the recipient rats with spleen cells from the donor strain and passively with hyperimmune serum 11 and 10 days before transplantation of a cardiac allograft, respectively. When subtherapeutic doses of CS (1.5 mg/kg/d for 7 or 14 days) were injected intramuscularly after, but not before transplantation, into enhanced hosts, permanent graft acceptance was observed. Adoptive transfer of splenic T cells of CD8 or CD4 phenotype from long-term (> 200 days) graft recipients prolonged donor-specific test graft survival in naive rats and delayed rejection in reconstituted B rats from 7 days to 21 to 23 days (742).

Sequential use of CS and ATG was shown to prolong skin allograft survival in rhesus monkeys. ATG was injected on days −1 through 3 posttransplant followed by a 3-week course of CS (tapered intramuscular doses from 20 to 10 mg/kg/d) beginning 17 to 20 days posttransplant. Mean graft survival time was 50 days, which represents a 57% increase compared with the survival in monkeys treated with ATG only (919). The same group has also succeeded in inducing allogeneic unresponsiveness to renal allografts in the rhesus monkey with a regimen consisting of various adjunctive immunosuppressive agents. ATG was administered daily on days 1 to 5 posttransplant and donor bone marrow cells were infused intravenously on day 12 into the recipients. CS was given initially at a lower dose of 10 mg/kg/d for 30 days and then at a higher dose of 25 mg/kg/d until day 150. Low-dose prednisone (0.1 mg/kg/d) was given in combination with CS. The median kidney allograft survival time in six recipients was 180 days compared with 70 days for ATG plus donor marrow cells. Detectable lymphocyte-mediate cytolysis was prevented with this quadruple regimen. Therefore it seemed likely that maturation of T_C lymphocyte was inhibited, especially since the frequency of donor specific T_C precursor cells was not diminished in the peripheral blood (917, 918).

The combination of CS and anti-IL-2R MAbs offers a relatively specific means of immunosuppression as both agents act on the immunological activation of lymphocytes (fig. 6). The two entities prevent activation and proliferation of the alloreactive lymphocytes in the induction phase of graft survival and additionally spare T cells with specific suppressor functions in vivo. An important study by Hancock et al. (384) has documented the presence of small numbers of activated IL-2R$^+$ intragraft T cells as well as macrophages during rat cardiac rejection, and shown how CS, and to a lesser extent anti-IL-2R MAb therapy, inhibits this in situ activation and prolongs graft survival. Graft survival in rats receiving therapy with anti-IL-2R MAb for 10 days was prolonged

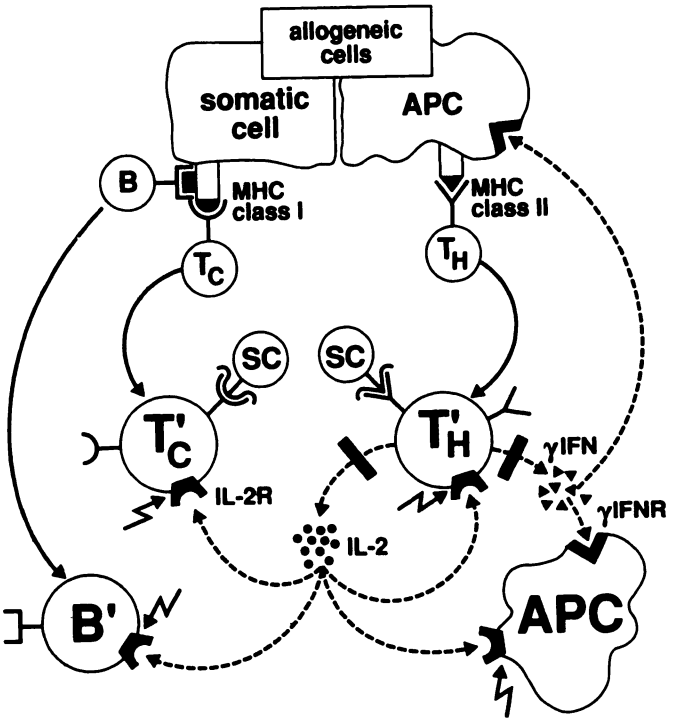

FIG. 6. Diagrammatic depiction of the synergistic action of CS (thick bar) in combination with anti-IL-2R monoclonal antibody (arrow) on activated T and B lymphocytes (antiidiotypic antibodies) and on antigen-presenting cells (APC). The sparing effect of CS on antiidiotypic suppressor cells (SC) is tentatively indicated (see text).

significantly (929). Administration of anti-IL-2R MAb decreased but did not prevent macrophage activation (A1–3$^+$ marker) or fibrin deposition, although IL-2R$^+$ cells were absent from grafts during the 10 days of MAb treatment. In contrast to IL-2R MAb therapy, CS-treated animals did not reject their grafts at all (CS at 15 mg/kg/d for 7 days injected intramuscularly). CS is known to block both IL-2 and γIFN production, although it has no effect on the actual binding of IL-2 to its receptor. No IL-2R$^+$ cells were seen in CS-treated grafts which is consistent with the blockade of T-cell activation (384). In addition, CS also diminishes, directly or indirectly, macrophage accessory cell function, partly by blocking the macrophage procoagulant response that results from T cell allogeneic stimulation (396) or inhibiting the expression of the A1–3 activation antigen and partly by greatly diminishing the macrophage-induced intragraft fibrin deposition. Thus, inhibition of alloreactivity by influencing different steps in the immune cascade with simultaneous preservation of T_S may explain the reasons for the synergy between CS and anti-IL-2R MAb modalities administered both in clearly subtherapeutic doses as demonstrated in a rat cardiac (929) and renal allograft model (908). Moreover, treatment of normal rats with anti-CD4 MAbs (MRC OX-35), beginning on the day of grafting, prevents heart graft rejection across a full MHC-haplotype mismatch. However, in the very stringent grafting test of transplantation of DA skin to high-responder LOU recipient rats, adjunctive therapy with CS, in addition to anti-CD4 MAb, is required to induce prolonged survival. A 30-day course with both agents will result in permanent tolerance (> 100 days) in 20% of the treated rats (401). Similar results were obtained by Auchincloss and Winn (33) who demonstrated that suboptimal doses of CS were synergistic with an anti-CD4 MAb, but not with an anti-CD8 MAb, in allogeneic skin transplantation in mice. The synergism between CS and anti-CD4 MAb was even more impressive in a rodent skin xenograft model (756a). There is no doubt that this form of therapy is of potentially high clinical interest.

CS and adjunctive suppression of APC function. An important immunoregulatory role is played by macrophages, dendritic and other accessory cells which act as APC and which are also designated as passenger leukocytes in allograft transplantation (948, 557, 261). Since it is known that i) dendritic cells within an allograft are able to present alloantigens to host T cells (283), ii) dendritic cells may trigger rejection when administered at or after transplantation (572), and iii) CS exerts in vivo only a marginal direct effect on the APC (529, 677, 924), it follows that combining CS, which mainly interferes with the T cell, with any agent or measure that prevents the function of the APC might result in a potentiating effect.

Ultraviolet (UV)-irradiation can prevent active antigen presentation and has been used in conjunction with brief peritransplant immunosuppression with CS. Hardy and coworkers (385) have shown that this combination can induce either permanent or prolonged pancreatic islet allograft and xenograft acceptance in rodents. UV-B (ultraviolet B) irradiated donor-specific blood transfusions were also used with peritransplant CS treatment in the rat. CS was injected intramuscularly at a dose of 20 mg/kg/d on days 0, 1, and 2 posttransplant. This combination induced prolonged heart allograft survival in a histoincompatible, strong responder host and this effect was donor specific (732). Fetal islet allografts treated in vitro with UVB irradiation and cultured in high oxygen environment were accepted by haploidentical outbred rabbits only if the recipients were given a high peritransplant oral dose of CS (30 mg/kg/d) or chronic low-dose CS (10 mg/kg/d) (247). The mechanisms underlying the in vivo immunologic unresponsiveness induced by pretreatment with UV-irradiated donor-specific blood transfusions and peritransplant CS may include inactivation without elimination of class II APC, generation of specific serum suppressor factor(s) and/or antiidiotypic antibody, and induction of donor-specific suppressor cells (730).

Cytotoxic anti-Ia (class II) MAbs have been used to treat in vitro the allograft before transplantation in various experimental models. This procedure can be combined with organ culture techniques which have proved very successful in reducing graft immunogenicity, especially of endocrine organs, by emptying the graft of its passenger leukocytes (557, 122, 223). The effect of short-term CS, low temperature culture, and anti-Ia MAb on prevention of rejection of rat islet allografts was investigated (912). The results indicate that rejection of rat islets transplanted into a strain with strong immune responsiveness can be prevented by a combination of temporary immunosuppression of the recipients with CS (30 mg/kg/d subcutaneously on days 0, 1, and 2) and pretreatment of the donor islets with either cross-reactive anti-Ia antibodies or culture of the islets at 24°C for 7 days (alteration of APC) before transplantation. A similar study has been performed in allogeneic islet transplantation in the dog. Intramuscular CS administration was begun 3 to 5 days before islet transplantation and the dosage was adjusted (5 to 10 mg/kg) to maintain serum trough levels between 150 to 300 ng/ml; treatment was discontinued after 30 days. It was concluded that low-dosage CS acts synergistically with the in vitro treatment of islets with anti-Ia MAb to prolong islet allograft survival in outbred dogs with induced diabetes mellitus (12). Pretransplant culturing of rat parathyroid allografts for 7 days at 37°C, followed by treatment of the graft with anti-Ia serum plus complement to eliminate passenger leukocytes and treatment of parathyroidectomised, hypocalcaemic rats with subcutaneous doses of 30 mg/kg/d CS for 3 days prior to transplantation,

resulted in 67% (six of nine) of the recipients having functional parathyroid allografts after 1 year. Controls given CS and transplanted with fresh, untreated glands showed a median survival of 81 days (84).

Attempts were made to flush canine renal allografts with a solution containing CS before transplantation and to treat the recipients afterwards with minimal immunosuppression. One group reported significant prolongation of kidney survival (798), whereas another group could not reproduce the former results (468). Improved survival of venous allografts in the dog was also achieved following pretransplant, in vitro graft treatment with CS followed by mild systemic immunosuppression with AZA of the recipients (47). It was postulated that the CS molecules bind to the surface of the endothelial cells (which may act as APC) of the graft, the initial site of graft-host confrontation, with possible masking of surface antigens and subsequent interference with recognition and early immune response.

CS in combination with TLI. Total lymphoid irradiation (TLI) consists of administering high doses of ionising irradiation to lymphoid tissues while protecting non-lymphoid tissues. Radiation is given in divided doses over an extended period and appears to result in both passive and active suppression which leads to long-lasting tolerance, especially when combined with adjunctive chemical immunosuppression and/or injection of donor lymphoid cells. TLI causes marked lymphocytopenia without late malignancies, depression of cell-mediated immunity including DTH, a decreased response of blood lymphocytes to mitogens, and in particular abolition of MLC responsiveness. TLI stimulates the development of powerful specific T_S cell activity directed against donor cells in the MLC, but presensitisation is not erased (see IV.A.1. and also IV.C.2.f.). An interesting comparative study by Haas et al. (370) on the effects of CS (15 mg/kg/d intramuscularly) versus TLI (20 × 1Gy) on peripheral blood T-cell subsets in cynomolgus monkeys showed that CS therapy augmented CD8 lymphocytes during prolonged allograft survival and that TLI persistently depressed CD4 reactive cells. With rejection, however, CS-suppressed animals exhibited a fall in CD8 lymphocytes, whereas this was not observed in TLI-treated monkeys (compare also 680). It was concluded that, while TLI and CS both increased relative levels of CD8 T-cell subsets during stable allograft function, each appears to exert its effect through very different mechanisms and that these two modalities of suppression may show additive or synergistic effects when used in combination (370). Indeed, a series of experiments performed in rodents, dogs, and monkeys have consistently demonstrated that CS utilised in conjunction with TLI results in synergism and is highly effective in prolonging both allo- and xenografts.

Three different groups have observed that low doses of CS have a synergistic effect with TLI in a rat model of cardiac transplantation. In the experiments reported by Rynasiewicz and coworkers (808) low-dose TLI (5 × 200 cGy) given pretransplant and combined with subtherapeutic doses of CS (1.25 mg/kg/d intraperitoneally) significantly prolonged heart allograft survival. Initiation of TLI posttransplant combined with a 1-week course of CS treatment was not effective, but continuous low-dose CS therapy in combination with postoperative TLI (9 × 200 cGy) resulted in a synergistic effect (808, 65). This protocol has potential for clinical application. Another study demonstrated that the synergism of preoperative TLI (3 × 200 cGy) and CS (2 mg/kg/d intramuscularly) was present even if transplantation was delayed after cessation of TLI. It did not show statistical benefit from addition of splenectomy; however, long-term survival did result when splenectomy was added to the 14-day delayed operation group (228). These data indicate that, in this rat model, transplantation can be performed at delayed intervals following TLI if CS is used as adjunctive immunosuppression. A third study also confirmed that the use of low-dose CS (5 mg/kg/d intramuscularly) for the first 14 days posttransplant, when combined with peritransplant TLI (single dose Pd-H of 13 mCi/kg on day −4), was a highly effective and safe method for prolonging heart allograft survival (549). Most remarkable results were obtained in the situation of heart xenotransplants in the hamster-to-rat model. TLI administered preoperatively over 3 weeks (total dose 15 Gy) and combined with continuous CS therapy (5 or 10 mg/kg/d intramuscularly) starting on the day of surgery allowed successful long-term survival over 100 days without any treatment-related deaths (527). However, very poor results were obtained in another concordant combination of cardiac xenografts, i.e., the rabbit-to-rat model (526). It has already been mentioned (IV.C.1.d.) that CS in combination with TLI led to the development of a state of prolonged immune suppression permitting the growth of tumour xenografts from murine and human origin in the rat (438).

The combined use of TLI (10-week course of 1.8 Gy) and CS (5 mg/kg) plus methylprednisolone (2.5 mg/kg) given intravenously (beginning 3 to 10 days before transplantation and daily thereafter) can inhibit the capacity of specifically hyperimmunised recipient dogs who received renal allografts to muster a secondary humoral response to the DLA antigen(s) used in the sensitisation process; such treatment also abrogates the ability of the recipients to reject renal allografts bearing the same DLA specificities in accelerated fashion (775).

The effectiveness of CS and TLI alone, and CS in combination with TLI was assessed in a primate segmental pancreatic allotransplantation model. Continuous administration of CS (25 mg/kg/d orally) or administration of fractionated TLI (10 × 800 cGy) alone resulted in mean graft survival of 22 and 13 days, respectively. In the group of fifteen chacma baboons that received TLI

(8 Gy) combined with CS (25 mg/kg/d orally for 5 days followed by 10 mg/kg/d intramuscularly until rejection), six had graft survival of over 100 days (mean survival = 52 days) (243). It is difficult to explain why another study has revealed a counterproductive effect of CS (17.5 or 30 mg/kg/d orally, or 2.5 or 5 mg/kg/d intravenously for 14 days posttransplant) plus prednisone (20 mg/d) combined with TLI (total dose 800 cGy) in kidney transplanted baboons (680). The latter finding is especially disconcerting since the same group reported good results in clinical transplantation using preoperatively fractionated doses of TLI (total dose 800 cGy) and CS (starting dose 6 mg/kg/d orally) plus prednisone (20 mg/d) (681). This protocol yielded for the forty-eight immunologically evaluable patients a one-year patient survival rate of 98% and a one-year first cadaver graft survival of 89% (680).

CS in combination with blood transfusion. The striking improvement effect of pretransplant blood transfusions on graft survival is well-known but ill-understood. It has been clearly demonstrated that CS does not override the transfusion effect, i.e, the success rate among patients treated with CS is significantly superior for those who were receiving transfusions than for the non-transfused recipients (733). Most experiments in mice, rats, rabbits, dogs, and monkeys strongly support the notion that donor-specific blood or donor lymphocytes given together with CS shortly before transplantation have a clear beneficial influence on subsequent allograft survival, whereas third-party antigen has little or no effect.

Gorczynski et al. (320) have shown that donor-specific transfusions before transplant decreased both the frequency of reactive cytotoxic precursors and their proliferative potential after activation. Further manipulations, like the addition of CS (single dose of 60 mg/kg intraperitoneally immediately prior to skin grafting) or ALS, aimed at preferentially sparing or enhancing the activity of T_S cells, prolonged skin graft survival in pretransfused mice, and led to the presence of T_S cells in the spleen of such mice, which were active upon adoptive transfer. Similar in vivo experiments with LEW rats, which had been treated with donor leukocytes (10^8 cells) and CS (about 100 mg/kg/d intramuscularly on days 0, 1, and 2) and which were bearing long-term W/F cardiac allografts, demonstrated that these recipients possessed suppressor cells in their spleen that were capable of prolonging donor-type graft when adoptively transferred to unmodified hosts. These circulating T_S cells were of CD8 phenotype and were also capable of suppressing MLC response to donor spleen cells (731).

Combined pretransplant donor-specific blood transfusions and low-dose CS constitute a very effective conditioning treatment for preventing donor-specific alloreactivity. Martinelli and coworkers (614) found that ACI and BUF cardiac graft survival in LEW hosts conditioned with specific or nonspecific blood transfusions (1.5 ml) and a 5-day postoperative course of CS (20 mg/kg/d intramuscularly) were indistinguishable from graft survival in untransfused hosts, indicating no interaction between transfusion and CS under those conditions. In contrast, the effect of a postoperative 5-day course of CS (10 mg/kg/d) was extended by conditioning the recipients preoperatively with both donor-specific blood transfusion (day −8) and CS (10 mg/kg/d on days −8 to −4). More remarkably, a posttransplant 30-day course of subtherapeutic doses of CS (2.5 mg/kg/d) resulted in long-term prolongation (> 100 days) of ACI grafts in many hosts conditioned with donor-specific transfusion plus CS, while the majority of controls conditioned with nonspecific transfusions plus CS or CS alone rejected their grafts within 3 weeks. These authors further demonstrated that heat-treatment of allogeneic blood eliminated the humoral cytotoxic responses to donor-specific transfusions and actually enhanced their beneficial effects in terms of graft survival; these same effects were strongly increased by CS (615). Niessen et al. (700, 699) reported that donor-specific pretransplant blood transfusions (1 ml on day −7) accelerated rejection of cardiac allografts one way in the rat strain combination, yet in the reverse combination a single transfusion resulted in indefinite survival. Posttransplant CS (15 mg/kg/d intramuscularly on days 0 to 7) overcame the sensitisation in the former case and did not abrogate the beneficial effect in the reverse situation. However the same authors also made the unexpected observation that CS (5 to 15 mg/kg/d intramuscularly on days −8, −7, and −6) given concomitantly with the donor-specific blood transfusion on day −7 resulted in a negative effect, since the moderate prolongation was less than that obtained with CS alone (701). In contrast, donor-specific blood transfusions (on days −21, −14, and −7) combined with concomitant CS (5 mg/kg/d injected subcutaneously from day −28 until −5) in recipient rats induced a state of tolerance which was associated with indefinite (> 100 days) renal graft survival, excellent graft function, and prolongation of subsequent donor skin grafts (612). Extension of these experiments disclosed a dramatic synergistic beneficial effect of prior multiple donor-specific or nonspecific transfusions to CS as opposed to AZA (613).

Donor-specific blood transfusions were used in the hamster-to-rat cardiac xenograft model. The test group of recipients received transfusions of 1 ml on days −21, −14, and −7, followed by triple immunosuppression with CS (20 mg/kg/d), AZA (2 mg/kg/d), and methylprednisolone (1 mg/kg/d) intraperitoneally beginning on day −1 and continued until rejection. This group showed the shortest survival with rejection within 11 minutes. This regimen without peritransfusion immunosuppression leads to sensitisation and enhanced antibody-mediated response resulting in hyperacute rejection. In contrast, the non-transfused but immunosuppressed group had a

bimodal survival with an average of 5 (n = 7) and 56 days (n = 6) (489).

Experiments performed with skin allografts in rabbits showed that allograft enhancement was optimally achieved by perioperative donor-specific blood transfusion (20 ml) and a concomitant single dose of CS (20 mg/kg intramuscularly). It was found that the CS-dose-dependent regulation of the transfusion-induced allograft benefit is not at variance with the clonal deletion hypothesis, where the graft prolongation obtained with the optimal concentration of CS and blood could be taken to indicate that 20 mg/kg CS is sufficient to delete the transfusion-induced sensitising clones without damage to the clones responsible for mediating allograft enhancement (872). Similar studies with renal allografts in rabbits confirmed the previous results, since CS not only abrogated the sensitising effect of donor-specific blood transfusion but, in combination with donor-specific transfusion, synergistically enhanced renal allograft survival between outbred rabbit strains (291).

In the dog, the blood transfusion effect (100 ml at 4, 3, and 2 weeks pretransplant) only showed up in recipients treated with AZA and prednisolone (starting on the day of operation) and not in those on CS (10 mg/kg/d for 28 days posttransplant; first 4 days intramuscularly, thereafter orally) (703, 702). These results were later confirmed by another group in so far as donor-specific transfusion (100 ml) 1 day prior to transplantation combined with a standard course of CS (17.5 mg/kg/d orally until rejection) also starting on day −1 actually decreased kidney survival to 20 days compared with 52 days for CS therapy alone (218). However, when CS (20 mg/kg/d orally for 6 days) was given together with donor-specific blood (50 and 30 ml on days −10 and −7) prior to kidney transplantation, there was clear indication that CS enhanced the donor-specific transfusion effect on canine renal engraftment (723). Others have also demonstrated that CS (15 mg/kg/d intramuscularly for 6 weeks) given concomitantly with donor-nonspecific transfusions (5U over a 5-week period) to beagle dogs before transplantation will both prolong renal allograft survival and decrease the sensitising effect of transfusions (553). These experiments were extended by adding low-dose CS for continuous posttransplant therapy. CS given prior to renal transplantation, with or without donor-nonspecific transfusions in DLA haplotype/MLC-reactive beagle dogs, resulted in prolonged graft survival with lower doses of CS (2 to 5 mg/kg/d intramuscularly) required posttransplantation (135). Finally, Borleffs et al. (114) have well documented that the beneficial effect of nonspecific blood transfusions (3 × 20 ml at biweekly intervals, last one 2 to 3 weeks before transplantation) was not altered by middle- to long-term treatment with CS (10 to 25 mg/kg/d intramuscularly starting on the day of grafting) in the rhesus monkey.

It is worth mentioning that the effect of repeated administration of 15 Gy irradiated donor buffy coat cells during the first 2 weeks following T-cell-depleted bone marrow transplantation in rabbits treated subcutaneously with decreasing doses of CS reduced the rate of rejection without increasing the GvH reaction. It was observed that T cells irradiated with 15 Gy no longer proliferate but remain viable and release IL-2 (331). A similar protocol used in patients appears to restore engraftment without losing the benefit of reduced GvH disease (337).

CS in combination with infusions of other leukocytes. Blood transfusions have also been replaced experimentally by infusions of bone marrow or spleen lymphocytes; but, as previously mentioned, injection of extracted donor antigens have also been used. Thus, CS does potentiate allograft unresponsiveness induced by bone marrow transfer combined with ALS in mice, dogs, and primates (for review see 661). In the mouse model, the timing of introduction of CS relative to bone marrow infusion seems important (661). Recent experiments in renal allografted dogs, conditioned with ALS and donor-specific bone marrow (harvested from ribs; infusion on day +10), showed that addition of a short course of low-dose CS (3.2 mg/kg/d from day 30 to 60 posttransplantation) significantly increased the number of long-term unresponsive animals (661). It was also found that CS and low-dose prednisone given for varying time periods acted synergistically with donor bone marrow and ATG to increase the incidence of long-term survivors in monkeys (917; see also above: CS in combination with ALS/ATG). However, postoperative donor bone marrow infusion on day 3 or 7 in rats after a 3– or 6–day course of peritransplant CS (8 mg/kg/d intramuscularly) did not show any significant increase in cardiac allograft survival over their respective controls (920). In contrast, vascularised hind limb allografts in rats led to development of donor-host lymphoid chimerism and, depending on the CS treatment protocols used, subsequently to indefinite composite tissue acceptance (408). Preoperative treatment of LEW rats with CS (10 mg/kg/d orally for 14 days) plus DA spleen lymphocytes (10^8) on days −14 and −7 markedly prolonged the survival of subsequent DA renal allografts, but neither CS alone nor splenic lymphocytes had any effect in preventing rejection. Chimerism was not detected and this pretreatment protocol exhibited specific unresponsiveness in terms of rejection of a third-party (PVG) renal allograft, but not in terms of the lymphocytotoxic antibody response to this graft (437). In another study donor spleen cells (10^8) were given intravenously immediately after heart transplantation in rats. A single dose of CS (25 mg/kg) was injected intramuscularly on day 2 after grafting. This perioperative treatment protocol prolonged median graft survival up to 37 days as compared with 14.5 days in controls treated with CS only. This effect was cell dosage dependent and also donor-specific, since third-party

spleen cells had no effect. Interestingly, when blood (1 ml) was used instead of spleen cells, no prolongation was found; this may be due to insufficient antigen dosage (982). In a different approach, it was shown that donor-specific auxiliary spleen allograft rejection could be prevented by CS treatment (10 mg/kg/d orally for 14 days) and that 50% of rats with long-surviving spleen allografts (previously made diabetic with streptozotocin) were accepting subsequent islet allografts placed under the kidney capsule from rats of the same donor strain. The acceptance of islet grafts was increased to 100% by a further short course of CS (10 mg/kg/d for 5 days) treatment postoperatively (340). All the above results, including those from blood transfusions, indicate that both the cell load and the timing of infusion and CS treatment are critical variables in establishing a successful protocol.

CS in combination with bromocriptine. Recent findings have documented that prolactin is involved in the modulation of immune responsiveness. Apart from the well established effects of prolactin on growth, reproduction and osmoregulation, it has been shown that hypophysectomised rats fail to mount an immune response and that treatment of these animals with either prolactin or the structurally closely related growth hormone restores immune responsiveness (for review see 802, 566, 414). Moreover, specific binding sites for prolactin have been demonstrated on human peripheral lymphocytes from which prolactin is displaced by CS, but not by a biologically inactive analogue. It has, therefore, been suggested that prolactin may be involved in the maintenance of T-cell immunocompetence, possibly by exerting a trophic effect, and that the immunosuppressive effects of CS may be mediated by the competitive displacement of prolactin from binding sites on lymphocytes (415). Very recently, Mukherjee et al. (677a) have discovered that prolactin induces IL-2R on the surface of lymphocytes in vitro.

An intriguing finding is the episodic increase in serum prolactin concentration occurring 5 to 6 days before a positive endomyocardial biopsy (> 3 times higher than in biopsy negative cases) and which appears to predict cardiac allograft rejection in the CS suppressed patients (567). Similar data had been previously obtained in a rat cardiac allograft model (D. F Larson, SRI Hanover; personal communication). Pretreatment of mice with the prolactin-secretion inhibitor bromocriptine (5 mg/kg/d subcutaneously for 7 days) markedly reduced lymphocyte responsiveness as assessed in an ex vivo MLC assay (415). CS was administered in combination with bromocriptine to rodents and the potentiating effect assessed in several immunological test systems. It is essential to treat the animals both before and after antigenic challenge with bromocriptine in order to have their prolactin level well depressed at the time of immune reactivity. Thus, rats were treated with CS (5 mg/kg/d orally from day 0 to 13) in combination with bromocriptine (5 mg/kg/d subcutaneously from day −7 to 13), and they received a kidney allograft on day 0. The allograft survival exceeded 250 days in all five animals, whereas no prolongation was observed with either drug alone (411). While low dose CS (1 mg/kg/d) or bromocriptine mesylate (5 mg/kg/d) alone had no effect on allograft survival, a combination of the two drugs demonstrated highly significant prolongation of graft survival in a rat heart plus lung en bloc transplant model (246). Remarkable results were also obtained in rats receiving pancreas allografts and treated with suboptimal doses of CS (2.5 mg/kg/d injected intraperitoneally until rejection and starting at day 0) and concomitant bromocriptine (2.5 mg/kg/d subcutaneously starting at day −5). Combination therapy prolonged survival to 39 days compared with 11 days for CS alone and 9 days for bromocriptine (263). In a murine model using newborn heart allografts placed under the ear skin, the recipient mice were treated with CS (18 mg/kg/d subcutaneously from day 0 to 17) in combination with bromocriptine used in a microencapsulated form and administered at a dose of 1.6 mg/kg/d starting at day −7 and lasting until day 17. Graft survival reached 21 days in the combination therapy and indicated a superadditive effect, since CS alone resulted in a survival time of 16 days and bromocriptine alone showed no effect (8.6 days compared with 8.5 days in untreated controls) (D. F. Larson, SRI Hanover; personal communication). In the localised GvH reaction in rats, the combination of CS and bromocriptine resulted in an additive effect (411). Moreover, the combination of low-dose CS (2 mg/kg/d subcutaneously) and bromocriptine (1.8 mg/kg/d subcutaneously) also produced an additive effect in the experimental uveitis model in rats (745). These potentiating effects of CS and bromocriptine have so far been restricted to experimental models in mice and rats. In contrast, CS (5 mg/kg/d orally) administered in combination with bromocriptine (10 mg/kg/d every 2 weeks intramuscularly) to diabetes-prone BB rats from the 40th day of age onwards was not more effective than CS alone in preventing development of diabetes, in spite of the prolactin plasma levels being significantly lower in the bromocriptine-treated groups (601).

Clinical results of a small open trial in rheumatoid arthritis patients treated with CS (mean dosage of 5 mg/kg/d) in combination with bromocriptine (starting at 1.25 and increased up to 6.25 mg/d) failed to show any significant difference in clinical and laboratory variables compared with the same patients under CS therapy only (227). The failure may well be due to subtherapeutic bromocriptine dosage. In a different clinical study fourteen patients with chronic sight-threatening intermediate or posterior uveitis, that had failed on corticosteroid therapy, were treated with 4 mg/kg/d CS plus 2.5 mg of bromocriptine 3 or 4 times daily. Ten out of fourteen

patients had significant improvements in vision or in inflammation after 6 months of therapy (746).

If the above data demonstrate that a reduction of serum prolactin levels by bromocriptine leads to a decrease of lymphocyte responsiveness toward antigenic stimulation, it can also be shown in the opposite direction that administration of a prolactin-releasing compound (SDZ 25-240 at 2.5 mg/kg/d subcutaneously) does restore lymphocyte responsiveness to antigen in CS-suppressed animals. Stimulation of prolactin secretion by SDZ 25-240 did completely abolish the inhibitory effect of CS treatment in the PFC assay in mice and in the localised GvH reaction in rats (415). However, SDZ 25-240 was not able to counteract the immunosuppressive effect of CS (10 mg/kg/d orally for 14 days) in rats bearing renal allografts (P. C. Hiestand, Sandoz, Basel; unpublished results).

Summary. Specific unresponsiveness or tolerance toward the graft without the need for permanent immunosuppressive drug therapy remains the goal in clinical transplantation. This state can be easily obtained in the rat species, at least with some organ and tissue allografts, but is rather exceptionally achieved in higher species. Bone marrow transplantation may represent an unusual situation, since the immune competence has to be newly established in the host. Therefore, as an intermediate clinical goal, one strives to reach a relative unresponsiveness toward the graft by searching for synergistic effects of appropriate combinations of drugs and/or means, which would allow for reducing the dose requirements substantially and, additionally, for keeping long-term maintenance immunosuppression at a minimal, well-tolerated level; i.e., exploiting at best the suppressive effects while avoiding the toxic effects. Experimental results demonstrate convincingly that the use of CS has led to major advances in this direction.

Several factors may contribute to the relative adaptation of the graft by eventually reducing its immunogenicity. Major factors could be the replacement of the endothelial system in the graft, which might potentially function as APC, by the host's own cells; suppression of lymphokine production, which in turn would inhibit activation of effector cells by preventing expression of IL-2R induced by IL-2 as well as MHC class I and II products stimulated by γIFN; sparing of the development of specific suppressor mechanisms; and reduction of the function of APC below a critical level (see also fig. 5). CS appears to contribute directly or indirectly to each one of these factors and, therefore, CS monotherapy was proven suitable to maintain long-term allograft acceptance at remarkably low doses. These low doses are known to be ineffective for suppressing a de novo immune response. However, in the early posttransplant period, addition of other means acting synergistically with CS, as for example steroids, MAbs, TLI etc., may help reduce the dose requirements of each suppressant and thus minimise their respective adverse effects.

The different attempts to achieve synergism in various experimental models and the respective effects of each component on the mechanisms promoting unresponsiveness have been analysed. The most promising protocols are obviously those in which each suppressive component used is complementary to another, i.e., each one acting on a different step in the activation cascade. Although the extrapolation of the experimental data, especially that derived from rodents, to the clinic is not easy, several of these combination regimens are already being assessed in patients (cf. for review ref. 930).

d. CS-INDUCED TOLERANCE IN THE RAT. The maintenance of specific tolerance may involve either a passive mechanism, which functionally eliminates reactive cells (clonal deletion), or an active mechanism which requires the function of cells or antibodies capable of inhibiting the graft-specific response responsible for rejection (suppression). The ideal immunosuppressive drug should suppress rejection without interfering with the process of tolerance induction and maintenance. CS appears to fulfill these criteria only in the rat, but since this species possess an unusual immune system, including its response to transplanted organs, it is not possible to extrapolate results obtained in the rat directly to man without experimenting in larger animals. In contrast to the latter, rodents do not express MHC class II antigens in the vascular endothelium (756) and are much less sensitive to irradiation (953).

Rejection of an orthotopic renal allograft in the rat is readily suppressed by a short course of CS given orally and a demonstrable dose response is evident. The lymphocyte-mediated cytotoxicity is little altered (433). The specificity of the suppression achieved in this model is partially specific, in the sense that rejection of third-party kidneys occurs in animals 100 days after the induction of tolerance, and partially unspecific, because the lymphocytotoxin response to these third-party allografts is suppressed (432).

Just as with the kidney, survival of heterotopic heart allografts is also prolonged indefinitely by a short course of CS, and indeed this model was used in the first instance to demonstrate the efficacy of CS in an organ allograft model (532). White and colleagues have shown that the kinetics of unresponsiveness induced by a 14-day course of CS in this model varies according to the time after transplantation at which it is tested (682). The results of this fundamental experiment are summarised in figure 7. Skin grafts from the heart donor or a third-party donor were performed at several different times after heart transplantation. The fate of the skin and heart grafts demonstrated that the properties of the unresponsive state induced by short-term CS treatment changed with time so that three different stages could be recognised. Stage 1 coincides with the period of CS

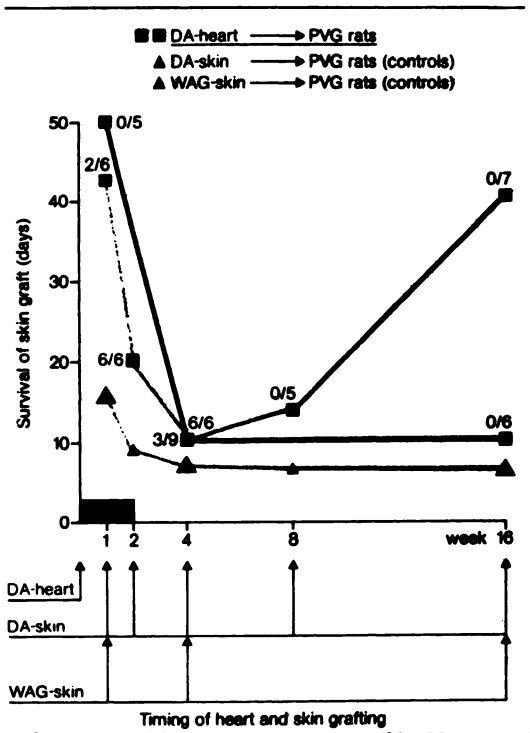

FIG. 7. Summary of the experiments reported by Nagao et al. (682) on the tolerance induction by CS. Rats were grafted with allogeneic hearts and/or skin in the above indicated strain combinations. Squares represent rat groups receiving allogeneic hearts and which were challenged with skin grafts at the intervals indicated. Solid squares stand for DA heart into PVG and challenged with DA skin. Open squares indicate PVG rats also receiving DA hearts, but being challenged with third-party WAG skin. The triangles represent controls receiving skin grafts only. The denominator indicates the group size, while the numerator shows the number of hearts rejected following the skin challenge. All rats, irrespective of the type of organ grafted, were treated with CS for 2 weeks at the beginning of the experiment.

therapy. This early unresponsiveness is relatively stable since in only two cases is the rejection of a skin graft followed by the loss of a heart graft and the skin graft survival is very prolonged. This first stage unresponsiveness is not donor specific in that third-party skin grafts are equally protected from rejection. Stage 2 (weeks 2 to 4) could represent a transitional phase characterised by a marked decline in unresponsiveness. Skin graft survival is not prolonged and rejection of a DA skin graft is inevitably followed by the loss of the DA heart graft. Three out of nine DA hearts are also lost after rejection of third-party WAG skin grafts. Stage 3 unresponsiveness develops from 8th to 16th week post-transplant and the recipients become specifically unresponsive to skin grafts from the DA heart donor strain only, whereas they reject WAG skin in normal acute first-set fashion. This type of unresponsiveness—or rather tolerance—is highly stable since rejection of the heart can not be induced by skin grafts or by transfer into the recipients of large numbers of syngeneic normal or specifically immune lymphocytes.

In further experiments it was shown that a 2-week treatment schedule of CS at 15 mg/kg/d was twice as effective as a 1-week schedule at the same dose. However, even with the latter schedule, the rejection rate is still one third, with the majority of grafts rejecting over a relatively short risk period between days 40 and 60 in which CS blood levels fall below 300 ng/ml (586). When the treatment protocol was devised so as to maintain the CS blood levels above 250 ng/ml until day 60, a long-term (200 days) success rate for heart grafts of 100% was obtained (588). This can be achieved with an intermittent treatment schedule in which the recipients are treated on days 0 to 6, 21 to 27, and 42 to 44 (total of 17 days) with oral doses of 15 mg/kg/d of CS. A time span of at least 8 weeks seems required for this tolerance state to mature. The length of this risk period will possibly change from species to species and probably with the responder status of the recipient. In pigs with orthotopic heart graft the risk period extends to about 120 days (983), and in dogs with a kidney graft up to 1 year (195). Clearly, if such a phenomenon were also to occur in clinical patients, it would be of prime importance to determine over what time-scale immunosuppressive therapy should be maintained.

Immunogenetic disparities representing one or more regions of the MHC in congenic rats differ in their abilities to induce rejection. Unresponsiveness to heart grafts can be induced in the presence of class I or minor antigen disparities, even without immunosuppression. This is in contrast to rats mismatched for class II incompatibility, or for major, or major plus minor incompatibilities, in which situations immunosuppression is required (587). It was also investigated whether CS per se is important in prolonging specific organ acceptance or if the persistence of a graft by itself is sufficient. Skins grafted across a class I incompatibility are all rejected with a mean survival time of 12 days. However, when a short course of CS treatment was added, 80% of skins grafted across the same incompatibility are accepted for 100 days (589). In contrast to skins, hearts grafted across a class I disparity are not rejected, but in these recipients tolerance does not develop as a consequence of a resident graft, since they are unable to accept donor-specific skin grafts for more than 25 days. However, when a short course of CS is administered, not only do these recipients continue to retain their skin or heart grafts long-term, but they are now also able to accept subsequent skins from the donor strain for over 100 days. This phenomenon of tolerance is specific in that third-party grafts are promptly rejected. Previous experiments (492) had also disclosed that the continuous presence of the heart graft was required for the establishment of tolerance. (A failing of these data is that the initial heart graft was not removed from long-term-tolerant rats, but rather during the risk period.) However, results obtained in two rat models of heart and pancreatic islet allografts by Haug and coworkers (392) further confirmed that CS requires the presence of a graft capable of initiating an immune

response for inducing a state of tolerance. From this important work (587, 589) it can be concluded; First, that in the induction of tolerance to organ grafts, the presence of class II is not necessary; second, that despite the fact that in this strain combination, class I-mismatched hearts are not rejected, there is enough immunogenicity present for tolerance to be induced; and last, that CS contributes actively to the development of tolerance.

e. ANALYSIS OF MECHANISMS PROMOTING UNRESPONSIVENESS. Although unresponsiveness most often occurs in the rat, it does occasionally also develop in other species. We have already described under what conditions which organs are accepted long-term in certain species (see part IV.C.1., particularly C.1.a.). We shall now attempt to analyse the possible mechanisms which favour the development of non-specific and specific unresponsiveness.

The primordial question to be addressed is how an allograft is being rejected, because otherwise there would be no way to identify the hypothetical targets of CS' action. We have presented earlier the immunological basis of alloreactivity in general terms (IV.C.2.a.). Now we need to differentiate among certain critical events occurring during rejection and those which are observed when the allograft is tolerated. Thus, the recirculation process of leukocytes plays a vital role by maximising cellular interactions in organ transplantation. The lymphocyte migration patterns reflect the differentiation of uncommitted cells into specific effector cells, the dissemination of sensitised lymphocytes, and their selective migration to critical host areas. Although the role of T cells as mediators of acute immune responsiveness has been well established, it is poorly understood how sensitised lymphocytes are attracted and accumulate within allografts: Is it because of specific alloantigen recognition or are they retained indiscriminately after entering from the circulation? Rat studies were performed using in vitro, differentially radiolabelled, sensitised lymphocytes and the mixed suspensions were adoptively transferred into recipients of two hearts or skin allografts of differing genotypes. Preferential accumulation of specifically sensitised cells occurred consistently in the appropriate allograft; however, the immunospecificity of the cellular accumulation was relatively small. Thus, the cellular interactions induced by the recruited antigen-specific lymphocytes presumably invoked nonspecific mechanisms for the accumulation of other uncommitted T cells. These, in turn, localise indiscriminately, probably as part of a general inflammatory response, and, when activated, produce lymphokines chemotactic for other effector lymphocytes and mononuclear cells important in the efferent arc of immunity (for review see 546).

The pivotal role for antigen-specific T_S in the development and acquisition of unresponsiveness has been demonstrated by the transfer of splenic T cells or their CD8 fraction from CS-treated grafted host rats into naive recipients in which they doubled donor strain, test graft survival (94). Both T-cell fractions migrated in a similar manner, primarily into lymph nodes and away from spleens of test animals. Such traffic of cells carrying memory of prior immunological manipulation may contribute to the improvement of test graft survival by stopping temporarily the maturation of host T effector cells and of host B lymphocytes. In contrast to $CD8^+$ T cells, transfer of T_H (CD4) from CS-modified hosts never improved test graft survival in normal rats (94). Moreover, transfer of T_H-from CS-treated hosts into B (T-cell depleted) grafted recipients produced allograft rejection of otherwise indefinitely functioning transplants, at a rate comparable to that of specifically sensitised T_H (543). Critically, migration patterns of these cells were similar to T_H from unmodified grafted hosts, supporting the notion that T_H cells present in CS-modified grafts recipients were fully potent. Studies were also designed to recreate immune responsiveness in CS-treated graft recipients. Surprisingly transfer of alloimmune lymphocytes could not produce allograft rejection (545). However, pretreatment of CS-treated rats with CP, which results in abrogation of CP-sensitive T_S, allowed subsequently transferred sensitised cells to produce consistently acute graft rejection. Since CS does not alter the immunogenicity of the graft over time (94), a preferential and high accumulation of these transferred lymphocytes at the graft site was observed (545).

Chisholm et al. (166) have analysed several immunological parameters in cardiac allograft tissue taken from recipient rats during uninterrupted graft rejection and compared it with those of recipients in which rejection was prevented by CS. The difference seen between rejecting and CS-maintained nonrejecting graft tissue were evident at all periods after grafting tested and showed that the extent of intragraft leukocyte infiltration was substantially less in CS-treated rats, although this difference was much more marked at later stages. Similarly, the extent to which the infiltrate comprised lymphocytes with immunological specificity for the antigens of graft was significantly less in CS-maintained nonrejecting grafts (186). Additionally, phenotype analysis of the T cells present in rejecting, compared with nonrejecting grafts, revealed a preponderance of CD8 over CD4 T cells in rejecting grafts (166). These findings were confirmed in another study in which the phenotype of leukocytes infiltrating a rat renal transplant at day 5 was determined. In untreated rejecting grafts CD8 cells accounted for about 60% of the infiltrate and many were $IL\text{-}2R^+$. In contrast, CD4 cells predominated and accounted for 60 to 80% of the total leukocyte infiltrate in CS-treated rats (634). In syngeneic control grafts, reflecting nonspecific inflammation as a result of operation, $OX1/30^+$ (leukocyte-common antigen) cells reached 5%, which was significantly greater than the 1.4% infiltration

found in normal kidneys, but much less than the 42% seen in rejecting allografts. The predominant leukocyte subset was CD4 in the syngeneic and normal groups (634). Interestingly, in the cardiac allografts implanted 5 days previously, the fibrin deposition within the allografts—as a consequence of T-cell activation—was substantially reduced by CS treatment (186). Furthermore, CS treatment caused diminution in CD8 lymphocytes and A1-3$^+$ macrophages (macrophage membrane activation marker linked with procoagulant activity) in skin allografts, which in turn results in an absence of the widespread thrombotic and necrotising microvascular injury typical of acute rejection in untreated rats (360).

The in vivo effects of CS on the systemic GvH reaction (parental cells into F1-hybrid rats) were examined in both intact and duct-thoracic-cannulated recipients (166). It was concluded, that CS did not prevent alloantigen recognition, i.e., the sequestration in lymphoid tissues of alloantigen-reactive T cells, nor did it prevent activation of lymphocytes within the T-dependent areas of the lymphoid tissues. However, CS did inhibit, or at least delay, the release of activated cells into the circulation, and it was effective in preventing clinical GvH disease for as long as it was administered. Withdrawal of the drug resulted in overt, lethal GvH disease that was accelerated in tempo, presumably as a consequence of the T-cell activation that had occurred in the presence of the drug (166; see also second part of IV.C.1.e.).

The ability of long-term survivors (rats bearing cardiac or skin allografts; see 589) to respond in mixed lymphocyte culture (MLC) and to produce T_C cells against donor antigens has been examined. Using lymph node lymphocytes, no diminution in MLC response to donor cells and in T-cell cytotoxicity against donor targets was found. In contrast, the alloreactive repertoire of lymphocytes taken from the peripheral circulation showed a donor-specific hyporeactivity in both tests compared with naive controls (985). These results are in keeping with those obtained in different experimental models. Bradley et al. (124) were harvesting infiltrating cells on day 5 from rat renal allografts that were either untreated and rejecting or healthy, i.e., CS-treated or passively enhanced. Using a cytotoxicity assay, T cells from all three groups showed similar levels of nonspecific cytotoxicity against Y3 myeloma cells, but only untreated, rejecting grafts showed alloantigen-specific target cell lysis. These results suggest that specific cytotoxic T cells rather than nonspecific responses play an essential role in allograft rejection in the rat. Finally, Norin et al. (711) have examined the immunosuppressive effect of CS in a canine, single-lung transplantation model which allows the harvest by bronchoalveolar lavage of large quantities of intragraft lymphocytes for assessment of cell-mediated immune function. Thus, alloreactivity of intragraft and peripheral blood lymphocytes from CS-induced unresponsive as well as rejecting dogs was investigated. Evidence is provided that increased intragraft cytolytic T-lymphocyte activity is associated with lung allograft rejection and that diminished intragraft T_C-cell activity is found in tolerant lung allograft recipients. In vitro MLC studies are in agreement with the above in vivo observations, since MLC of lymphocytes from tolerant recipients and irradiated donor lymphocytes also resulted in decreased donor-specific T_c-cell activity compared with controls. Tolerant recipients retained a normal ability to respond to third-party alloantigens in MLC, thus indicating that the diminished donor response was specific. Unresponsiveness was, however, a selective phenomenon in that donor alloantigens in MLC induced a strong proliferative response of T cells from tolerant recipients. Therefore, the data suggest that the induction of diminished donor-specific cytolytic T-cell response, mostly directed against MHC class I molecules, is critical to the development of the tolerant state, whereas the inhibition of a donor-specific proliferative response, which is directed to MHC class II antigens and which is not suppressed, seems not important. This suggested dichotomy in the mechanism of these responses could account for the differential in vivo effect of CS on generation of specific T_C-cell reactivity compared with proliferative activity in tolerant allograft recipients (711). Unresponsiveness could occur at the level of the precursor T_C lymphocyte or at the level of cell regulation, e.g., T_S cells. One possible mechanism by which the alternative DTH-mediated graft rejection may not occur is that the transplanted organ may become depleted of cells bearing surface class II molecules (559), and this phenomenon may be enhanced by CS treatment (353).

CS is known to block the release of γ-IFN (interferon) and to prevent the induction of MHC products in vitro (353). This induction step may itself be critical to the recognition by T cells of cells which do not constitutively express abundant MHC antigens and which constitute the vast majority of cells in any tissue. CS may, through this particular step, be able in vivo to inhibit certain effector functions, such as T-cell cytotoxicity, which are themselves resistant to CS (96), but may be effective when critical levels of MHC products are lacking in the target tissue (382). Indeed, expression of MHC class I and II antigens in rejecting heart and kidney allografts in the DA to PVG rat strain combination was increased 5- to 30-fold, whereas CS-treated heart and kidney allografts showed no induction of class II antigens. MHC class I antigens were induced in spite of CS-therapy, but at levels lower than those seen in untreated allografts, and precisely the same pattern and degree of class I induction was seen in untreated isografts, which finding suggests that this was probably a consequence of the transplantation procedure (654). It was also noted, in the CS-treated heart allografts, that all donor interstitial dendritic cells had disappeared and been replaced by recipient interstitial dendritic cells by the end of the

second week after grafting (654). Results obtained in orthotopic rat liver allografts (DA to LEW) differ in that the induction of class II antigens on the normally class II-negative Kupffer cells was not inhibited with therapeutic doses of CS. Replacement of donor Kupffer cells by recipient macrophages started around day 10 and was complete by days 20 to 40 postoperatively. Although CS therapy reduced the level of class I expression, a definite but weak class I antigen expression was found on all liver cells (309). DA livers from long-term surviving, temporarily CS-treated LEW recipients, were orthotopically retransplanted to naive LEW rats without further immunosuppression. The in vivo relevance of graft adaptation was demonstrated by the fact that all LEW recipients accepted these DA liver allografts and went on to long-term survival (309). Renal allograft adaptation occurring in long-surviving F_1 recipient rats (CS treatment for 14 days) was demonstrated after their retransplantation into normal secondary AS recipients, because the kidneys survived indefinitely without further immunosuppression. In contrast to the fate of F_1 grafts, homozygous AUG kidneys were rejected when retransplanted to second naive AS recipients (376).

The expression of MHC class I antigen on vascular endothelium of mouse skin allografts in vivo is variable and is under influence of the immune status of the recipient. Treatment of recipients with CS was accompanied by low levels of class I antigen expression in the grafts, and similarly low levels were measured in grafts carried by nude recipients in the complete absence of rejection. An increased class I antigen expression in the donor skin was observed after withdrawal of CS-therapy or during first-set rejection (215, 87). The expression of MHC class I and II antigens rises in the kidneys of MRL-lpr/lpr mice as nephritis appears and is coincident with an increase in specific mRNA (messenger ribonucleic acid) for MHC class I and II genes and for β_2 microglobulin. The sites of tubular MHC expression correspond closely to the sites of peritubular Ig deposition. CS given for 6 to 8 weeks (50 to 200 mg/kg/d orally or 30 to 50 mg/kg/d intraperitoneally) reduced the peritubular Ig deposits, renal class I and II antigen expression and specific mRNA for MHC and β_2-microglobulin genes, but did not reduce anti-DNA antibody levels in serum (381). These results raise the possibility that the increase in renal MHC expression not only accompanies the renal lesions, but may play a role in their pathogenesis. Furthermore, mice given two intraperitoneal injections of LPS showed approximately 10-fold increases in MHC class I and II products in kidney, heart, and pancreas. LPS induced class I and II antigens also in nude mice and in mice with severe combined immunodeficiency, indicating that T cells are not required. Interestingly, the effect of LPS is inhibitable by CS and by a MAb against γIFN indicating that γIFN is required for the MHC induction and that the pathway is T-cell-independent and CS-sensitive (470, 380). From the above observations, it can be concluded that CS may exert part of its immunosuppressive effect by blocking the induction and possibly the 'normal' expression of MHC products in tissues.

Part of the maintenance of unresponsiveness seen in CS-treated animals may be due to sparing or activation, or both, of T_S cells (for review see 542). Wang and coworkers (970) have demonstrated that mice sensitised with alloantigens and treated with CS were incapable of generating T_C lymphocytes in vitro; this effect was long-lasting and specific, because lymphocytes from these animals could not be reactivated upon exposure to the same alloantigens in MLC, but their response to a third-party remained intact. The suppressor cells appeared to be T lymphocytes, because treatment with anti-Thy-1.2 antibody and complement abrogated their suppressor activity. Furthermore, these T_S are radioresistant, CP-sensitive, and undetectable in animals receiving CS only, i.e., without alloantigens, implying that CS does not induce, but rather permits the development of T_S generated by allosensitisation (406, 545, 250, 492, 392). Adoptive transfer studies were performed to investigate the functional significance of the T-cell subsets mediating suppression. Survival of test grafts was prolonged significantly when cells infiltrating grafts and spleen were transferred during the inversion of $T_H : T_{S/C}$ ratio, i.e., at 7 days posttransplantation. Before that period, test graft survival was shortened in a second-set manner (23). Prolongation of graft survival was also achieved with inocula of peripheral blood cells obtained from the CS-treated recipients bearing long-standing well-functioning grafts (23).

A recent study confirmed suppressor activity in a rat skin allograft model and has shown that effector function, increasing after discontinuation of maintenance CS, can be again inhibited by T_S cell activity after reinstitution of the drug (928). Induction of a relative allograft unresponsiveness has further been demonstrated in rats utilising the combination of donor-specific blood transfusion (5 ml on day −1) or donor histocompatibility antigen extracted from spleen cells (5 mg/kg/d orally on days −1, 0, 1) which caused synergistic immunosuppression. The mean survival time of BUF rat renal allografts was prolonged in W/F recipients from 7 to 44 or 26.5 days, respectively (CS given alone = 12 days). Two supplemental cycles of CS on days 10, 11, 12 and 20, 21, 22 in recipients conditioned with extracted donor antigen prolonged survival to 67 days (1002). The timing of antigen administration as well as the number of treatment cycles were critical, since with one perioperative CS treatment cycle the shift of donor antigen infusion from day −1 to day 1 resulted in a prolongation of mean graft survival from 23 to 57 days. However, perioperative injection of donor antigen on day −1 or 1 was equivalent when CS was given in three cycles (on days −1, 0, 1; 7,

8, 9; and 14, 15, 16), because survival was further prolonged to 89 or 82 days, respectively (CS alone: 19 days) (1008). This state of unresponsiveness seemed mediated by splenic and peripheral blood T_S cells which were not adherent to plastic or nylon-wool, displayed CD8 phenotype, and were CP-sensitive (1007). Adoptive transfer of T_S cells from antigen-CS-treated hosts prolonged the survival of donor BUF, but not third-party BN, grafts in secondary W/F recipients (1007). Moreover, these T_S cells had a synergistic effect with a perioperative, 3-day course CS administration into the secondary hosts; mean survival was 24 days compared with 12 days for CS alone. The combination of the donor antigen plus CS regimen with T_S cells injected one day posttransplant caused an even greater prolongation of graft survival of 34 days compared with 23 days for antigen-CS-treated hosts (1009). The presence of T_S cells both in the spleen and in situ in renal allografts with prolonged survival in primary hosts suggested that local mechanisms may augment systemic elements to control generation of alloimmunity (1010, 1011). Analysis of the frequency of alloantigen-specific T_C cells present in the lymphoid organs of the W/F recipient rats, following treatment with donor antigen combined with CS, led to the conclusion that both T_S cell activation and clonal deletion participate in the induction and maintenance of specific unresponsiveness under the above protocol (461). The ability of extracted donor antigen to potentiate the suppressive effect of CS has also been documented in the heterotopic rat cardiac allograft model and the histocompatibility antigen extract was shown to contain both class I and II determinants (217).

Other groups using similar adoptive transfer models arrived at similar conclusions (170, 814, 376, 941). Lancaster et al. (562) have examined the properties of the spleen-derived rat T_S cells and have detected a subpopulation that proliferates in vitro when stimulated by irradiated syngeneic T blasts reactive to MHC alloantigens of the kidney donor strain. Since comparable proliferation was not induced either by syngeneic blasts reactive to a third strain or by polyclonal syngeneic blasts, it follows that this subpopulation is anti-idiotypic, with specificity for the idiotypes carried by syngeneic T cells stimulated by the kidney allograft (see also fig. 8).

Hall and coworkers (377, 374, 375), using adoptive transfer assays and irradiated rat recipients, purified lymphocyte subpopulations mediating suppression and showed that radiosensitive CD4 T cells of the helper/inducer subclass, when injected alone, failed to restore rejection, and were also able, when injected with normal lymph node cells or the CD4 cells separated from them, to prevent these cells from effecting rejection. CD8 T cells of the cytotoxic/suppressor subclass, B cells, and serum from rats with long-surviving cardiac grafts all failed to inhibit the allograft responsiveness of normal lymph node cells, and thus were not identified as mediators of the state of specific unresponsiveness. It is possible that CS-treatment, which initially inhibits T_H response to alloantigen, which is responsible to induce graft destruction, either allows cells of this subclass to transform into antigen-specific suppressor cells, or permits the generation of a new population of CD4 cells with suppressor potential. The paradoxical finding that cells from rats with specific unresponsiveness (long-surviving allografts, lack of capacity to initiate rejection in original or adoptive host) have normal reactivity in GvH assays and MLC when reexposed to graft-strain alloantigens remains unexplained (see above, IV.C.2.e.). In addition, removal of the graft allows CD4 cells to regain reactivity within 8 days (374). Inhibition of T_H function (e.g., IL-2 secretion) apparently does not account for CS effects in vivo, because T_H cells from CS-modified recipients can reestablish immune responsiveness in T-cell-deprived (B) rats. Administration of these cells induces both rejection of indefinitely surviving cardiac allografts in B rats, and a shift from depressed to normal IL-2 release in the untreated and repopulated B hosts, respectively (94, 543). Moreover, repeated injections of exogenous IL-2 from conditioned medium failed to modify the immune unresponsiveness of CS-treated hosts, but succeeded in augmenting immune responsiveness towards cardiac allografts in B rats (545).

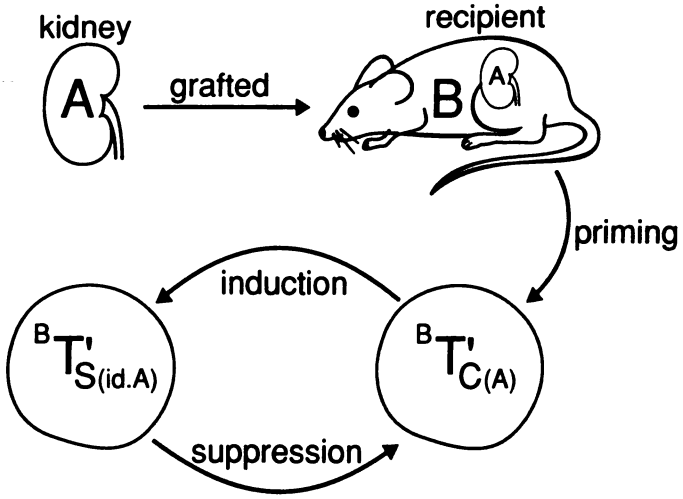

FIG. 8. Tolerance induced in the presence of CS is maintained by active suppression, i.e., specific T_S.

Direct evidence has been provided that CS-treated and allografted rat recipients contain CP-sensitive T cells that suppress the action of passively transferred, specifically sensitised splenic lymphocytes. Moreover, splenic lymphocytes or thymocytes, as well as thymic grafts from CS + CP treated recipients, apparently lost suppressor activity and failed to transfer specific unresponsiveness into normal untreated animals (545, 542). Thymic extirpation in prospective CS-treated mouse recipients prior to transplantation of skin allografts invariably results in rejection once CS is being discontinued (576). However, thymectomy after grafting and completion of the CS

regimen does not influence indefinite graft acceptance. This finding emphasises the close interdependence between the "central" thymus and "peripheral" allograft. Thus, it appears that T_S are "schooled" in the thymus, then accumulate within the graft to ensure its acceptance by the host (544, 546).

It further seems that the type of suppressor cells generated may vary with different models. Yoshimura et al. (1012) used a mouse model in which B6 mice were sensitised with a single intraperitoneal injection of 10^7 allogeneic P815 cells combined with a 5-day course of 50 mg/kg/day CS given intramuscularly. Spleen cells harvested 10 days after treatment did not exhibit any cytotoxic activity, but did nonspecifically suppress induction of T_C when added in vitro to normal B6 mouse lymphocytes and irradiated stimulator cells. The T_S cells were non-adherent to plastic or nylon-wool, sensitive to 15 Gy, and Thy-1 positive. These splenic T_S cells displayed a marked suppression on cytokine release (IL-1, IL-2, γ-IFN, and less on IL-3) when added in vitro to stimulated normal spleen cells. The immunosuppressive efficacy of these antigen-nonspecific T_S cells was further demonstrated in two in vivo systems (adoptive transfer of T_S inhibited development of T_C cells in normal B6 mice to allogeneic tumour cells; suppression in normal B6 mice to develop DTH responses to allogeneic spleen cells). This T_S cell population appears to mediate its suppressor effect by soluble factor(s), since culture supernatants from these T_S cells inhibited in vitro T_C lymphocyte generation.

There is controversy in the literature concerning the expression of cell surface phenotype of the T_S cells, the specificity of their suppressive activity, and their resistance/sensitivity to CP and irradiation. Some have claimed that the suppressor function was correlated with the CD8 phenotype in the rat (94, 546, 23, 1013, 1011, 731) while others found it in the CD4 subset (377, 375). This latter finding also occurred in enhanced allograft recipient rats (no CS used) (741). Hutchinson and Morris (453, 454, 455) reported to have identified three distinct T-cell subsets with allospecific suppressor function in a LEW to DA rat renal allograft model using adoptive transfer analysis. These T_S cells, which are all resistant to CS, were distinguished on the basis of their CP sensitivity or resistance, their cell surface phenotype (CD4 or CD8), and their antigen-binding or anti-idiotypic receptor specificity. The pattern of the genetic restriction of these subsets for major and minor alloantigens and the time sequence of their appearance is compatible with an inducer-transducer-effector pathway interpretation (480, 20). Furthermore, CS has been shown to potentiate skin and kidney allograft unresponsiveness induced by ALS and donor bone marrow in mice, dogs, and primates; very likely by its capability to prevent development of T_C cells while permitting uninhibited development of T_S cells (reviewed in 661). However, due to critical experimental variations among the different groups more in vivo animal work with CS is needed to resolve this problem; there is also a dire lack of data obtained in higher species.

Suzuki et al. (899) have studied the development of suppressor cells in the peripheral blood of recipient dogs receiving heterotopic cardiac allografts and being treated daily with an oral dose of 20 mg/kg of CS. The development of specific and radiosensitive (20 Gy) suppressor cells was observed posttransplantation as demonstrated in ex vivo MLC, although the allografts were eventually rejected. Moreover, IgG fractionated from a recipient's serum exerted a dose-dependent inhibitory effect on the recipient and donor MLC only, strongly suggesting the presence of anti-idiotypic antibodies (899). Deeg and coworkers (201) have investigated the development of specific tolerance and immunocompetence in recipient dogs of marrow grafts from DLA-haploidentical littermates or completely allogeneic unrelated donors. GvH prophylaxis consisted of CS (initially intramuscularly, then orally; starting on day 0 with 15 mg/kg/d and continued, at gradually reduced doses, until day 100) combined with MTX (0.4 mg/kg intravenously on days 1, 3, 6, and 11). Cells from haploidentical chimeras, obtained early after transplantation, non-specifically suppressed donor cell proliferation. Later on, lymphocytes from GvH-free dogs showed specific suppression of donor cells, while lymphocytes from chimeras with GvH disease continued to show nonspecific suppression. The time course of appearance and disappearance of suppressor cells was similar to that observed by Tutschka in the rat model (941; see IV.C.2.f.). Cells from completely allogeneic dog chimeras both with and without GvH disease never suppressed donor cells specifically. Both specific and nonspecific suppressor cells were enriched by nylon wool adherence, expressed T-cell markers, and were not affected by indomethacin. By 1 year after transplantation, chimera lymphocytes no longer showed suppression. In cell-mediated lymphocytolysis assays, lymphocytes from all chimeras, regardless of GvH disease, failed to generate T_C cells against host target cells. While haploidentical chimeras exerted cytotoxicity against third-party targets, completely allogeneic chimeras, which have major defects of cellular immunity, did not. Histopathological studies revealed slow thymus reconstitution in all chimeras. However, while healthy haploidentical chimeras eventually showed thymic histology normal for age, completely allogeneic chimeras did not. Furthermore, the data presented are compatible with the notion that clonal abortion is involved in the development of tolerance in this dog model (201).

We are not aware of further in vivo investigations concerning T_S cells in higher species, except the following three studies all performed in humans. Kerman et al. (503) have evaluated postoperatively CS-treated (in combination with prednisone) recipients of a primary cadav-

eric renal allograft for their donor and nonspecific responsiveness. Patients with post-transplant T_H : T_S cell ratios < 1.0 for the first 0 to 30 days post-transplantation had significantly better 1-year serum creatinine levels, fewer rejection episodes, less immune graft losses, lower post-transplant panel MLC as well as donor stimulation indices, and a lower post-transplant: pre-transplant donor MLC ratio compared with recipients with a T_H : T_S ratio > 1.0. Many of these low T_H : T_S ratio patients displayed T_S cell activity, i.e., T-donor specific and T-non-specific MLC suppressor activity. Additionally the majority also exhibited non-specific adherent monocyte MLC suppression. The observed T suppressive activity was not solely due to CD8 cells, but also to CD4 cells. Finally, there was a significant correlation between the display of T_S cell activity in patients who were matched with their donors at the HLA-DR locus versus no T_S activity in patients unmatched with their donors at the class II locus.

One group has assessed the effect of CS on suppressor cell function in patients with primary biliary cirrhosis (695). Concanavalin A-induced T_S cell regulation of immunoglobulin-producing cells using an haemolytic plaque assay was investigated and the effect of CS after in vivo and in vitro administration was studied. The results show that these patients have defective concanavalin A-induced suppressor cell activity and that this can be corrected in vivo by treatment with CS. The relevance of the improvement in this defect for the pathogenesis and progression of the disease has not yet been established. Furthermore, the assay employed measures only one aspect of suppressor activity and the system used is antigen non-specific. However, correction of the T_S cell defect by CS is expected to improve immunoregulation and thus retard progression of the disease.

Results of another study indicate a reproducible defect in concanavalin A-induced suppressor activity in patients with progressive multiple sclerosis (48). Suppressor and cytolytic cell functions (both expressed by CD8 cells) were evaluated in a rigidly defined group of patients with multiple sclerosis who were being treated with CS. Suppressor function was assessed using both a concanavalin A suppressor assay and a pokeweed mitogen-induced IgG secretion assay. Treatment of these patients with CS did not restore concanavalin A-induced suppressor activity to control values or reduce levels of pokeweed mitogen-induced IgG secretion, but did dramatically inhibit CD8 cell-mediated alloantigen directed cytolytic activity; this latter effect was reversed by addition of exogenous IL-2 to cultures.

To conclude, the discrepancy between in vitro results, obtained under artificial and controlled conditions and with isolated cell populations, and in vivo experiments, in which the various cell-types are constantly interacting at multiple levels in a dynamic and complex environment, never becomes as obvious as when trying to analyse the intricate steps promoting functional unresponsiveness. Many apparent contradictions may not be real, but resolve as immunological knowledge advances. Thus, the process of recirculation of leukocytes allows for cellular interaction and selective migration of antigen-specific as well as inflammatory cells and their accumulation to critical host areas. There are clearly much fewer cells infiltrating an allograft with CS treatment and the infiltrate shows a preponderance of CD4 T cells over CD8 lymphocytes. T cells expressing suppressor functions play a pivotal role for maintaining unresponsiveness in CS-modified allograft recipients. The state of tolerance is dependent on the persistence of alloantigens and MHC class I antigens may be more important than the class II products which are more easily depressed by CS. Since enhanced MHC product expression correlates with rejection and relapse in autoimmune diseases, the ability of CS to effectively reduce their expression must be implemental in the induction and maintenance of unresponsiveness. Retransplantation experiments indicate at least a partial graft adaptation in long-term survivors. Replacement of APC such as dendritic and Kupffer cells has been documented. As shown in the systemic GvH reaction CS does not inhibit activation of lymphocytes in the lymph-nodes, but it does inhibit their release into the circulation. Surprisingly, lymph node cells do not exert donor-specific suppression when added ex vivo in a MLC; however, there is a donor-specific hyporeactivity of lymphocytes in the peripheral circulation. This hyporesponsiveness pertains only to the cytotoxic reactivity, but not to the proliferative capacity. Certain experiments in T-cell depleted rats also suggest that the T_H cell function may not be inhibited in vivo. We are fully aware of the controversy raging among immunologists regarding whether T_S cells exist or not. While remaining open-minded, we have nevertheless attempted to summarise in table 5 the partially controversial data concerning T lymphocytes expressing suppressor characteristics as found in vivo or ex vivo in allograft recipients. It should be obvious that the scarce in vivo studies in CS-treated higher animals do not allow a definitive assessment of the clinical relevance of the many but often contradictory results with T_S cells in rodents. A further caveat concerns suppressor mechanisms in transplantation, where there is consensus that alloantigens and CS treatment may induce T cells with suppressor function, and those observed in autoimmune diseases, where CS therapy may potentially prevent the emergence of suppressor activity, as will be discussed in part IV.D.5.

f. INTERFERENCE OF CS IN THE REGULATION OF TOLERANCE TO SELF AND NON-SELF. CS is not only able to induce unresponsiveness to non-self, but it can, under defined conditions, also break tolerance to self. Lafferty and coworkers (556) have suggested a solution to these paradoxical effects called the CS anomaly. According to

TABLE 5

Tentative characteristics of T cells expressing suppressor activity (T_S)

1. Induction in vivo requires both allosensitisation and CS.
2. Development around day 5 to 6 postgrafting in thymic cortex and splenic red pulp.
3. First present in spleen and infiltrating allograft, later in peripheral blood.
4. Demonstrated as functional in rodents, dogs, and humans by several techniques (adoptive transfer in vivo; ex vivo or in vitro MLC).
5. T lymphocytes as determined by T-cell markers; consisting of possible different subsets: CD4 or CD8.
6. All types are resistant to CS; usually sensitive to CP; radioresistant or -sensitive in rodents, but radiosensitive in dogs and humans.
7. Partly specific suppression: anti-idiotypic receptor specificity; partly antigen non-specific: inhibits release of lymphokines.
8. Conclusion from points 5, 6, and 7: lack of a single entity of T_S cell.
9. Hypothesis: development of different T_s subsets is compatible with an inducer-transducer-effector pathway.

their hypothesis (figure 8), unresponsiveness or tolerance which develops to tissue A transplanted to recipient B in the presence of CS is maintained by suppressor cells $^{B}T'_{S(Id.A)}$, which are specific for the idiotype carried by the alloreactive T cells $^{B}T'_{c(A)}$, and which do not depend on lymphokines for their induction. T_S cells with anti-idiotypic specificity have been demonstrated in rats receiving kidney allografts and rendered tolerant by short-term CS administration (562). The establishment of the negative feedback loop regulating alloreactive cells requires that the production of both $^{B}T'_{c(A)}$ and $^{B}T'_{s(Id.A)}$ occur under conditions that block effector function of $^{B}T'_{c(A)}$. CS is ideal for this purpose, because it suppresses effector function of alloreactive T cells without inhibiting T-cell priming in vivo (see also table 1). This is the form of tolerance resulting from negative feedback mediated by T_S cells which are specific for the idiotype of alloreactive T cells.

However, there is another, different form of tolerance which develops early in neonatal development or during bone marrow reconstitution of the irradiated immune system. Natural suppressor (NS) cells, which have a "null" phenotype are found in all environments where tolerance induction may occur. NS cells show no antigen specificity and their optimal activity is linked to the function of T cells (603). When T cells are stimulated by auto- or allo-antigens in an environment rich in NS cells, the lymphokines they release stimulate the suppressive action of NS cells (fig. 9). This increased NS activity in its turn acts as a negative feedback loop to inhibit the development of fully functional T cells. Interestingly, because of the apparent inability of NS cells to kill target cells, clonal abortion may not occur. This model of NS-mediated tolerance, therefore, postulates the continued existence of potentially functional auto- and allo-reactive T cells (603). In the presence of CS the process of NS cell activation by stimulated T cells will be suppressed, because the drug inhibits lymphokine synthesis. Even though priming may occur, CS also inhibits the alloreactive effector function and thus no GvH reaction will develop. However, when the drug is removed at a late stage of haemopoietic repopulation, primed autoreactive cells which were not functionally deleted, will swing into action and initiate the syngeneic or autologous GvH disease.

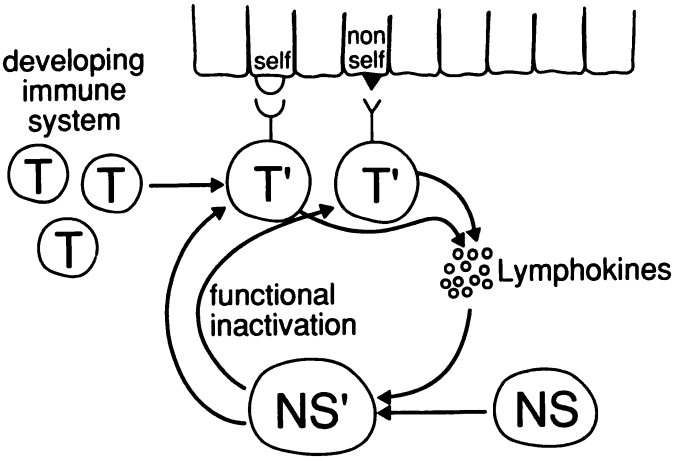

FIG. 9. Natural suppressor (NS) cell-mediated tolerance occurs during the development of the immune system (see text).

The phenomenon of syngeneic GvH (275; cf., last part of IV.C.1.e.), whether induced by CS or another drug, may be viewed as part of a more fundamental biological event, namely that of autoregulation in self and non-self discrimination. Sakaguchi et al. (811) have shown that various organ specific autoimmune diseases could be produced in mice by depleting CD5 T cells, including CD4 and CD8 T cells, but leaving Thy-1^{+}, CD5^{-} T cells in the immune system. They recently demonstrated that CS also selectively abrogated these CD5 T cells in the murine thymus (812). In addition, they showed that organ-specific autoimmune diseases such as gastritis, oophoritis, thyroiditis, or insulitis developed in athymic nu/nu mice after engraftment of the thymus from euthymic nu/+ mice treated with CS. The development of autoimmune disease in the nu/nu mice was prevented by inoculation of thymocyte suspensions prepared from normal nu/+ mice, but not by thymocyte suspensions from CS-treated nu/+ mice. Cotransplantation of normal nu/+ mouse thymus with CS-treated thymus also suppressed the development of autoimmune disease. Inoculation of spleen cell suspensions prepared from normal adult nu/+ mice prevented autoimmune disease, but inoculation of those from newborn nu/+ mice did not. Thus, CS appears to interfere selectively with the thymic production of certain T_S cells controlling self-reactive T cells, allowing the latter to expand and cause autoimmune disease (812). It was recently demonstrated that neonatal administration of CS (10 mg/kg/d intraperitoneally) to

BALB/c mice, particularly during the first week postpartum, causes the elimination of CD4 T cells and CD8 T cells in the thymus and consequently their depletion from the peripheral lymphoid organs, which later results in various organ-specific autoimmune diseases. Development of autoimmunity was prevented when CS-treated newborn mice were inoculated with splenic T cells from normal syngeneic mice. However, removal of the thymus immediately after neonatal CS treatment produced autoimmune disease with a higher incidence and in a wider spectrum of organs. Administration of CS to adult mice does not cause autoimmune disease (813). Hiramine and coworkers (421) have shown that intraperitoneal CS administration to normal, unprimed BALB/c mice at the dose of 20 mg/kg/q.o.d. for 3 weeks markedly reduced thymus weight as well as the size and cellularity of the thymic medulla with relative preservation of the cortex. Analysis of T-cell subsets by MAbs and flow cytofluorometry indicated a preferential impairment of the CD4, $CD8^-$ subset residing in the thymic medulla, whereas CS is much less effective against the immature CD4, CD8 subset present in the cortex and the $CD4^-$, CD8 subset.

Another interesting observation is that CS does alter the induction of allospecific tolerance in vivo (321). CBA mice can be made hyporesponsive to A/J alloantigens by either neonatal inoculation of CAF_1 hybrid lymphoid cells or by intravenous injection of adult mice with A/J bone marrow cells. Specific alloreactivity can be demonstrated by assessing in vitro induction of anti-A/J T_C lymphocytes or in vivo skin graft rejection. When CS is administered in a single intraperitoneal dose (75 μg at birth or 40 mg/kg per adult mouse) at the same time as the tolerance inducing regimen of F_1 or allogeneic lymphoid cells or 3 or 7 days later, it abolished the hyporesponsiveness normally induced by these injections. It seems clear that the in vivo mechanisms which underlie induction/maintenance of tolerance/unresponsiveness in the models described are complex ones. However, it is also evident that the in vivo effect of CS on allograft responsiveness in these particular models is not easily understood from the data existing on the effects of CS in vitro. Mayumi et al. (622) showed that survival of B6 skin grafts was prolonged in C3H mice by priming with viable B6 spleen cells and CP treatment, when the mice received combined treatments with splenectomy, adult thymectomy plus splenectomy, and/or a short course of a larger dose of CS. However, in normal mice (with both thymus and spleen) that had been primed with viable B6 spleen cells, CS-treatment resulted in shortened skin survival time (same as untreated controls).

Since tolerance is achieved with relative ease after allogeneic bone marrow transplantation, the establishment of such tolerance requires a profound reorganisation of the entire immune system. Previously foreign antigens are now seen as being part of self, and vice-versa self-antigens might become regarded as foreign. If that were correct, such a reorganisation of the immune system might not be confined to allogeneic bone marrow transplantation but might equally occur after syngeneic and autologous marrow grafting. Thus, immunologic tolerance to alloantigens should be inducible in recipients of syngeneic marrow transplants, and autoaggression should occur in such a system as well. Indeed, it has been experimentally demonstrated in rats that this reorientation of the immune system, in which the thymus plays a central role, can lead to allotolerance and/or autoaggression.

Tolerance induction to skin allografts in irradiated (10 Gy TBI) and CS-treated recipient Lewis rats of syngeneic bone marrow transplants was attempted. The syngeneic chimeras were skin grafted on the day of marrow transplantation and received 20 mg/kg/d CS subcutaneously for 20 days. These first skin grafts from several histoincompatible strains survived between 87 and 120 days. The operational tolerance was shown to be specific, since only third-party test skin grafts were rejected (941). Further testing showed that the tolerogenic period was limited to about 12 days after irradiation and marrow transplantation. Ex vivo MLC for testing the presence of suppressor effects performed on day 12 suggested a non-specific suppression in all alloantigen combinations and specific suppression on day 40 (941). In addition, adoptive cell transfer studies were done to prove the influence of suppressor cells. Secondary Lewis recipients were irradiated (4 Gy TBI) and grafted with skin allografts from relevant donor strains and injected with 30×10^6 spleen cells from the corresponding alloantigen tolerant syngeneic chimeras. Adoptive transfers with spleen cells harvested either on day 12 or 40 were successful in the majority of irradiated secondary hosts (941). Interestingly, this protective effect of the adoptive transfer into secondary hosts was not affected by infusing chimeric spleen cells to which normal nonsensitised syngeneic spleen cells or thymocytes had been added. However, if a thymus implant was performed in the secondary hosts or if their thymus was shielded during irradiation, the suppression of the chimeric spleen cell transfer was abolished (941). The thymus appears to play a crucial role in this reprogramming of the immune system leading to allotolerance.

The presence of the thymus is also essential in the CS-induced syngeneic or autologous GvH reaction in which autoaggression results from the preceding reorientation of the immune system which is disturbed in the presence of the drug (314, 879, 165, 69, 941, 116, 605, 127, 137). Acute syngeneic GvH disease can be adoptively transferred to secondary recipients if they have been irradiated with 4 Gy TBI and if they are injected with at lest 30×10^6 spleen cells harvested from rats with clinical symptoms of acute GvH disease. Adoptive transfer fails in unirradiated rats or where the thymus has been shielded during TBI. Implantation of a normal syngeneic thymus

after TBI in the secondary recipient also prevents the development of the disease following cell transfer (941). Sorokin et al. (879) have confirmed these data. In addition, cotransfer of an excess of lymphoid cells from normal donors blocked the disease in the secondary recipients.

Why does immunosuppression with CS (and a few other drugs) during the emerging repertoire modify the immune system in such a way as to cause an autoimmune-like GvH reaction? It has been observed that CS profoundly disturbs normal maturation and differentiation of T and B lymphocyte subpopulations. The data indicate that after discontinuation of CS, the syngeneic GvH reaction is primarily acute, with epithelial infiltrates of $CD4^-/CD8$ T cells and lamina propria infiltrates that include double-labelled cells consistent with immature thymocytes. Thereafter follows a rapid transition to chronic syngeneic GvH disease in which the residual mucosal infiltrate is now dominated by double-labelled T cells or thymocytes while the lamina propria infiltrate has more mature helper-phenotype T cells (71). Another study demonstrated that the combination of lethal irradiation, syngeneic bone marrow transplantation, and CS treatment selectively suppresses the repopulation of T_H cells, as monitored in peripheral blood. This suppression of T_H cell regeneration is maintained as long as CS is given, since these cells recover only after withdrawal of the drug. Their reappearance in the peripheral circulation coincides with the development of syngeneic GvH symptoms, suggesting a role of these T_H cells in initiating or causing the disease (116). Aberrant MHC expression has also been found in this model; especially the thymic medulla of these rats, which is normally strongly class II positive, is atrophied and depleted of class II positivity. There is a marked induction of class II antigens and hyperexpression of class I antigens on the non-lymphoid target tissues of GvH symptomatic animals, including tongue, skin, oesophagus, and serous salivary gland. Strong class II antigen induction is seen on many hepatic sinusoidal cells, resembling Kupffer cells. Since unaffected tissues, i.e., heart, pancreas, adrenals, etc., show no differences from normal controls, it seems that the non-lymphoid target tissues may be selected for immune damage by aberrant MHC antigen expression (749).

Finally, spleen cells from CBA/N mice developing a systemic autoimmune disease after daily injection of CS during an autologous bone marrow reconstitution were transferred into unmanipulated syngeneic recipients (605). Adoptive transfer allowed the development of CD5 B cells which also expressed CD11b differentiation antigen. Expansion of formerly absent CD5 B cells is paralleled by a severe reduction in common $CD5^-$ B cell development in the recipients which indicates that precursors for CD5 B lineage do exist in CBA/N mice (208). Furthermore, transfer of thymocytes from such treated donor mice are also able to promote selective development of CD5 B cells in the syngeneic recipients (mentioned in 208). This would imply that the lack of CD5 B cells in immunodeficient CBA/N mice is not the consequence of a primary defect in their precursors, but rather a failure, in xid strains, of the regulatory mechanisms allowing the initial expansion of CD5 B cells in neonates and their subsequent maintenance or, alternatively, the existence of a suppressive mechanism, released by CS treatment, specifically blocking CD5 B cell development in xid mice (208).

Therefore, it is obvious that CS exerts marked effects on the thymus. A single oral administration to mice results in thymus weight reduction and produces a glucocorticosteroid-like effect (805). Systemic treatment over a few weeks also causes involution of the thymus with a normally reversible loss of the medullary epithelium (69, 70, 544, 421). The destruction of the medullary microenvironment, which may be due to a cytotoxic cell-mediated immune reaction (69), is accompanied by a atrophied and depleted MHC class II positivity (749, 127, 304). In contrast, the cortex of CS-treated rodents is relatively intact (70, 421); a normal cortex was also reported in CS-treated chickens (986). These thymic changes suggest that the failure to achieve self-tolerance may be due to the inability of developing thymocytes to encounter class II antigens in the thymic medulla. Accordingly, CS depletes in these CS-treated rats the thymic T_H subset ($CD4, CD8^-$) which is class II restricted, while sparing the T_S subset ($CD4^-$, CD8; class I restricted) and immature thymocytes (CD4, CD8) (127).

These effects of CS on T-cell development in the thymus were further investigated in the mouse by Jenkins et al. (469) to elucidate the physiologic events underlying this induction of a T-cell-mediated autoimmunity. Two major effects were demonstrated: First, CS interferes with the development of mature TCR-$\alpha\beta^{hi}$ single positive cells without affecting the development of $\gamma\delta$-T cells. Second, CS blocks the deletion of the potentially autoreactive T-cell clones among the small number that do mature in the presence of CS. These results provide a potential explanation for the paradox of CS-induced autoimmunity. CS prevents the transition of 90% of cells to the single positive mature stage (CD4, $CD8^-$ or $CD4^-$, CD8). In the small subset that does mature, clonal deletion is prevented by interference with TCR-mediated signal transduction or by reduction of class II molecule expression on bone marrow-derived elements. The autoreactive T cells then seed to the periphery. Their self-reactivity, however, is not manifested until the withdrawal of CS, which relieves the block in lymphokine production and initiates the autoimmune process (469). Similar results were reported by Gao et al. (304) who, in addition, demonstrated that CS-treated mice show incomplete deletion of T cells expressing TCR molecules reactive to self H-2 I-E molecules. Marcos et al. (605) have also shown in their CBA/N

mouse model (irradiation, autologous reconstitution, CS treatment) that these mice exhibit, both in thymus and periphery, a preferential expansion of Thy-1$^+$ T-cell subpopulations characterised as being negative for both CD4 and CD8 markers and that mature T cells are generated in the absence of double positive common thymocytes. Very recent data from Kosugi et al. (533) confirm that CS, in addition to its immunosuppressive effect, can arrest T-cell differentiation. This developmental arrest resulted in the complete absence of functional single positive T cells in the thymus and spleen of syngeneic bone marrow transplanted mice subsequently treated with CS (30 mg/kg/d intraperitoneally for 6 to 7 weeks). In contrast, CS had no detectable effect on B-cell differentiation and/or the generation of CD4, CD8, or CD4$^-$, CD8$^-$ thymocytes; these immature T cells expressed normal functional activity when stimulated with anti-CD3 MAb.

Autoimmunity

1. CS as a new approach to therapy of autoimmune disease (ADs). Autoimmunity is frequently viewed as an abnormality of self-regulation of the immune response, and autoimmune disease (AD) as the result of autoimmune attack. The failure of normal mechanisms of maintaining unresponsiveness to self, either by specific deletion of self-reactive clones or by their active suppression, results in immune dysregulation and potential self-aggression (see C.2.f.). Spreading crossreactivity of antibodies primarily directed against foreign (viral, bacterial) material or release of sequestered antigens into circulation are but two out of several theories advanced to explain the paradoxical loss of tolerance to self. The immune reaction in tissues having incorporated a virus into their genome, which is traditionally viewed as dysregulated and autoaggressive, may indeed represent an appropriate and protective response in tissues disrupted by an independent primary lesion. This reaction may be no different from the immune response to conventional infection, which, although frequently damaging to the host tissues, is nevertheless readily accepted as protective (for review see 844, 174).

Concepts of AD have evolved with the changing understanding of the immune response. In recent years, attention has been focusing mainly on two quite different theories, one postulating clonal deletion of autoreactive cells and the other concentrating on the dynamic regulation of antiself immune reactions. Both concepts, far from being mutually exclusive, may actually operate in a complementary fashion. Studies using MAbs against products of certain Vβ genes expressed on T cells specific for defined class II MHC-associated antigens (489 a, 489 b), or constructing transgenic mice expressing some T-cell receptor specific for a minor histocompatibility antigen (517 a), have provided convincing evidence for a functional T-cell tolerance by clonal deletion in the thymus. Considering the other concept postulating the regulation of antiself immune reactions, it is now clear that self-reactive B cells and some self-reactive T cells persist in the body, and it seems that antigen-presenting cells are fully capable of presenting self-antigens in the same manner as foreign antigens. The critical event in the induction of AD, according to this theory, is the quantitative balance of active suppression versus the induction of self-reactive help (791).

It is helpful to regard ADs as a spectrum with organ-specific disorders such as thyroiditis, myasthenia gravis, and juvenile diabetes at one pole, and non-organ-specific disorders like systemic lupus erythematosus and rheumatoid arthritis at the other. An important feature is the overlap within ADs at each pole (788). Almost certainly, these ADs have a multifactorial basis (genetic, infectious, hormonal, and stress) and the actual contribution from each factor may vary in different patients (906). (Other recent and excellent reviews dealing with basic autoimmunity are the following: 479, 445, 117, 55, 37).

Clinicians and basic researchers are confronted with a large number of unsolved problems in the field of autoimmunity. Any drug capable of shedding explanatory light on the complexities of disturbed autoregulation and at the same time offering genuine possibilities of treatment will meet with spontaneous interest. In view of its presumed mechanism of action, which induces immunosuppression and modulates immune-mediated, chronic inflammatory reactions, it was evident that CS should be tested in experimental and clinical ADs (833, 479, 558). CS has been investigated in both induction and genetic autoimmune models in several animal species (table 6). It has been used preventively to inhibit the onset of the disease, i.e., when it is administered before or from the start of sensitisation. It has also been used for therapeutic treatment, i.e., when drug administration starts only after sensitisation has occurred or is delayed until the first pathological symptoms are appearing. Although these models are helpful in understanding autoimmunity, their extrapolation to the human counterpart must be made with caution. Nevertheless, CS has also shown promising results in a number of clinical ADs, the most important being uveitis, psoriasis, nephrotic syndrome, and rheumatoid arthritis (479).

Since the field of application of immunointervention in clinical ADs is rapidly growing, in terms of both target diseases and therapeutic agents, the risk/benefit ratio of immunosuppressive therapy must be objectively evaluated. There should be a clear distinction between benefit and therapeutic effect. Benefits expected from immunotherapy in ADs can be classified under three headings: Short-term prevention of potentially fatal autoimmunity (e.g., lupus nephritis, aplastic anaemia, multiple sclerosis); long-term prevention of life threatening complications (e.g., juvenile type I diabetes); immediate improvement in the function of a given organ whose deficiency is associated with a major handicap or discomfort

TABLE 6

Summary of results obtained with cyclosporine in experimental autoimmune models

Autoimmune models[a]	Species	Preventive vs. therapeutic treatment[b]	References
Induced animal models			
Freund's adjuvant arthritis (rheumatoid arthritis)	Rat	+/+	106; 108; 112; 182; 210
Collagen type II arthritis (rheumatoid arthritis)	Rat	+/+	483; 398; 484; 482; 108
	Mouse	+/+	900; 428
Streptococcal cell-wall-induced arthritis (rheumatoid arthritis)	Rat	+/+	1005; 988
Allergic encephalomyelitis (multiple sclerosis)	Mouse	+/ND	839
	Rat	+/+	106; 112; 108; 717; 92; 579; 801, 420; 762; 265; 64
	Guinea pig	+/+[c]	92; 292; 779
	Monkey	+/+[c]	108; 98; 92
Allergic neuritis (Guillain-Barré syndrome)	Rat	+/+[c]	514; 388; 687
	Guinea pig	+/+[c]	514
Autoimmune uveitis (human uveitis)	Rat	+/+	719; 720; 159; 721; 658; 158; 716; 718; 154; 157; 509; 657; 296; 130
	Guinea pig	+/+	592; 593; 893; 600a; 707a
	Rabbit	+/ND	493; 494
Autoimmune glomerulonephritis (autoimmune nephritis)			
Acute serum sickness nephritis	Rabbit	+/+	691; 693
Chronic serum sickness nephritis	Rat	+/+	694; 858
Antibody-induced glomerulonephritis	Mouse	+/ND	836
	Rat	+/−	934; 933; 994; 914
Heymann nephritis	Rat	+/−	207; 155
Interstitial nephritis	Rat	+/+	916; 313; 859
Mercuric chloride-induced	Rat	+/+	29; 50
Diabetes (type I insulin-dependent diabetes)			
Streptozotocin-induced	Mouse	−/−	530; 846; 591; 462; 465
Endomyocarditis virus-induced	Mouse	−/−	964; 324
Autoimmune thyroid disease (Graves' and Hashimoto's thyroiditis)	Rat	+/+[c]	629; 390
	Mouse	+/−	967
Allergic orchitis	Guinea pig	+/ND	427
Autoimmune myocarditis (congestive cardiomyopathy)	Mouse	−/−	664; 724; 257; 256; 255
Autoimmune myasthenia gravis (myasthenia gravis)	Rat	+/+[c]	229; 365; 630; 631; 951
	Rabbit	+/ND	951
Autoimmune hemolytic anemia (autoimmune hemolytic anemia)	Mouse	+/ND	185
Genetic animal models			
Spontaneous autoimmune diabetes (type I insulin-dependent diabetes)	BB/W rat	+/−	569; 584; 126; 467 998; 999; 373
	NOD mouse	+/−	505; 287; 665; 972; 909; 288
Spontaneous autoimmune thyroiditis (Hashimoto's thyroiditis)	Obese chicken	−/−	987
Spontaneous posterior uveitis (vitiligo)	SDS chicken	+[c]/ND	277; 748
Murine autoimmune lupus (systemic lupus erythematosus)	NZB/W mouse	+/+[c]	460; 473; 472; 366; 362; 367; 729
Murine autoimmune lpr disease (SLE, arteritis, arthritis)	MRL/lpr mouse	+/+	326; 67; 669; 364
Clinical model			
Psoriasis	Man	ND/+	887; 115; 45; 959; 302; 301; 672; 248; 697

[a] The human correlates to the animal models are indicated in the parentheses.
[b] Positive sign (+): beneficial effect; negative sign (−): detrimental effect of CS; ND: experiment not done.
[c] Relapse of symptoms following discontinuation of CS treatment.

(e.g., rheumatoid arthritis, uveitis, psoriasis) (36). Immunotherapy aims at stopping or decreasing the autoimmune process and will only become manifest if i) no irreversible damage has occurred and ii) the immune process is still aggressive (36). It is, therefore, important to understand the natural history of "pattern" of the disease. The first pattern would be those ADs in which there is an explosive onset with much inflammation, and a rapid loss of end organ functions, with little chance of recovery over the shorter period. In this situation there

is a short time span for immunointervention (e.g., Graves' disease, aplastic anaemia, systemic lupus). The second pattern is one in which, over a long period, there is a chronic inflammatory disorder in which there is a slow loss of function. In view of the chronicity of the disease, the therapeutic effect on the disease has to be measured on the basis of preserved function and, secondarily, on evidence of reduced chronic inflammation (e.g., uveitis, glomerulonephritis, nephrotic syndrome). The third pattern, is one of relapsing/remitting disease, which may appear superficially the same as the second pattern, but is in fact one of remission and recrudescence, suggesting that there is autoimmunity alternating with autoregulation. In this situation, where autoregulation does occur spontaneously, immunointervention tries to prevent autoimmunity while preserving autoregulation (e.g., multiple sclerosis, myasthenia gravis, diabetes mellitus type I, rheumatoid arthritis). Depending upon the periodicity it may be necessary to use an induction type of immunosuppressive regimen, which is converted to a maintenance type of therapy once remission is obtained (887). These different patterns of ADs delineate fundamentally different approaches to i) induction immunosuppressive therapy, ii) maintenance immunosuppression, and iii) promotion of autoregulation. It further seems that relapse/recrudescence may require an approach similar to that used to overcome a rejection crisis (887). In conclusion, immunointervention should not be preferentially used in advanced cases which need high drug dosage and whose clinical response will, at best, only be partial, but should, on the contrary, be used to treat earlier cases which would probably need lower dosage and show a more clearcut benefit for a smaller risk. It is against this background that the clinical results obtained with CS in ADs should be viewed in order to objectively judge their relevance.

Although there are many ongoing clinical trials with CS in several ADs, it is still important to review the effect of the drug in various animal models for the following reasons. First, CS is often used clinically to treat patients who have failed to respond to various other treatments. These patients may belong to the subset who would not respond to treatment of any kind and who may also have irreversible pathological damage even when the immune response would be suppressed. In animal experiments, treatment with CS may be started at various stages after the onset of disease to provide a theoretical knowledge of how late into the clinical course of a disease the drug can produce an effect. Secondly, the heterogeneity of patients in term of age, sex, HLA type, and clinical picture, in addition to the heterogeneity of the treatment protocols, may provide a very confusing picture. Animal experiments produce much more controlled situations. Finally, various manipulations of the animal models may provide some insight into immune defects in the various diseases. These manipulations are not ethically possible in patients.

Autoimmunity can be achieved experimentally by breaking tolerance through bypassing various immunoregulatory pathways. This can be achieved in most normal strains of animals by introducing an autoantigen rendered more immunogenic (e.g., by the addition of FCA). However, in most cases, such diseases are self-limiting and closely resemble the normal immune response to other heterologous antigens. The prerequisite to maintain a state of autoimmunity is to create some inherent (i.e., the genetic susceptibility of selected strains of animals to immune dysregulation) or induced (e.g., immunisation with self antigens, thymectomy and/or whole body irradiation) abnormalities of the immune system. The genetic models, however, should not be regarded as the only ones relevant to the investigation of immunomodulatory drugs for the human conditions. The induction models may be useful not only to elucidate certain mechanisms involved in autoimmune pathology, but also to study the effects resulting from therapeutic modifications. Because the current thinking is that human autoimmune diseases might be due to multiple causes in genetically susceptible individuals, the most appropriate approach would still command the use of both induced and genetic models.

Although animal models are helping us to understand autoimmunity, their extrapolation to the human condition must be made with caution. These experimental models of ADs suffer from the disadvantage that many are artificially induced and that the correlation with the human counterpart of even the genetic models remains in dispute. Thus, it can be seen from table 6 that both the induced and spontaneous models of uveitis and nephritis are susceptible to CS treatment. In contrast, only the induced, but not the spontaneous thyroiditis responds to CS therapy. Similarly, streptozotocin- and virus-induced diabetes are refractory to CS, whereas spontaneously developing diabetes both in BB rats and NOD mice does promptly react to prophylactic treatment with CS. With increasing knowledge of the mechanisms governing the development of autoimmunity in these models, it becomes more obvious why certain models relative to others have a better predictability for some human ADs.

The efficacy of a drug to modify the course of an autoimmune disease can be tested in at least two ways. It can be used preventively to inhibit the onset of the disease, that is sensitisation; on the other hand, it can be used for therapeutic treatment, that is after sensitisation to treat pathological symptoms. Length of treatment, discontinuation, or reinstitution of drug administration, and other parameters (e.g., solvent, dose, route of administration) may be adjusted in several ways (109). Table 6 summarises the results obtained with CS in all

the experimental autoimmune models in which it has been studied.

2. *Results of CS in induction models.* a. EXPERIMENTAL ARTHRITIS MODELS. An experimentally induced condition with T cell-dependent autoimmune character is Freund's adjuvant arthritis (FAA) in rats, which shows close similarities to several forms of human arthritis. Injection of mycobacterial adjuvant leads to T-cell activation and proliferation and to subsequent massive mononuclear cell infiltration of the joints which ultimately results in complete joint destruction. CS strongly reduced the chronic inflammatory symptoms when given either preventively (days 1 to 18 after adjuvant injection) or therapeutically (days 14 to 20) (106, 108, 112). Several other natural cyclosporins exerted a comparable activity to CS as e.g., (Thr^2)-CS, (Val^2)-CS, (Nva^2)-CS, and ($Nva^{2,5}$)-CS (412). The derivative (Val^2) dihydro-CS also showed interesting suppressive effects in FAA and collagen arthritis (108). However, CS and all tested derivatives were inactive in acute inflammatory reactions; thus, they had no significant effect in models like carrageenan paw oedema and granuloma pouch in the rat, no antipyretic effect in the yeast fever model in rats, and did not inhibit UV-erythema in the guinea-pig (H.U. Gubler, SRI Berne; unpublished results). In contrast to most antiinflammatory drugs, the cyclosporins do not induce stomach ulcers in the rat (idem).

Since there is evidence for inflammation-mediated osteopenia in the rat, the therapeutic effect of CS on arthritis-related bone resorption in rats suffering from FAA was investigated. Treatment with CS (5 to 15 mg/kg/d orally for 10 days) resulted in a significant dose-dependent regression of articular swelling and cartilage degradation, concomitant with improvement in bone density; the protective effect of CS on articular damage was supported by progressive improvement in total epiphyseal glycosaminoglycan content. CS-induced blockade of T-cell activation via inhibition of lymphokine production will interrupt the immune-mediated inflammatory process and, in turn, lead to reduction of phlogogenic cytokine release such as IL-1, which is likely to be a mediator of bone and cartilage breakdown in arthritic disease (210). CS has also been used in the naturally occurring retrovirus-induced caprine arthritis model in which advanced symptoms, especially severe inflammation-induced damage of the knee-joints, were reversed under continuous drug treatment (5 mg/kg/d) orally for 17 weeks) as demonstrated by X-ray (103). Therefore, the findings of Movsowitz et al. (671) indicating that CS (7.5 and 15 mg/kg/d orally for 14 to 28 days) administered to normal adult male Sprague-Dawley rats resulted in a significant increase in bone remodeling with striking bone loss is unexpected and clearly contrasts with clinical observations (see also references cited in 671).

Another group (182) has studied the efficacy of CS and MTX in the treatment of rats affected with FAA, as measured on day 17 by reduction of paw inflammation, lymphocyte activating factor activity and the acute phase response (plasma fibronectin, C-reactive protein, albumin and iron). CS (3 to 5 mg/kg/d orally on days 3 to 17) or MTX had a strong therapeutic effect on all parameters measured. In comparison, the nonsteroidal antiinflammatory drugs, aspirin and phenylbutazone, significantly inhibited paw inflammation, but did not decrease the high rate of lymphocyte activating factor activity, nor alter the acute phase response in FAA rats. It is concluded that since CS and MTX have been reported to be effective in rheumatoid arthritis patients, their activity on these immunological and serological parameters may be useful in identifying potential antirheumatic agents and distinguishing them from standard nonsteroidal antiinflammatory drugs (182).

Collagen arthritis can be induced readily in many strains of rats by immunising them with heterologous or homologous native type II collagen emulsified in Freund's incomplete adjuvant. This disease, which is characterised by the development of both cellular and humoral immune responses to type II collagen, can be transferred by sensitised spleen and lymph node cells as well as IgG antibodies to type II collagen. These findings are consistent with the proposal that collagen arthritis is the result of immunologic hypersensitivity to type II collagen. The pathogenetic difference between collagen arthritis and FAA using CS was studied. Daily treatment with CS (25 mg/kg/d subcutaneously) for 14 days completely suppressed the development of collagen arthritis and FAA in rats during the observation period of 45 days. To study whether the unresponsiveness produced by CS was antigen specific, the CS-protected rats were rechallenged with either type II collagen or FCA after discontinuation of CS treatment. Type II collagen-immunised, CS-protected rats did not develop arthritis in response to reimmunisation with type II collagen, but they did develop FAA in response to a subsequent injection of FCA, and vice-versa. The results indicated that specific immunologic unresponsiveness could be induced by CS in these two experimental models of polyarthritis and that there is no cross-reactivity between type II collagen and the mycobacterial cell wall components (483). The antiarthritic effect of CS was mediated by systemic and local effects on the immune system since depression of disease was associated with a significant decrease in serum antibody against type II collagen and a decrease in lymphocyte accumulation in arthritic joints (398).

Kaibara et al. (484) have also studied the importance of antigen-specific antibodies and complement in this model. They produced collagen arthritis by passive transfer of a serum concentrate from immunised donors to immunologically naive recipients as well as CS-treated, type II collagen-tolerant rats. In addition, significant enhancement and a longer duration of the passively

transferred arthritis were induced in the latter rats when the transfer concentrate was administered while CS was being given continuously. These results show that collagen arthritis can be induced by humoral immunity alone and further suggest that a cellular suppressor system sensitive to CS might participate in the regulation of arthritis once initiated (484). Kaibara and coworkers (482) had previously investigated the effects of CS treatment in three different regimens: i) only during the induction phase of immunity (prevention), ii) only during the immediate preclinical phase of collagen arthritis, and iii) on the established disease (therapy). The preventive treatment with CS (15 mg/kg/d orally for the first 14 days) suppressed the development of arthritis as well as antibody and DTH responses to type II collagen. In contrast, when CS administration was started only during the immediate preclinical phase (days 7 to 13) or after onset of disease symptoms (days 14 to 20), a significant enhancement of the disease was observed. This paradoxical enhancement was accompanied by an increased DTH skin reaction, while antibody responses were either suppressed or unaffected. These results appeared to be attributable, at least in part, to a suppressive effect of CS on a population of T_s cells, thus resulting in a T-cell-mediated helper effect. Borel et al. (108) have reported that CS (30 mg/kg/d orally on days 14 to 20) given therapeutically exerted only a weak to moderate inhibitory effect on the oedematous swelling of the paws. However, at the end of this therapeutic treatment, when the skeleton of the tarsi was evaluated by X-ray and compared with those of the solvent-treated controls, a clear prevention of cartilage destruction closely resembling the normal state was observed.

The effect of CS on collagen-induced arthritis was also studied in mice. Prophylactic CS treatment (100 mg/kg/d subcutaneously on days 0 to 13) suppressed development of arthritis and the immunological response to native type II collagen. Furthermore, treatment with CS (days 21 to 34), started on the same day as the booster injection with type II collagen (reinoculation is required to produce a high incidence of arthritis in mice), also resulted in inhibition of development of arthritis and immunity to collagen. However, therapeutic treatment (days 35 to 44) with CS did not affect the clinical course of the disease or the immune response to collagen (900). In an attempt to study the requirements of immunocompetent cells in maintaining and perpetuating the ongoing inflammation once the arthritic disease has initiated, it was shown that CS therapy (75 mg/kg/d intraperitoneally for 1 to 2 months starting 35 to 42 days after first immunising dose) produced a marked beneficial effect in mice with established collagen-induced arthritis (428). Despite controversial results, it seems that under certain conditions CS is able to affect positively collagen-induced arthritis in both rats and mice (108, 428).

Systemic administration of an aqueous suspension of cell wall fragments from group A streptococci and various other bacteria into susceptible rats induces an acute, self-limited polyarthritis, which is followed by the development of a chronic proliferative and erosive polyarthritis as well as the formation of hepatic granulomata. Since the role of T lymphocytes in this model is controversial, the effects of CS were studied. Continuous daily treatment with CS (25 mg/kg/d intramuscularly), begun either 24 hours before or 7 to 12 days after streptococcal cell wall injection and continued for 6 weeks, resulted in significant inhibition of the chronic proliferative, erosive synovitis (but not of the acute arthritis), and total inhibition of hepatic granuloma formation. When CS was stopped at 6 weeks, its effect continued for at least another 5 weeks, demonstrating long-term benefit without continuous administration of the drug. In contrast, CS given only during the acute phases of the experimental disease (days 1 to 12) did not inhibit the development of the chronic disease. These data provide evidence of an important role for the T_H cells in this model (1005). Euthymic LEW rats, when injected with streptococcal cell walls, exhibited rapid onset development of acute exudative arthritis coincident with enhanced synovial expression of Ia (class II) antigen. By 21 days after injection, the expression of Ia was markedly increased compared with basal conditions and paralleled the severity of the later developing proliferative and erosive disease. Immunodeficient athymic and CS-treated rats developed only the early phase arthritis, which was again paralleled by synovial Ia expression; however, chronic expression of high levels of Ia antigens was not observed. The results indicate that the rapid onset enhanced Ia expression is thymus independent, whereas the markedly enhanced chronic phase Ia expression is thymus dependent (988).

Concerning the therapeutic effects of CS in the treatment of rheumatoid arthritic patients, all studies so far performed indicate that this drug is effective (226, 1006, 1014, 599, 642). However, because of renal and other side effects, CS has to be used at low-dose and, to be more effective, in combination therapy, but without non-steroidal antiinflammatory drugs. General considerations and guidelines for establishing treatment protocols have been made by experienced investigators (642, 17, 289, 36; see also part IV.D.1).

b. EXPERIMENTAL NEUROLOGICAL AUTOIMMUNE MODELS. Active experimental allergic encephalomyelitis (EAE) can be induced in susceptible animals by a single injection of CNS tissue or myelin basic protein (MBP) emulsified in FCA, whereas passive EAE is achieved by cellular transfer of EAE-reactive cells. In the active EAE a monophasic acute paralytic disease appears in susceptible rat strains about 10 to 14 days post-sensitisation. They recover within 7 days, but in other species the attack is usually lethal. Depending on the sensitisation procedure and genetic background a chronic relapsing-

remitting form (CREAE) may develop (266). While acute EAE presents pathology similar to acute human inflammatory CNS (central nervous system) disease, the neuropathological changes and disease course observed in CREAE are more typical of multiple sclerosis. EAE seems primarily to be a T-cell-mediated disease in which immune reactivity to MBP is an important factor in the pathogenesis. Trafficking of MBP-sensitised T cells into the CNS from peripheral blood in animals affected with EAE has been demonstrated. EAE is the experimental autoimmune model on which most work with CS has been carried out.

Wistar and LEW rats sensitised with guinea pig spinal cord in FCA and treated with CS (15 mg/kg/d or higher, orally on days 0 to 20) did not develop EAE during the period of drug administration, whereas all placebo-treated controls showed some degree of paralysis, most of them with a high clinical and histologic grade of EAE (106, 717, 100, 92). The perivascular inflammatory changes were prevented at a dose of 20 mg/kg/d. If CS administration was delayed by 4, 6, or 8 days after sensitisation, development of EAE was still prevented, and in the therapeutic group, in which drug administration started at day 10, i.e., at the time of early signs of EAE, the duration and severity of the paralytic disease were greatly reduced with 50 mg/kg/d given orally. The extent of CNS changes was also attenuated. Thus, CS affected later stages of EAE and had a beneficial effect when used therapeutically (100, 108, 717, 92). When studying the modulatory effect of CS on mononuclear cell distribution during EAE, it was found that CS (25 mg/kg/d intraperitoneally) given at the time of induction of the disease (days 0 to 6) inhibited lymphocyte proliferation in vivo, lymphocyte trapping into the draining lymph nodes, and delayed the subsequent infiltration of cells into the CNS and other inflammatory sites. When given around the time of disease manifestation (days 7 to 13), CS also reduced the rate of cell accumulation into the CNS and foot, but was without effect in modifying lymphocyte trapping in the draining nodes, despite the fact that this was still an ongoing process (801).

In addition, another form of active EAE in the rat—hyperacute EAE induced by simultaneous injection of *B. pertussis* vaccine—was suppressed by CS (50 mg/kg/d orally for the first 12 days) when used preventively (579).

Essentially similar effects of CS were found in the acute model in guinea pigs (92). CS (35 mg/kg/d orally) administration commencing with antigen injection conferred almost a complete protection, especially in females. In addition, a significant therapeutic effect was shown when CS was started at the onset of disease; however, in this situation the improvement was more pronounced in males.

Acute lethal EAE also develops in rhesus monkeys within 20 to 50 days after intracutaneous injection of calf spinal cord emulsified in FCA. Administration of CS (50 mg/kg/q.o.d. intramuscularly from day 0 to 56) inhibited the development of EAE in four out of four animals. Upon cessation of treatment, two monkeys subsequently developed EAE which was immediately reversed after only four intramuscular injections of the same dose of CS. The follow-up of the history of these monkeys showed no relapse up to 200 days. Interestingly, a second antigenic challenge at this time resulted in hyperacute, lethal EAE 8 to 10 days later (98). Since all protected monkeys developed a genuine secondary reaction pattern upon antigenic rechallenge, this indicated that priming must have occurred in the presence of CS in all animals. Similarly, in another group of four monkeys, considerable improvement was seen in two animals on treatment with CS at the onset of neurological signs. The failure in response by the other two monkeys was attributed to the slow absorption of the drug when given by intramuscular injection (92). A very recent experiment has shown that intravenous infusion of CS (20 to 30 mg/kg/q.o.d.) had a marked therapeutic effect (J. F. Borel, Sandoz, Basel; unpublished data). The significant therapeutic benefit may result from preventing further recruitment of T cells which perpetuate the inflammatory process. Another series of ten rhesus monkeys was treated therapeutically during fifteen relapses with the CS derivative (Val^2) dihydro-CS (108). The main conclusions are that i) the time of onset and the course of EAE in the rhesus monkey remains unpredictable; ii) provided therapeutic treatment starts immediately after the onset of first signs with high doses, the progression of the disease can usually be halted and even very ill animals will be rescued; iii) when treatment is continued long enough, most animals will show temporary remittence; and iv) EAE is not self-limiting in the monkey. Despite successful remittence, relapses will occur sooner or later after discontinuation of the drug (108).

When therapeutic treatment with CS (50 mg/kg/d orally) was started in rats showing pronounced symptoms of EAE and the treatment period was shortened to only 5 days, eleven of twenty-one recovered animals relapsed, in contrast to one of nineteen treated with (Val^2)dihydro-CS (same dose) (152). We have often observed that when CS treatment was stopped, many rats would show transient and mild symptoms of EAE, and that the disease then did respond to further treatment with CS (92). The influence of treating rats preventively for shorter (0 to 16 days) or longer (0 to 43 days) periods with CS (50 mg/kg/q.o.d. orally) on the relapse rate was investigated during an observation period of 82 days. In the 16-day prophylactic treatment, the effect of CS was mainly to delay the onset of EAE by about 14 days, corresponding roughly to the period needed for the symptoms to occur in controls. This delayed appearance after discontinuation of short-term drug treatment may be caused by antigen persistence, because the rats were completely protected from the disease when the treat-

ment period was prolonged to 43 days. (Val²)dihydro-CS seemed to protect most animals equally well with either treatment schedule. Using the therapeutic treatment starting on day 9 until 24, both drugs suppressed the onset of EAE and, additionally, prevented severe relapses, although a few rats displayed weak, but transient symptoms such as flaccid tail (108).

The above results may also occur because a suppressor mechanism fails to develop after the onset of the disease, since CS prevents the initial step of antigen sensitisation in this model. Additional evidence for CS preventing the occurrence of a suppressor mechanism has been gained from a recently developed CREAE in the LEW rat (266). Disease developing after preventing treatment with either CS (25 mg/kg/d orally for 14 days) or (Val²)dihydro-CS (idem) was predominated by chronic and hyperacute attacks, in contrast to the relapsing course of controls. This pattern was also the result after CS was given therapeutically (for 15 days at the onset of first attack), whereas (Val²)dihydro-CS eliminated all further attacks in the majority of rats and only minimal transient disease occurred in the remainder. Both drugs were beneficial during administration in CREAE; however, in contrast to CS, (Val²)dihydro-CS possesses an important curative action when applied therapeutically (265). Further studies on the immune regulation in this CREAE model suggest the existence of an organ-dependent and time-dependent balance between effector and suppressor populations (168).

Suppressor cell regulation of EAE has long been suspected because LEW rats, which spontaneously recover from active disease, are resistant to reinduction of active EAE, even though effector T-cell lines can be rescued from these recovered rats. Recently T_s-cell lines were generated from the draining lymph nodes of rats immunised with MBP one month beforehand. To select for T_s cells rather than T_H cells, the lymph node cells were grown in vitro for 7 days in the presence of MBP and CS and these selected T_s cells, when admixed with MBP-specific T_H cells, were shown to prevent adoptive transfer of EAE. These suppressor T lymphocytes appear to be of the CD4 phenotype (249). These findings provide direct evidence that in vitro CS is not affecting the development of T_s cells in EAE. However, they are difficult to reconcile with some previous results and also with the next two following reports.

When used as the primary immunogen in guinea pigs, the tolerogenic MBP-derived peptide S42, which is highly cross-reactive with the encephalitogenic synthetic peptide S3, suppresses clinical disease induction upon later immunisation with MBP. When animals were first sensitised to S42 antigen and CS (17 mg/kg/d intraperitoneally) administered on days 0 to 4, only one of the four guinea pigs escaped protection after an MBP challenge 60 days later. If, however, the S42 injected animals received a single dose of CS on day 15, three out of four animals developed EAE after MBP challenge (292). These results suggest a blocking effect of CS on developing or developed disease-suppressing cells. This effect was in contrast to the reversibly affected DTH skin test to S42, because CS administered in a single dose on day 15 inhibited the T_{DTH} lymphocytes only temporarily (on day 16); the full DTH responsiveness was recovered a few days later (292). When a CREAE was induced in young strain 13 guinea pigs and CS (25 mg/kg/d orally) was administered from day −1 until days 12 to 39, the acute phase of EAE was totally suppressed in four out of ten and partially suppressed in six out of ten animals. In these animals surviving the treated induction of EAE, clinical disease appeared or increased in severity between 3 to 10 days after cessation of CS treatment (779). The suppressive effect was also accompanied by a lack of increase in blood-cerebrospinal fluid barrier permeability to proteins and a lower leukocyte count in the cerebrospinal fluid (779, 778). Polman et al. (762) have reported that prophylactic low-dose CS (2 to 4 mg/kg/q.o.d. subcutaneously for 22 days) treatment induced a relapsing form of EAE in all LEW rats examined. During the initial attack, paralysis was noted to be mainly flaccid, whereas during relapses a spastic paraplegia was more prominent. In contrast to the mild lesions observed in untreated EAE rats, the histological findings in the CS-treated animals with clinical relapses were impressive. An interesting explanation of these results is based on the failure of CS to pass the blood brain barrier (575, 835), thus giving immunoregulatory mechanisms within the CNS the opportunity to escape the drug's immunosuppressive effect (762).

A CREAE can be induced in 8-week-old SJL/J mice by injecting an encephalitogenic emulsion on days 0 and 7 and a third injection on day 70 which precipitates an attack with high mortality (62%) after 7 to 9 days. CS was given intraperitoneally at doses of 25, 100, and 250 mg/kg 1 or 3 times per week starting from day 40 post sensitisation and continuing over the next 17 days. In the CS-treated mice, there was a dose-dependent shortening of the length and severity of the attack elicited by challenge with the third injection as well as a significant reduction in mortality. In addition, a decrease of lymphocyte-derived chemotactic factor released from PHA-stimulated spleen cells of mice with CREAE and treated with CS was observed when compared with normal mice and mice with CREAE but treated with vehicle alone (839).

Since CS inhibits mitogen and antigen-induced proliferation, it was of interest to determine whether CS would have an effect on the in vitro conditioning step required for passive transfer of EAE with immune spleen or lymph node cells. The effects of CS on active and passive EAE are summarised in fig. 10. Spleen or popliteal lymph node cells from rats treated preventively with CS failed to transfer adoptive EAE, which indicates that such cells

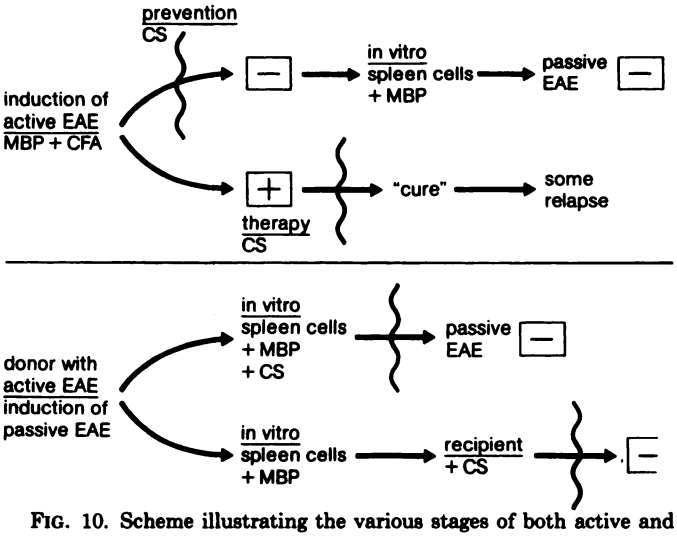

FIG. 10. Scheme illustrating the various stages of both active and passive (adoptive) experimental allergic encephalomyelitis (EAE) that are susceptible to inhibition by CS. MBP: myelin basic protein (Reproduced with permission from reference 108.).

do not become sensitised under this condition. When spleen or lymph node cells derived from actively sensitised rats were cocultured with con A or with the MBP antigen in the presence of CS (100 to 1000 ng/ml), they were suppressed and could not passively transfer EAE. When such cells were cultured with antigen, but without CS, they normally would induce adoptive EAE in a normal recipient. If, however, such recipients were treated with CS (50 mg/kg/d orally) from the day of cell transfer, they were protected and remained free of disease during the 15-day treatment period (806, 91, 420).

Sensitisation of most species of laboratory animals with peripheral nerve tissues produces an autoimmune demyelinating disease, experimental allergic neuritis (EAN). When administered prophylactically to guinea pigs and rats, CS (50 mg/kg/d orally for 28 days) totally suppressed the development of EAN during the period of its administration. When CS (same dose started on day 22 for 1 or 3 weeks) was given therapeutically after the onset of neurological signs, it effectively prevented further deterioration. This effect was more pronounced after treatment for 3 weeks than only 1 week. After discontinuation of CS treatment there was, with both regimens, a latent period before clinical symptoms of neuritis developed. This latent period was similar to that observed in the development of EAN in normal control animals and was probably due to the continued presence of antigen at the injection sites. After primary treatment of EAN with CS, animals that relapsed did not respond to further treatment with CS (514). Similar results were also reported by another group (687). Finally, the effects of CS on the clinical, electrophysiological and morphological expression of EAN were examined after adoptive transfer of the disease in LEW rats by injection of lymphocytes from a T-cell line reactive to the neuritogenic P2 protein. CS (1.5 mg/kg/d intraperiotoneally) injected from day 0 until sacrifice suppressed development of EAN (388).

In conclusion, there is a major difference between EAE and multiple sclerosis (MS) (141). In EAE the sensitised effector lymphocytes are derived from peripheral T cells which were activated by MBP or spinal cord antigens processed by peripheral APCs. The disease can be prevented or remittence induced by immunosuppressive drugs, including CS, which block the immune reactivity at the periphery. In contrast, the immune response in MS occurs within the CNS where sensitisation to MBP initiates a demyelinating process which is caused by endogenous activation of specific lymphocytes, their clonal expansion and further recruitment of T cells from the peripheral blood, and which escapes the control by endogenous suppressor cells. Consequently, the concentration of lymphocytes bearing activation markers is considerably greater in the spinal fluid than in the blood. Treatment with CS, which is not centrally active since it does not cross the blood-brain barrier in therapeutic amounts (835, 141), may be rather ineffective in suppressive relapses in CREAE and MS where memory T cells are presumably already present in the CNS (196). There has indeed been some debate whether CS should be used in MS (59, 799). The results from two clinical trials are not convincing for the benefit of CS (490, 504). A clinical study has confirmed the above considerations by demonstrating that peripheral blood lymphocytes from CS-treated MS patients had significantly lower proportions of IL-2R$^+$ cells compared with untreated patients. This inhibition, however, was not reflected in the cerebrospinal fluid lymphocyte populations from CS-treated patients and underlines the urgent need for an immunosuppressive drug which can enter the CNS in sufficient concentrations to inhibit local T-cell activation (140). Another study indicated that CS treatment of MS patients reduced the cytolytic function of CD8 T cells from baseline normal values, but did not alter the con A suppressor cell defect (48). The relevance of these findings for the course of MS is not known.

c. EXPERIMENTAL AUTOIMMUNE UVEITIS. The term uveitis denotes any type of intraocular inflammation without specifying the most affected structure in the eye nor the cause: Infections or autoimmune. The S-antigen, a glycoprotein, is found in the mammalian retina and in the pineal gland of most vertebrates as well as in many invertebrates. Immunisation with μg quantities of S-antigen mixed with FCA at a site far from the globe induces a severe bilateral uveitis. Patients with posterior uveitis have positive in vitro cell-mediated responses to this S-antigen, and the clinical picture seen in experimental animals resembles the disease seen in man. CS proved to be an ideal immune probe for testing the hypothesis of T-cell involvement in experimental autoimmune uveoretinitis (EAU). Since EAU appears to be a relevant model for human disease, the efficacy of

CS in this model bears importance for human ocular inflammatory disease (717).

It was the group of Nussenblatt and coworkers (719, 720, 159, 721, 658, 158, 716, 718) who first described and thoroughly investigated the immunosuppressive effects of CS in the EAU in the LEW rat. Using varying doses of subcutaneously injected CS, it was seen that 10 mg/kg/d from day 0 to 14 protected all rats from clinical signs of EAU (719). However, when therapy started one week after sensitisation (7 to 14 or 7 to 21 days), the dose of 40 mg/kg/d was effective in totally preventing the expression of EAU in all rats. The histologic examination of the eyes of animals treated with suboptimal doses (10 mg/kg/d) demonstrated a shift from an acute inflammatory episode to a more indolent granulomatous one (719, 718, 717). Marked changes occurred at the level of the draining lymph node. They were smaller than those from controls and both T and B areas appeared hypocellular. A statistically significant lower number of T-helper/inducer cells was found in the lymph nodes of S-antigen immunised and CS-treated rats (720). S-antigen reactive cells, which are found circulating in immunised rats by days 11 to 12, i.e., shortly before the development of the clinical symptoms, were not observed in the CS-treated group. In vitro proliferative responses of lymphocytes from draining lymph nodes of CS-treated rats had a peak on day 13 or 14 as compared with days 8 to 10 in untreated rats. Both lymphocytes from lymph node and peripheral blood from CS-treated animals exhibited a greatly diminished in vitro response to various mitogens (720). CS caused a profound reduction of T-cell infiltration to the target organ in treated rats. The marked reduction of CD8 cells in all stages of EAU may be a secondary result related to the initial inhibition of CD4 cells by CS which blocks the expansion of the inflammatory process. Therefore, the T_s cells would not be recruited to the eyes and play their down-regulating role and as a result, the high T_H:T_s ratio might continue throughout the course of EAU as observed (158). Furthermore, CS treated rats (15 mg/kg/d subcutaneously from day 0 to 21) produced anti-S-antigen antibodies in a similar titre as seen in controls, except with a marked delay in the peak titres (159), but higher doses of CS were inhibitory (497).

Comparative studies were carried out with several other immunosuppressive and antiinflammatory agents for testing their preventive and therapeutic effects on EAU (658, 154, 497). The data provide information concerning the unique effect of CS and underline its superiority over the other tested agents. Moreover, a 2% CS solution in oil applied topically 4 times a day for 14 days effectively prevented the expression of EAU, but also resulted in circulating CS levels in the therapeutic range; thus making it impossible to evaluate whether the effect of topical therapy was central or local. However, intraocular levels were extremely low and outside the accepted therapeutic range. Intravitreal CS therapy appeared to protect eyes from EAU without producing significant circulating CS levels. Therefore, a local CS preparation with enhanced penetration into the globe may be a practical approach to therapy in the future (716, 493, 494).

Expression of IL-2R and MHC class II antigens by the T cells, which constitute the major cellular component of the ocular infiltration during peak ocular inflammatory activity in EAU, was investigated in sensitised and CS-treated (10 mg/kg/d intramuscularly) rats. CS treatment resulted in a marked inhibition of IL-2R expression on proliferating T cells and a moderate inhibition of class II antigen expression by lymphoid and non-lymphoid cells in EAU (157). However, in other experiments using rats with endotoxin-induced uveitis, the expression of class II antigens on the epithelium of the ciliary body and iris was not significantly suppressed by CS (20 mg/kg/d intramuscularly on days −1 and 0) (509).

Similar to the experiments with EAE, the transfer of EAU can be successfully achieved by utilising either freshly explanted lymph node or spleen cells from S-antigen-immunised rats after in vitro priming with antigen or mitogen for 48 h. Treatment of the recipient rats with CS (10 mg/kg/d) effectively prevented the development of passive EAU (717). The efficacy of CS on suppressing in vitro activation of primed autoimmune rat T_H cells was assayed. These lymphocytes are a long-term cell line specific to the retinal S-antigen and can adoptively transfer EAU, after in vitro reactivation with antigen, to naive syngeneic hosts. Antigen-driven production of IL-2 and proliferation were inhibited in a concentration-dependent manner by CS (IC50 at 0.5 to 2 ng/ml). Similar concentrations also significantly suppressed the generation of high- and low-affinity IL-2R by T_H cells in response to antigen. In contrast, the in vitro activation of the uveitogenic potential of these S-antigen specific T_H cells, incubated with antigen in the presence of CS (10 ng/ml) and adoptively transferred into untreated recipients, was not affected by CS (154). It was conclude that triggering of the pathogenic potential of primed autoimmune T_H lymphocytes can take place in the presence of CS and in the absence of cellular proliferation. When released from CS influence, the suppressed cells can proliferate and passively induce EAU in the syngeneic hosts. These results are at variance with others obtained in the EAE model (91, 806) which may be explained by the fact that much higher CS concentrations (100 ng/ml to 1 μg/ml) were used during the in vitro reactivation procedure.

Finally, it was demonstrated that CS treatment initiated at the time of S-antigen sensitisation was capable of inducing antigen-specific suppressor cells (657, 296). The majority of LEW rats immunised with S-antigen and treated with CS (10 mg/kg/d intramuscularly) from day 0 to 14 failed to develop EAU when reimmunised

with S-antigen on day 30. In contrast, similarly treated rats were fully susceptible to induction of EAE when immunised with MBP, or even to EAU when immunised with another retinal antigen. Further in vitro and in vivo cell transfer experiments led to the conclusion that specific unresponsiveness plays a role in the suppression of EAU by CS and that the unresponsiveness is mediated in part by antigen-specific suppressor cells (657, 296).

The findings relative to the effect of CS in the EAU model in the rat were confirmed in the guinea pig. Guinea pigs sensitised to S-antigen were treated from day 0 with CS (25 mg/kg/d orally). CS profoundly reduced DTH skin reactions to S-antigen and PPD (purified protein derivative), and prevented vitreal inflammation at 17 days and retinal damage. Lymphocytes from the draining lymph nodes, but not spleens of immunised animals, exhibited a proliferative response to S-antigen which was suppressed by CS administration (592). Using this same model it was also demonstrated that preventive CS treatment prevented both intraocular inflammation and MHC class II expression on ocular tissue despite a choroidal infiltrate of predominantly $T_{c/s}$ cells. By using cultures of guinea pig and rat retinal pigment epithelium cells, it was shown that CS blocked the production by mononuclear cells of MHC class II-inducing lymphokine; CS itself having no effect on class II antigen expression induced on these cells by preformed lymphokine or rat rγ-IFN (593, 592). The therapeutic efficacy of CS was also examined in guinea pigs with already developed EAU. Treatment with CS (10 mg/kg/d intramuscularly for 14 days) began 16 days after S-antigen administration, i.e., after development of clinically evident uveitis. Clinically and histopathologically, animals treated with CS had a significant reduction of intraocular inflammation compared with controls. Corticosteroids alone had a less beneficial effect, and combined use of the two drugs at the single dose tested did not demonstrate synergism (893). Both preventive and curative treatment with CS (10 or 20 mg/kg/d subcutaneously) were confirmed as being effective in guinea pigs immunized with autologous retina (707a).

Kaswan et al. (493, 494) have thoroughly studied the effect of topically applied CS in the experimental model of immunogenic uveitis induced by intraocular injection of human serum albumin (HSA) in rabbits. The acute nonspecific inflammatory response that immediately follows intravitreous injection was not affected. However, the severe panuveitis that develops during the second week was markedly reduced. Topical CS treatment consisted of 10 μl of 2% CS in olive oil, applied to the dorsal limbus of both eyes every 6 hours and beginning 1 hour after HSA injection. The safety and efficacy of topical CS application in this model suggested that this procedure may be an effective route of therapy for certain immune-mediated forms of uveitis in man.

Uveitogenic antigens such as bovine opsin have been used to induce a retinopathy with less inflammatory response than that seen in S-antigen-induced EAU. Development of opsin-induced EAU in rats was inhibited by CS (3 and 18 mg/kg/d intramuscularly starting at day 0) as measured by lowered antibody response and elimination of measurable lymphocyte transformation in vitro (130). Furthermore, CS (10 mg/kg/d intraperitoneally on days 0 to 14) injected in rats reduced the clinical and pathological signs of experimental retinal vasculitis (882).

In summary, CS has proven to be effective both preventively and therapeutically in the treatment of experimentally induced ocular inflammatory diseases. This compound has also acted as an immune probe, furthering the understanding of the role of T cells in EAU, and by extension, in human ocular disease. The efficacy of CS on the inducer fraction of T cells with a resultant alteration in the kinetic response to the S-antigen seems well established. Its effect on the suppressor/cytotoxic fraction of T cells will be discussed later (see IV.D.5.). The straightforward effects of CS applied topically may yield promising clinical results (493). The drug has proved to be also effective in patients suffering from uveitis and Behçet's disease and has recently been registered in several countries for this indication (715).

d. EXPERIMENTAL IMMUNOLOGICAL NEPHRITIS MODELS. There are several ways for experimentally inducing immune-mediated nephritis mainly in mice, rats, and rabbits. Glomerular proliferation in acute serum sickness (ASS) nephritis in rabbits is predominantly the result of influx of monocytes into the glomerulus and is associated with proteinuria. Serum sickness was induced in rabbits by a single injection of bovine serum albumin with *E. coli* endotoxin. When CS (15 or 25 mg/kg/d) was injected intramuscularly, starting either 2 days before or at the time of induction of ASS, proteinuria was profoundly reduced and glomerular proliferation was inhibited, indicating that T cells may play a role in mediating the ASS nephritis. Even when rabbits were first treated with CS (25 mg/kg/d) 5 days after the induction of nephritis, proteinuria and glomerular proliferation were similarly inhibited (691). It was subsequently observed that these treatment regimens led in those animals to the development of glomerular capillary thrombosis and cortical infarction, lesions not seen in the unmodified ASS, but reminiscent to the pathological appearance found in the haemolyticuraemic-syndrome. Signs of this renal injury were haematuria, transient proteinuria, glycosuria, and oliguria which occurred during the rapid phase of antigen elimination when immune complexes were being formed (692). Further investigations showed that muscular arteries of the heart and splanchnic organs developed an arterial injury in which there was extensive fibrinoid necrosis of the vessel wall, but little or none of the mononuclear cell reaction that is normally associated with arteritis of ASS. A microvascular injury also oc-

curred which led to interstitial haemorrhage in the gastric mucosa and multifocal necrosis in heart and liver. These lesions were seen with equal frequency in all groups of rabbits with ASS who received CS, irrespective of whether they also received endotoxin (693).

A model of chronic serum sickness (CSS) can be established in rats by injecting them intraperitoneally with bovine serum albumin 5 days a week. Albuminuria increased gradually over the first 80 days and persisted thereafter. On day 146 rats were given CS (25 mg/kg/d intraperitoneally for 3 days) and albuminuria fell from 1.7 to 0.45 mg/ml. On day 150 the renal histology was examined and the glomeruli showed no significant abnormalities. A striking feature of this model was the abrupt onset of microscopic haematuria with CS treatment (694). The suppressive effect of CS (20 mg/kg/d intramuscularly) on the development of CSS nephritis in the rat was also demonstrated by another group. When CS was given in the preimmunisation stage, neither induction of glomerulonephritis nor specific antibody response was observed. In contrast, when CS was administered in the stage of continuous sensitisation (1 month later), mild CSS nephritis as well as specific antibody response developed (858).

Another type of experimental glomerulonephritis producing glomerular injury and proteinuria can be induced by injection of antiglomerular basement membrane antibody or immune complexes. Using a mouse model of passive antiglomerular basement membrane nephritis, it was shown that preventive CS therapy (75 mg/kg/d orally from days 1 to 4) had a profound antiproteinuric effect. It was suggested that this effect was not related to immunosuppression, but rather mediated by a reduction of the glomerular filtration rate and probably also by a change in glomerular permselectivity (836).

Development of autologous or active antiglomerular basement membrane glomerulonephritis requires two events: Production of an antigen-specific immune response and triggering of an inflammatory response within the glomeruli. The injury in the autologous rat model of antibody-induced glomerulonephritis has been shown to be mediated by macrophages. The T-cell infiltrate consisted mainly of T_H cells, was maximal 24 hours after induction of disease, and clearly preceeded the peak of influx of macrophages and glomerular damage. Suppression of T-cell function using CS (25 mg/kg/d subcutaneously beginning 1 day prior to immunisation) prevented T-cell accumulation and the subsequent macrophage induced injury (934). However, treatment started after the antibody response was established, failed to alter antibody production, its glomerular deposition or the outcome of the disease. CS treatment did not influence passive (heterologous) glomerulonephritis, occurring 24 hours after intravenous administration of sheep anti-rat glomerular basement membrane globulin to unimmunised rats. Thus, CS is able to block the disease by preventing an active antibody response, but has no effect when the antibody response is established or glomerular injury is passively induced (933). Another very similar study reported that CS given prior to or at the onset of immunological insult induced extensive glomerular infiltration with polymorphs and glomerular thrombosis, lesions not seen with unmodified nephritis or in rats receiving CS alone (994). The reasons for these contradictory results remain unclear.

The influence of CS on the development of renal injury in the in situ immune complex glomerulonephritis in rats was examined. CS (15 or 25 mg/kg/d orally in two divided daily doses) inhibited proteinuria without affecting immune complex deposition, glomerular cell accumulation, and proliferation. When CS administration was stopped, proteinuria appeared or increased, indicating that cellular and tissue damaging inflammatory processes were not irreversibly prevented, but only suppressed as long as CS was present (914).

Single footpad immunisation of rats with crude preparations of renal epithelium together with FCA leads to a membranous type of chronic glomerulonephritis known as Heymann's nephritis with lesions similar to those observed in human membranous glomerulonephritis. Treatment with CS (15 mg/kg/d intramuscularly from day 0 to 14) markedly inhibited the development of disease to a subsequent nephritogenic challenge. This tolerance, however, could not be transferred by lymphoid cells from CS-treated rats, but could be transferred by lymphocytes derived from the thymus or spleen of high antigen-dose tolerant rats, suggesting that CS prevented the occurrence of a suppressor cell (CD8) responsible for high-dose tolerance (207). The effects of CS treatment (8 mg/kg/d subcutaneously) on active Heymann's nephritis starting at different times after induction were studied. When given at the time of antigen injection, CS blocked both free antibody and circulating immune complex formation. Immunopathology and renal function tests remained normal despite a rise in both these parameters to control values after CS was discontinued. When given at a later stage of the disease process, there was no evidence of any pathological and functional disease amelioration, despite some suppression of both antibody and complex formation (155).

In a similarly induced tubulointerstitial nephritis rat model, CS (10 mg/kg/d orally), when given before or at the time of onset of disease, completely inhibited the expression of nephritis, in spite of autoantibodies against tubular basement membranes persisting in the circulation and in the kidney (916). These results were essentially corroborated by another group (313). These authors showed, in addition, that a delay in CS treatment (20 mg/kg/d subcutaneously) until day 10 after immunisation, i.e., when antibody response is established, still was able to drastically reduce the levels of serum antitubular basement membrane IgG and abrogated the interstitial

inflammatory cell response, in spite of persistent kidney-bound antibodies (313). This therapeutic effect of CS in interstitial nephritis in rats was also confirmed by others (859), because administration of CS (6 mg/kg/d subcutaneously) after the development of interstitial nephritis, i.e., from day 15 to 36 after immunisation, arrested the progression of the histological lesions.

The mercuric chloride-induced autoimmune glomerulonephritis in rats is biphasic and characterised by lymphoid hyperplasia and glomerulonephritis. All manifestations of mercuric chloride-induced disease were prevented in rats treated concurrently with CS at either 7 or 10 mg/kg/d orally. The initial phase of nephritis could be completely suppressed with a short, 15-day course of CS. The later phase of the disease could be tempered by CS therapy starting on day 10 after the first mercuric chloride injection and continuing for 60 days. CS appeared to interfere with the polyclonal activation of B cells observed in the mercuric chloride-induced autoimmune nephritis, accounting for its striking preventive and curative effect in this model (50). CS treatment (20 mg/kg/d orally at day 0 to 10) during mercuric chloride administration completely prevented the autoimmune phenomena of glomerulonephritis and, moreover, a prolonged unresponsiveness to mercuric chloride was induced lasting for at least another 5 weeks. This unresponsivness could not be adoptively transferred with peripheral lymphoid cells from CS-treated rats (in contrast to untreated donors in remission), but could be broken by reconstitution with naive lymphocytes. These results suggest that the tolerogenic effect of CS in mercuric chloride-induced autoimmunity is not mediated by active suppression; instead, the observed unresponsivness might be due to direct functional deletion of autoreactive T lymphocytes (29). Since a dissociation between the development of proteinuria and synthesis of antiglomerular basement membrane autoantibodies was demonstrated, it is suggested that a CS-sensitive cellular effector-mechanism might be involved in the induction of proteinuria in this model (29).

In conclusion, CS treatment was effective both in preventing development of nephritis in all models tested and in improving the symptoms of established nephritis in most models, the exceptions being Heymann's nephritis and the antibody-induced glomerulonephritis. It appears that the drug interfered mainly with cellular-effector mechanisms involved with proteinuria, since a rapid reversal of proteinuria could be obtained in several instances despite the persistence of autoantibodies and circulating immune complexes. However, preventive CS treatment was also effective in suppressing autoantibody formation. Interestingly, CS treatment induced a state of unresponsiveness in two conditions, Heymann's nephritis and mercuric chloride-induced glomerulonephritis. In both cases tolerance could not be transferred with lymphocytes derived from CS-treated animals although this was possible with cells from appropriate control donors (see also IV.D.5.). In several of these nephritis models CS was successfully used as a probe to elucidate a T-cell involvement at some stage of their development. These various experimental models are thought to bear some relevance with clinical nephrotic diseases. There are many reports of clinical trials demonstrating the efficacy of CS in these diseases (305, 601, 907, 395).

e. EXPERIMENTALLY INDUCED DIABETES. In some strains of mice and rats, multiple low-dose injections of the diabetogenic agent streptozotocin produce diabetes with concomitant inflammatory lesions of the pancreatic islets (insulitis). Immune reactions appear also to be involved in the induction of this experimental diabetes which shares several features with insulin-dependent diabetes type I in man (IDDM). Several studies have investigated the influence of CS on the course of multiple low-dose streptozotocin-induced diabetes in mice. Different protocols were used (day 0 = first injection of streptozotocin): 10 or 60 mg/kg/d CS subcutaneously on days 0 to 20 or 5 to 20 (530); 50 mg/kg/d CS orally on days −4 to 7 (846); twice 5 mg/kg/d CS intraperitoneally on days 7 to 60 or 7 to 60 (591); 20 mg/kg/d CS intraperitoneally on days 0 to 10 (462); 10 or 50 mg/kg/d CS intraperitoneally on days 0 to 5 (465). All these studies concur that CS treatment resulted in a dose-dependent enhanced hyperglycemia, hypoinsulinemia and β-cell destruction following low-dose streptozotocin injections. The mechanism by which CS aggravates this type of diabetes is unclear. In the above studies, in which control mice received CS alone, a mild to moderate direct toxicity of CS on the pancreatic islet cells was reported. Sestier et al. (846) observed a decreased glucose tolerance and Iwakiri et al. (462) observed a deterioration of β-cell function but without morphological changes or development of hyperglycemia. Jansson and Sandler (465) observed an increased vascular permeability in the islets which was reversible after discontinuation of CS. They did not exclude that this increased capillary leakage induced by CS may contribute to the hyperglycemia caused by steptozotocin treatment, because this would allow an increased permeation of inflammatory cells into the islets and a subsequent release of inflammatory mediators such as IL-1. The controversial issue of a direct cytoxic effect of CS on β-islet cells has been discussed in part IV.C.1.b.

Low-dose streptozotocin-induced diabetes in rats treated with CS (10 mg/kg/d intraperitoneally) was not aggravated, but since CS was used in saline the drug was not dissolved and therefore not effective (508). Another study in the rat demonstrated the influence of streptozotocin-induced diabetes on the pharmacokinetics of CS. The diabetic condition clearly altered CS kinetics as reflected by the increase in half-life and reduction in clearance. This abnormality could be corrected by insulin treatment (234). However, the experiment was not con-

ceived for showing the influence of CS on multiple low-dose streptozotocin-induced diabetes in the rat.

In the encephalomyocarditis virus-induced diabetes in mice, involvement of genetic factors has been demonstrated, but the relative roles of infection and autoimmunity in the induction of insulitis are less well established. CS was administered intramuscularly at a dose of 4 times 200 mg/kg/week on days 0 to 14 or 14 to 28 or 21 to 35. Both early or late CS therapy increased mortality and frequency of diabetes in female mice and did not influence these parameters in males despite a reduction of pancreatic inflammatory lesions. The results argue against an autoimmune pathogenesis for diabetes in this model and suggest that the virus plays the major role in β-cell damage (964). Moreover, the potential involvement of the immune system in the pathogenesis of encephalomyocarditis virus-induced diabetes in a susceptible and a relatively resistant mouse strain using CS was investigated. Treatment with CS (10 mg/kg/d intraperitoneally) from 3 days before virus inoculation until 5 days afterwards resulted in increased severity and incidence of diabetes in these mouse strains (324).

f. OTHER INDUCED, MAINLY T CELL-MEDIATED MODELS OF AUTOIMMUNITY. Induction of experimental autoimmune thyroiditis (EAT) by early thymectomy and subsequent sublethal TBI results in a reliably reproducible model of EAT with progressive thyroid gland lymphocytic infiltration leading to follicular obliteration and associated circulating antithyroglobulin antibodies. Eight weeks after their last dose of irradiation, female PVG/c rats commenced CS therapy (10 mg/kg/d orally) and were treated for varying time intervals thereafter. This therapeutic treatment reversed the T_H:T_s ratio and was associated with a significant improvement in the disease process. These drug-induced alterations lasted only as long as CS was administered and thereafter reverted towards the severity of disease seen in untreated control animals (629). CS (same dose) given preventively, i.e., beginning immediately after thymectomy (at 3 weeks) and continued for 28 days, significantly lowered the levels of serum thyrotropin and autoantibody titres at 11 and 15 weeks, and also markedly reduced the histological grade of thyroiditis. Therefore, CS given for a sufficiently long period of time (>2 weeks) decreased the severity of EAT, and when administered for the whole induction period (through thymectomy and irradiation), though not preventing the development of EAT, not only decreased its severity but also delayed its onset. Withdrawal of CS caused a progressive development of EAT (390).

EAT can also be induced in many strains of mice by immunising them with thyroglobulin emulsified in FCA. The disease is characterised by circulating thyroglobulin antibodies and by infiltration of the thyroid, chiefly with mononuclear cells, similar as seen in Hashimoto's thyroiditis in man. Mice were injected intraperitoneally with 50 mg/kg/q.o.d. CS starting at day −2 (before first antigenic challenge), or day 0, or day 8, and treatment lasted until day 16. The animals were sacrificed on day 21. The results showed that regardless of the time CS administration was started, the titre of thyroglobulin antibodies was not influenced by CS (this dosage is too low to affect antibody production; see IV.A.1,2, and 5.). However, preventive CS treatment decreased the degree of thyroid damage and the magnitude of the thyroid infiltration when administration was started before or on the same day as the antigenic challenge (967).

Experimental allergic orchitis (EAO) is a suitable model for analysing the immune pathogenesis of organ-specific ADs. T lymphocytes are required for the induction of EAO, although both cellular and humoral immunity are involved in producing the complete picture of the disease. CS (10 mg/kg/d intraperitoneally, intramuscularly, and subcutaneously, daily changing the route in turn) administration for 14 days, starting on the day of immunisation with testicular antigen in FCA, almost completely suppressed the induction of EAO, delayed DTH skin reactions to both testicular antigen and PPD, and antisperm antibody response in strain 13 guinea pigs when examined 2 weeks after immunisation (see IV.A.5.). The unresponsive state induced by CS was of a transitory nature, since after cessation of CS treatment, EAO-eliciting capability and cellular immune responsiveness recovered in a relatively short time, whereas restoration of antibody responsiveness was delayed. Transfer of lymph node cells taken from CS-treated, EAO-suppressed animals at two weeks postimmunisation into normal syngeneic recipients inhibited induction of EAO and delayed skin DTH response. The suppressor cell activity appeared partly antigen-specific (427).

To postulate that immunopathologic mechanisms that culminate in a chronic dilated cardiomyopathy are set in motion during acute viral myocarditis serves as an attractive basis for unraveling the pathogenesis of a similar biphasic chain of events in the murine model (792). The acute or initial phase is virally mediated and characterised by minimal necrosis and inflammation. The chronic or late phase develops after viral clearance has occurred, resulting in a smoldering, inflammatory reaction, incremental myocyte necrosis, and ultimately, clinical emergence of congestive heart failure several months later (for review see 578a). To test whether CS immunosuppressive therapy might ameliorate this process CS (25 mg/kg/d subcutaneously) was administered for 3 weeks, starting at 1 week after infection (with encephalomyocarditis virus) during viral replication, or at 3 weeks after infection, i.e., after the period of viral replication. CS treatment led in both instances to no benefit with regard to survival or pathologic indices of myocardial injury and was associated with impaired myocardial function and heart failure (664; see also IV.E.2.f.). The effects of CS (15 mg/kg/d intraperitoneally beginning on

day of infection) were also studied in the murine model of coxsackievirus B3-induced myocarditis. The mice concurrently infected and given CS had a high mortality rate (75% versus none) and a significantly attenuated mononuclear infiltrate in the presence of enhanced necrosis when compared with control infected mice. CS administration starting 1 week after infection caused a lower mortality rate (55%), but very similar histologic abnormalities. In contrast to negligible or no virus in the hearts of infected, but untreated mice, the CS-treated groups had easily detectable virus in their hearts 14 days after infection (724). Investigations performed by Estrin et al. (257) confirmed that severe myocarditis persisted in CS-treated BALB/c mice infected with coxsackievirus B3. In this mouse strain myocardial injury is mediated by CD8 T lymphocytes recognising normal myocyte antigens, making this an autoimmune disease. The autoimmune response cannot be inhibited by CS treatment (120 mg/kg/d subcutaneously started between days −2 to 4 and continued until day 8 to 10) of the infected animals, since mortality in treated mice was increased 2 to 4 times. Neither virus-specific antibody nor T_c lymphocyte response was affected, and maximal virus concentrations in the hearts of CS-treated and control animals were similar. However, allograft immunity in the same CS-treated mice was fully suppressed. Cardiac damage remains T-cell-mediated, because mice given both CS and rabbit-ATS failed to develop significant myocardial inflammation (257). These results suggest that CD8 T cells in BALB/c mice are completely CS resistant.

Further work by this group (256) demonstrated that two cytolytic T-lymphocyte populations arise in coxsackievirus-infected mice. One population belongs to the CD8 T lymphocyte subset (as found in BALB/c mice) and reacts specifically with uninfected heart cells (autoreactive T_c cells), whereas the other belongs to the CD4 T cell subset (as occurring in DBA/2 mice) and reacts with infected targets (virus-specific T_c cells). Although both immune T lymphocyte populations can induce cardiac inflammation in vivo, autoreactive T_c cells predominantly cause tissue injury. The virus-specific T_c cells could not be generated in cultures containing CS, but autoreactive T_c cells could. It was concluded that in this BALB/c model autoimmunity (mediated by autoreactive CD8 T_c cells) is CD4 T_H cell- and IL-2-independent as well as CS-resistant. In contrast, the IL-2-dependent virus-specific CD4 T_c cells in the coxsackievirus-infected DBA/2 mice, which presumably develop exclusively antibody-mediated myocarditis, are CS sensitive and the drug completely protected these mice from cardiac inflammation (255). Interestingly, the A/J strain develops myocarditis mediated by both CD8 and CD4 T cells, i.e., either subset being capable of independently inducing injury. Since CS inhibits one T subset only, it does not protect A/J mice from autoimmune myocarditis.

g. OTHER INDUCED, MAINLY ANTIBODY-MEDIATED MODELS OF AUTOIMMUNITY. The basic abnormality in myasthenia gravis is a reduction of acetylcholine receptors (AChR) at neuromuscular junctions, brought about by an antibody-mediated immune response. The preventive and the therapeutic effects of CS treatment on the autoantibody response in the experimental autoimmune myasthenia gravis (EAMG) model in the rat have been reviewed under IV.A.5. CS was capable of suppressing the antibody responses both to the hetero- (torpedo) and auto-antigens (rat AChR) during primary, ongoing, and secondary immune responses (229, 365). Prevention of EAMG was also achieved in the rabbit model when CS (15 to 30 mg/kg/d orally) was given concomitantly with the immunising dose for 1 to 3 weeks. Whereas all control animals died of EAMG within 45 days, eleven out of twelve rabbits treated with CS survived for over 3 months in spite of repeated booster injections of AChR. Anti-AChR antibody titres rose slowly in preventively CS-treated animals, but achieved the same levels of controls after 3 weeks of immunisation. Interestingly, the antibody titre did not correlate with the severity of the disease in CS-treated or in control animals (951). CS has also been reported to improve the clinical condition of patients suffering from myasthenia gravis (722, 932).

McIntosh and Drachman (630, 631) have recently succeeded in inducing suppressor cells specific for AChR. Draining lymph nodes were removed from 3 to 6 weeks-previously sensitised rats expressing EAMG. They were dissociated into single-cell suspensions and cultured in the presence of AChR and CS (200 ng/ml) for 7 days. These cells, when mixed with lymphocytes from rats with EAMG in vitro, strongly suppressed the antibody response to AChR and were antigen-specific, since they did not inhibit antibody responses to an unrelated antigen. Cells induced by this method exhibited cell surface markers (CD4 and CD8) characteristic of rat T_s cells, and they were adherent to nylon wool and plastic dishes. These cells released a suppressive factor into the culture medium.

Mice injected with rat erythrocytes produce erythrocyte autoantibodies, resulting in an autoimmune haemolytic anaemia model. Such mice also produce suppressor cells that inhibit autoantibody formation without inhibiting the net production of antibodies against rat erythrocytes. Cox et al. (185) showed that CS (50 to 100 mg/kg/d intraperitoneally starting at day 7 or 14) treatment during the induction phase of the response significantly inhibited autoantibody production. The CS-treated mice that failed to synthesise autoantibodies, though, produced suppressor cells that specifically inhibited autoantibodies. However, these suppressor cells, which were of Thy 1.2 phenotype, did not suppress antirat erythrocyte antibodies in adoptive transfer experiments.

3. Results of CS in spontaneous genetic models.

a. INSULIN-DEPENDENT DIABETES MELLITUS (IDDM) IN

THE BB RAT. An acute diabetic syndrome occurs spontaneously in a partially inbred colony of BioBreeding/Worcester (BB/W) rats. Salient features of the syndrome include genetic predisposition, abrupt onset of insulin-dependent, ketosis-prone diabetes between 60 to 120 days of age, associated with a lymphocytic insulitis with virtually complete destruction of the pancreatic β-islet cells. Cell-mediated immunity has been implicated in the pathogenesis of spontaneously diabetic BB rats which are considered an excellent animal model of type I IDDM. Diabetes-prone BB rats have several striking abnormalities in their T lymphocyte immune responses such as poor T-cell proliferation in response to alloantigen in vitro and failure to generate T_c cell responses. Recent work provides evidence that the defective T-cell responses are due to bone marrow-derived cells (APCs) that are not T-cell precursors and which influence the maturation of normal thymocyte precursors by residing in the thymus during T-cell maturation (310).

Laupacis et al. (569) first demonstrated the efficacy of CS in preventing the development of diabetes in the BB rat. CS started orally at a dose of 10 mg/kg/d on day 34 was later reduced to 8 mg/kg/d with continuous CS serum level measurements (>100 ng/ml). No CS-treated rats developed diabetes until day 121, but diabetes occurred in some animals (mostly females) after the drug was withdrawn (569, 888). Pretreatment of susceptible BB rats for 10-day intervals prior to 80 days of age with CS (20 mg/kg/d intraperitoneally) significantly reduced the frequency and delayed the onset of diabetes. The relatively narrow time frame of successful treatment (from day 60 to 70) suggested that effector cells responsible for β cell destruction may be activated during this period of time prior to the onset of overt hyperglycemia. This short-term therapy did not protect against the occurrence of lymphocytic thyroiditis or autoantibody production, suggesting that these BB immmunologic phenomena may be controlled by a distinct series of immunologic events (584). In another study, intermittent administration of CS was determined to be a biologically effective regimen in the prevention of spontaneous diabetes in the BB rat. Beginning at 30 to 49 or 50 to 55 days of age, treated animals received CS (15 mg/kg/d subcutaneously) for 2 weeks (induction phase) and then twice weekly (maintenance phase) until 160 days of age and all animals were followed to 275 days of age. Blood levels of CS and major metabolites were undetectable intermittently during the course of therapy. Major complications (nephrotoxicity, malignancy, infection) were not associated with this intermittent CS protocol which delayed and often permanently prevented a spontaneous onset of diabetes (126). Further investigations disclosed that prophylactic CS (10 mg/kg/d orally), started at 6 weeks of age and terminated at 21 weeks, completely prevented diabetes in the disease-prone BB rat. Protection against diabetes was lifelong (follow-up to 106 weeks of age), provided CS prophylaxis was initiated when insulitis was minimal or absent, and pancreatic insulin content was normal. This treatment protocol also inhibited lymphocyte infiltration in several organs against which there is autoimmunity in the BB rat (466). CS therapy initiated later, but still before the onset of symptoms (8 to 9 weeks), and terminated at 22 weeks, or CS prophylaxis started at the appropriate time (6 weeks) but terminated prematurely (17 to 19 weeks of age), were only partially or not effective, respectively. In the latter regimen diabetes developed after cessation of therapy (466, 467).

However, a similar study reproducing this protocol of administration (10 mg/kg/d orally from age 30 to 150 days) demonstrated that five out of twenty-four CS-treated rats became glycosuric during treatment, but none demonstrated weight loss, all required insulin only intermittently after onset, and all showed persistence of β cells (999). The incidence of hyperglycemia and glycosuria was unaltered by CS, although the diabetic syndrome was milder. CS induced hypoinsulinemic glucose intolerance in non-diabetes-prone BB rats. In contrast to the former studies in which the end point was the presence or absence of the classic syndrome of the diabetic BB rat, the present experiment has carefully examined the metabolic and cellular immunological concomitants in diabetes-prone BB rats; non-diabetic-prone BB rats were treated as controls. Thus, CS inhibited the normal rise with age of peripheral blood lymphocyte cell numbers identified with MAbs. CD5 (pan-T) and CD4 T_H cells were affected, and there was an increase in the large $W3/13^+$, $CD5^-$ population characteristic of diabetes-prone BB rats. CS also caused the appearance in both types of BB rats of CD4, $CD5^-$ and CD8, $CD5^-$ subsets; CD8 $T_{c/s}$ lymphocytes and Ia^+ cells were less affected (999).

In contradistinction to its prophylactic effect, CS (10 mg/kg/d orally) therapy started only when appearance of hyperglycemia was monitored in diabetes-prone BB rats, failed to induce remission in any of these rats, and did not affect their daily insulin requirements. After 9 weeks of CS treatment, there was essentially total β cell loss and the pancreatic insulin cell contents were less than 1% of normal levels. During the 9 weeks of CS treatment, there was a decrease in numbers of peripheral blood Ia^+ lymphocytes, an increase in CD8 $T_{c/s}$ and NK cells, but no change in the other subsets (998). Diametrically opposed results were obtained in newly diagnosed diabetic BB rats with short-term treatment for 10 days with a subtherapeutic dose of CS (1.5 mg/kg/d intramuscularly) in combination with the anti-IL-2R MAb ART-18, a treatment shown previously to eliminate specifically antigen-activated $IL\text{-}2R^+$ T lymphocytes while sparing suppressor cells. This combination therapy resulted in eight of eleven BB rats with mild hyperglycemia normalising their plasma glucose levels and seven out of

eight animals maintaining normoglycemia during the observation period of 120 days after beginning of treatment. The β islet cell volume density was maintained and there was an increase in pancreatic insulin content. Moreover, the glucose tolerance of successfully treated animals was not significantly different from that of normoglycemic BB rats during the whole observation period (373).

For the sake of completeness, we have to mention that CS treatment has been reported to induce dysmorphic changes in the BB/W rat as well as relative infertility (673). These serious findings were not observed by others in either BB or normal rats (see for instance reference 23 in 999; 807 and several references therein; S. Brüggemann, I. Kubli, B. E. Matter, B. Ryffel, H. Schön, and K. E. Suter, Sandoz, Basel; unpublished data).

b. INSULIN-DEPENDENT DIABETES MELLITUS IN THE NOD MOUSE. The non-obese diabetic (NOD) mouse is a strain in which insulin-dependent, non-obese, ketotic diabetes mellitus develops spontaneously in about 80% of female mice between 12 and 26 weeks of age. The disease process in this model is thought to be of autoimmune etiology and CD4 T-cell-dependent (971); however, it does not seem to be associated with insulin-specific, autoreactive T cells (452). A prophylactic effect of CS therapy on the development of diabetes in the NOD mouse was demonstrated by several groups (505, 286, 665). CS (20 or 40 mg/kg/d orally) was started at 4 weeks of age and continued for 5 weeks. The incidence of insulitis was significantly decreased, particularly in male NOD mice, whereas the incidence of anti-islet cell surface antibody was not altered. However, the incidence of insulitis and anti-islet antibody did not correlate (505). Subcutaneously injected CS (10 mg/kg) every 4th day from 8 to 26 weeks of age, followed by an observation period up to 5 months beyond therapy, resulted in a complete suppression of diabetes development in the female NOD mouse and abolished lymphocytic infiltration of the β islets against which there is autoimmunity. The effect of this remarkably low-dose CS treatment persisted week past the duration of therapy (287). Similar results were obtained in NOD mice aged 30 to 60 days and treated with oral doses of 25, 15, and 2.5 mg/kg/q.o.d. until 160 days of age. These mice showed neither a significant increase of plasma glucose levels nor the development of insulitis (665).

Therapeutic treatment with CS (25 mg/kg/q.o.d. orally) for 35 days, starting after development of glucose intolerance, did little to influence the course of the disease and no remissions were recorded (665). A pancreatic allograft into the NOD mouse represents a presumed first-set allogeneic response, as well as a possible second-set immune response to islets. To assess the effect of donor H-2 antigens and the influence of autoimmune disease on pancreatic graft survival, newborn pancreata from various strains of mice were transplanted into diabetic NOD mice treated with a CS (40 mg/kg/d orally on day 0 to 9) protocol that prevented skin allograft rejection. The grafts were then harvested at day 10 to histologically assess the graft viability. The results indicate that in diabetic NOD mice the CS dose controlling allograft rejection is incapable of controlling the recurrence of the antiislet autoimmune process. This autoimmune destruction of β cells in the NOD mouse is also restricted to H-2 haplotypes shared between the donor and the NOD mouse. Accordingly, CBA pancreatic grafts, incompatible at all MHC loci, showed the least lymphocytic infiltration, and good donor β-cell survival; while in the BALB/c allografts sharing class I MHC antigens and in NOD isografts, graft destruction was strongest (909). When NOD mice are grafted with cultured BALB/c islet tissue, the islet graft is destroyed by disease recurrence in the graft which is a CD4 T-cell-dependent process. CS (60 or 20 mg/kg/q.o.d. subcutaneously) alone was ineffective in controlling disease recurrence in the islet graft transplanted to actively diabetic NOD mice. Even when the CD4 T cells were eliminated from the diseased animals prior to islet tissue grafting and CS administration, it was not possible to maintain the graft viability with low-dose CS therapy (20 mg/kg/q.o.d.). However, contrasting results have been obtained (K. J. Lafferty, Denver; unpublished data) indicating that this is a controversial area at present. It may be concluded from these experiments that, although anti-CD4 treatment controlled the expression of the disease process and allowed survival and function of the islet graft, this treatment did not return diseased animals to the prediabetic condition in which the development of diabetes can be controlled by low-dose CS therapy (972).

A novel strategy to induce specific immunosuppression was investigated which was based on the hypothesis that specific suppressor cell activity could be increased by IL-2 if cytolytic/helper activities were simultaneously blocked by CS. Because a characteristic of NOD mice is an unusually high percentage of splenic T lymphocytes, splenic lymphoid cells from young not-yet-diabetic female NOD mice were exposed ex vivo to IL-2 plus CS (1 μg/ml) for 72 hours before their reinfusion into the same animal from which they were isolated. After this treatment only two of eleven mice became overtly diabetic during an observation period of 19 weeks (i.e., at 31 weeks of age), while eighteen of twenty-one age-matched control mice developed diabetes during the same period. These data suggest an ex vivo preferential IL-2 activation of specific suppressor cells for the autoimmune process with CS blockade of cytolytic/helper activities. Because the in vivo concentrations of CS with this procedure would be negligible, these findings may have implications for the potential nontoxic use of CS in human protocols as well (287).

Meanwhile, it has been documented in clinical trials that CS induces remission of IDDM after early interven-

tion. The positive effect is observed on residual insulin secretion leading to low- or no-need of exogenous insulin in a number of newly diagnosed younger patients. However, it has also been shown that this effect tends to decrease with time or continued treatment and disappears totally after discontinuation of the drug. This positive effect is usually seen with high blood concentrations of CS which in turn are related to an unacceptable risk of renal damage and other side effects, some of which may be irreversible (913, 580, 89, 197, 267, 518, 644). The major problems of Sandimmune therapy in type I IDDM are i) the maintenance of remission, ii) the avoidance of long-term side effects, and iii) by-passing the resistance to therapy. The risk to benefit ratio of CS in this indication is being further investigated (38).

c. SPONTANEOUS AUTOIMMUNE THYROIDITIS IN OS CHICKENS. The obese strain (OS) chicken is an experimental model with Hashimoto-like spontaneous autoimmune thyroiditis. OS chickens suffer from two defects in the glucocorticoid-mediated control of immune function (538). First the concentration of free, hormonally active corticosterone is diminished due to a marked increase in corticosteroid-binding globulin. Second, OS chickens lack the increase in plasma corticosterone that normally follows administration of antigen, suggesting that the immunoendocrine dialogue has been disturbed. The significance of corticosterone levels in this autoimmune model can be deduced from the finding that neonatal hydrocortisone treatment of OS chicks prevents the development of thyroiditis (260). This may indicate that T cells are not primarily involved in the induction of the disease (986). It may also explain why CS, administered in a dose effectively prolonging skin allografts in the OS chickens, consistently failed to prevent the development of thyroiditis or to alter the frequency and severity of this condition. CS was administered orally, starting post-hatching and continued until 3 weeks of age when the birds were sacrificed (987). Moreover, when given to OS embryos on days 15, 17, and 19 of incubation, CS entailed the development of significantly more severe disease and higher titres of autoantibody to thyroglobulin as compared with untreated controls (987). With the present knowledge on the etiology of this disease, which is obviously very different from the induced autoimmune thyroiditis in mice and rats (see IV.D.2.f.), there is no reason anymore to postulate an adverse effect of CS on precursors of T_s cells.

d. SPONTANEOUS POSTERIOR UVEITIS IN SDA CHICKENS. The Smyth delayed amelanotic (SDS) line of chickens displays symptoms commonly associated with human vitiligo. The SDA chicken is characterised by a spontaneous and progressive loss of melanin pigmentation in both cutaneous and ocular tissues in young animals. Ocular amelanosis is accompanied by an intense choroidal inflammation that appears to have an autoimmune component. Secondary involvement of the retinal pigment epithelium and photoreceptors leads to progressive retinal degeneration and blindness in most adult animals. CS therapy from day of hatch altered and/or arrested choroidal inflammation and amelanosis in a dose-dependent fashion, further strengthening the association between the SDA chicken syndrome and autoimmune activation (277).

More experiments were undertaken to investigate the role of CS in this animal model of spontaneous posterior uveitis. CS was injected intramuscularly at 40 mg/kg, 3 times per week beginning on day of hatch for either 4, 8, or 12 consecutive weeks. Integumental amelanosis was monitored weekly to determine onset and frequency of pigment loss. Chorioretinal amelanosis and inflammation were determined at 8 and 12 weeks of age. CS treatment significantly delayed the mean age of onset and incidence of integumental pigment losses; and the associated ocular pathology was also less severe in the treated chicks. Termination of CS administration resulted, however, in enhanced integumental and choroidal amelanosis, choroidal inflammation, and chorioretinal damage beyond that observed in nontreated controls. This rebound enhancement of symptoms occurred 4 to 8 weeks after drug withdrawal. These results suggest that discontinuation of CS therapy of this spontaneous AD may exacerbate associated symptoms (277, 748). Additional research will be required to determine which types of cellular components are affected by CS.

e. SPONTANEOUS AUTOIMMUNE LUPUS MODELS IN MICE. The New Zealand black/white (NZB/W) hybrid mouse spontaneously develops an autoimmune disease with characteristics that resemble systemic lupus erythematosus (SLE) in humans. By 2 to 3 months of age, a systemic (organ-nonspecific) immune complex disease develops with nephritis and proteinuria. This leads to a predictable course ending in chronic renal failure with a 50% mortality rate by 8 to 9 months of age. Chused et al. (171) have analysed the multigenic basis of AD in New Zealand mice (see also 915).

In a first experiment, we observed that CS (60 mg/kg/d in the feed) starting at 24 weeks and continued up to 75 weeks of age failed to prolong survival of NZB/W hybrid mice. However, this dose of CP markedly diminished proteinuria, to the same extent as CP (50 mg/kg/10 d intraperitoneally), during the entire observation period of 75 weeks (99). Another early study reported a dramatic diminution of anti-DNA antibody level in old (56 weeks of age) NZB/W hybrids, which remained low throughout CS therapy (about 120 mg/kg/d orally, 5 times/week) and persisted for 7 weeks after drug withdrawal. Glomerular deposits were decreased compared with control mice (460). The last early study showed that CS (100 mg/kg/d orally) significantly prolonged the life span of female NZB/W hybrid mice and reduced the levels of anti-dsDNA autoantibody. It also prevented glomerular damage and renal failure, despite the same

degree of cellular infiltration and Ig and complement deposition in the glomeruli as in control mice (473). These latter investigations were pursued and extended, but essentially confirmed the earlier findings (472). Surprisingly, the levels of rheumatoid factors were found to be increased in CS-treated mice.

More in-depth studies were performed in our laboratory by Gunn (362) and Gunn and Ryffel (367, 366, 100). Autoimmune NZB/W hybrid mice were treated with CS (100 mg/kg/d orally 5 times/week) for 12 weeks, beginning in young mice at 12 weeks of age and in old mice at 36 weeks of age, to assess the prophylactic and therapeutic effect of the drug, respectively. CS was effective in preventing autoantibody production (anti-DNA and anti-SE antibodies) in young mice and markedly reduced the autoantibody titres in old mice. These findings clearly established that autoantibodies, at least in this murine lupus model, can be controlled pharmacologically (362). Moreover, CS prevented the deposition of immune complexes in the kidneys and the subsequent development of glomerulonephritis and proteinuria in young mice. It also exerted a therapeutic effect by reducing proteinuria in old mice, even in those exhibiting an advanced stage of glomerular damage (367, 366). Renal histology revealed almost normal appearance of glomeruli with only minimal residual lesions after 12 weeks of treatment, i.e., at 48 weeks of age (100, 366). Further experiments dislosed that another CS congener, (Nva^2)-CS which is thought to possess reduced nephrotoxicity in rats, was also very successful in suppressing a strong ongoing immune response in the NZB/W hybrid mouse (368). Okudaira et al. (729) obtained similar results when treating 8 month-old NZB/W hybrid mice with CS (50 mg/kg/d orally 6 times/week). CS significantly depressed blood urea nitrogen levels and clearly prolonged the life span. Deposition of IgG and C3 was remarkably decreased, but there were concentrations of anti-DNA antibodies, and circulating immune complexes in the serum were not affected. Histological examination indicated a much milder glomerulonephritis in the CS-treated mice than in untreated controls (729).

The MRL/lpr mouse strain spontaneously develops glomerulonephritis, marked lymphoid hyperplasia, arteritis and chronic polyarthritis; there is a 50% mortality around 5 months of age (552, 915). A first report mentioned that short-term in vivo treatment of 3 month-old SLE lpr/lpr mice with CS (200 mg/kg/d intraperitoneally for only 10 days) significantly reduced serum levels of anti-DNA antibodies but had no effect on this strain's spontaneous massive T-cell proliferation (326). A similar CS treatment (but dose of 60 mg/kg/d) administered to MRL/lpr mice only modestly suppressed IgM levels and anti-DNA antibodies but in addition strongly reduced mesenteric lymph node weights (A. N. Theofilopoulos, La Jolla, CA: personal communication). MRL/lpr mice were treated with CS (25 mg/kg/d orally) from 6 to 22 weeks of age and CS blood levels within the therapeutic range were obtained. Treatment with this low dose did not alter autoantibody titres, slightly increased serum IgG levels, and had no effect on glomerulonephritis and glomerular proliferation. There was, however, a reduction in the amount of mesangial IgG deposits, in the extent of the interstitial and perivascular infiltrates, and in the frequency and severity of necrotising arteritis in the kidneys of CS-treated MRL/lpr mice. The most prominent effect of CS was an evident reduction in lympho-proliferation in these mice (67). The same CS regimen applied to BXSB mice (915) had apparently no effect on any of the parameters measured (67). Since the major abnormality in MRL/lpr mice seems to reside in the T_H circuit, while in BXSB mice this seems to be the B-cell circuit, these results with a clearly suboptimal dose of CS for modulating antibody production are not unexpected.

The lymphadenonopathy of the MRL/lpr mouse strain is primarily due to the expansion of an unusual $CD4^-$, $CD8^-$, $6B2^+$ T-cell population. CS (40 mg/kg/d intraperitoneally from 4 to 18 weeks of age) prevented lymphadenopathy and expansion of this unusual T cell population in the peripheral lymph nodes and also in the thymus. The increased expression of the c-myb and T-cell receptor β-chain genes associated with these unusual cells was also corrected. The finding of increased numbers of $CD4^-$, $CD8^-$, $6B2^+$ thymocytes in untreated mice suggests abnormal intrathymic differentiation in lpr/lpr mice, a defect that was corrected by CS (669). The CS-treated mice had also a marked decrease in arthritis and glomerulonephritis and significantly prolonged survival. These beneficial effects of CS occurred despite a lack of reduction in anti-DNA antibodies, circulating immune complexes, rheumatoid factor titres, or Ig concentrations. These results demonstrate that the B-cell hyperactivity of MRL/lpr mice can proceed even without the T-cell proliferative disease (669).

Ten weeks old MRL/lpr mice were treated with CS (100 mg/kg/d orally, 5 times/week) for 10 weeks continuously. This treatment strikingly reduced lymphoproliferation as measured by the weight of the submaxillary and axillary lymph nodes and the peripheral blood leukocyte counts. Spleen weight, however, was not affected. The only serologic parameter measured was the rheumatoid factor (RF). There was no difference in the levels of IgM-RF between treated and control mice. Paradoxically, the mice that were treated with either CS or (Nva^2)-CS (same protocol) showed a highly significant increase in the levels of IgG-RF (364). This finding has also been observed in the NZB/W F1 mouse (472).

In conclusion, CS exerts profound prophylactic as well as therapeutic effects in the different murine spontaneous SLE models. An interesting feature is that symptomatic improvement is not necessarily associated with a concurrent reduction in autoantibody levels. In clinical

SLE, nephritis has long been considered as one of the most ominous components; it is characterised by accumulations of immune complexes and lymphoid cells in several locations within the kidney. The extreme diversity of renal changes indicates that many variables and pathogenetic factors are likely to be involved (see Figure 3 in 46). There is clear evidence from pilot studies that CS may positively affect clinical SLE, especially in combination with steroids (642, 643, 379, 268). However, relapse of SLE in patients receiving CS has also been reported (604). Obviously, larger, randomised, controlled trials will be necessary to provide a firm basis for assessing the potential therapeutic value of the drug. It is worth noting that clinical improvement may occur in patients without concomitant reduction in autoantibodies and that the lymphocyte activity score (in vitro capacity of T cells to release IL-2 and amount of IgG production by B cells) is not immediate but takes a whole year to normalise (642).

4. Psoriasis, a clinical model of autoimmunity. Psoriasis has not yet been proved definitively to be a primary immunological disease, but circumstantial evidence supporting the immunological hypothesis is steadily increasing. It is a common and chronic disease with a complex heredity and is of unknown primary etiology. Studies directed at the detection of a biochemical defect in psoriatic skin have revealed a number of abnormalities related to transmembranous signal transducing systems (see for review 115). Among the abnormalities of the histogenetically different cell populations present in psoriatic skin lesions, there is an increase in an activation of many cell types, especially immunocytes. The skin immune system shows an increase in subepidermal dendritic cells and a high number of T cells infiltrating the epidermis. A wide variety of mediators (ILs and IFNs) that also play a role in keratinocyte hyperproliferation and inflammation is produced by cells of the skin immune system. The possible immunogens responsible for these events, however, have not been identified. The failure of a feedback inhibition of certain mediator effects is also not understood. CS has been reported to inhibit several activation pathways (115).

There is no experimental model available for studying psoriasis in an animal species. Although the phorbol ester-treated mouse skin is clearly an inadequate model for psoriasis, epidermal hyperplasia, vascular alterations, and neutrophil accumulation are common features of both systems. Drugs with antipsoriatic actions such as corticosteroids, retinoids, and CS clearly are effective in both systems. An additional link is protein kinase C activation, which occurs for unknown reasons in psoriasis and as a result of phorbol ester treatment in the mouse skin system. However, the mouse model represents an acute inflammation reaction, lacking the involvement of the immune system which appears to play an important etiologic role in psoriasis. Nevertheless, it has been demonstrated that CS and the immunologically inert congener, (D-MeVal[11])-CS, applied topically strongly inhibits the phorbol ester-induced ornithine decarboxylase activity by blocking its mRNA accumulation. CS also inhibited by about 50% the stimulation of IL-1α mRNA accumulation by phorbol ester. Histology of the treated mouse skin five hours after phorbol ester application and CS indicated that infiltration of the epidermis with neutrophils was prevented by the drug, thus suggesting that generation of a potent chemotactic stimulus by the epidermis had not occurred (248, 355).

Baker et al. (45) have shown that there is a preferential recruitment of $CD4^+$ T cells into psoriatic lesions. In chronic psoriatic plaques that have remained static in size for at least 1 year, increased numbers of $HLA\text{-}DR^+$ dendritic (Langerhans) cells were associated with approximately equal numbers of activated $CD4^+$ and $CD8^+$ T cells in the epidermis. It was postulated that the abnormal epidermal proliferation in psoriasis is mediated by factors released by interacting $CD4^+$ T lymphocytes and Langerhans cells. In the epidermis of patients total $CD4^+$ and $CD8^+$ T-cell numbers were substantially decreased after 12 weeks' CS treatment (45% and 65%, respectively). These reductions correlated with the decrease in PASI scores. Epidermal activated $CD8^+$ T cells were also markedly decreased (74%). In striking contrast, the number of epidermal activated $CD4^+$ T cells was little affected. Total numbers of epidermal dendritic cells in lesional skin decreased by a mean of 36%, especially during the latter part of the treatment. The effect of CS on epidermal dendritic cells was, however, highly selective, depleting only the DR^+6^- subpopulation. The reduction of these cells, which are absent in uninvolved psoriatic epidermis, correlated with that of the PASI scores. It was suggested that DR^+6^- cells are immature Langerhans cells recently recruited at an abnormal rate into the epidermis as part of the psoriatic process. It was finally speculated that CS exerts its therapeutic effects on psoriasis by inhibiting the release of epidermal proliferation factor and other interleukins by $DC4^+$ T cells in the epidermis. The rapid relapse of the disease after discontinuation of drug treatment supports the idea that activated $CD4^+$ T cells have been reversibly inactivated by CS (45).

Because the immunosuppressive activity of CS has been well-documented, its potent antipsoriatic effect (e.g., 959) has been advanced as an argument in favour of an immunopathologic origin of the disease. However, serious consideration should also be given to direct effects of this drug on epidermal cells and to the possibility that the cellular pathogenesis of this complex disease resides within the keratinocyte itself. CS has recently been shown to have direct antiproliferative effects upon cultured human keratinocytes grown in defined medium (697, 301). CS (1 to 10 μg/ml) and (D-MeVal[11])-CS (5 to 10 μg/ml) directly inhibited keratinocyte proliferation.

Although these concentrations appear rather high, they are to be considered as physiological, since after only 7 days of treatment psoriatic involved skin contained approximately 2.8 μg CS per ml (tissue wet weight) (276).

Therefore, human epidermis contains high concentrations of CS after oral administration which are within the range of those known to inhibit the growth of cultured keratinocytes. In contrast, others have found that CS did not inhibit keratinocyte outgrowth from pig epidermal explants (495) or had no direct effect on keratinocyte proliferation (498). The discrepancies may be due to differences in the systems and parameters used. It has also been reported that CS in high concentrations (5 and 10 μg/ml) directly inhibited accessory cell functions of epidermal Langerhans cells in vitro (302). Lower concentrations of 1 μg/ml or lower were not effective (302, 672). Since Langerhans cells exhibit potent accessory cell functions for the induction of contact sensitivity and DTH in vivo, their susceptibility to CS might constitute an important part of the drug's antipsoriatic effect.

It may be of anecdotal interest to recall that psoriasis was the first AD ever treated by CS. Already in 1979, Müller and Herrmann from the University Clinic in Basel reported four cases of psoriatic arthritis in which psoriasis showed a dramatic reduction after about 1 week of CS treatment but returned in all patients after stopping CS (674).

5. Interactions of CS with suppressor cells in autoimmunity. The problem of how CS affects suppressor cells in allograft models has been discussed previously in the analysis of mechanisms promoting unresponsiveness (see IV.C.2.e.). It seems generally accepted that antigen-specific T_s cells generated against alloreactive effector cells are resistant to the action of CS (542). A tentative summation of the numerous, often diverging findings reported in the literature is presented in table 5. Batchelor and colleagues (56) have recently reevaluated the evidence supporting the concept of antigen-specific T-cell suppression. They suggest that the structures recognised by these T_s cells are in principle the same as those recognised by other T cells, i.e., a MHC molecule holding a peptide in its binding cleft. In the particular circumstances of specific suppression, the peptide is derived from the variable idiotypic regions of the T-cell receptor of the target clone. T_s cells, belonging in both the CD4 and CD8 populations, may exert their suppressive functional effects simply by lysis or DTH-mediated elimination of the alloreactive target T cells; or, alternatively, the presentation by the latter T cells of their own idiotypes may lead to downregulation of the presenting T cell, for instance, the lymphokines produced by the responder T_s cell may be inhibitory to the presenting cell.

It seems difficult to envisage that suppressor cells developing in models of ADs should be at variance with those occurring in allograft reactivity, especially in the antigen-induced autoimmune models in which self antigens are manipulated so as to virtually be seen as alloantigens (173). In the spontaneous, genetically determined autoimmune models there is probably an immune imbalance taking place which compromises a preexisting regulatory suppressor mechanism. Immunosuppressive treatment may suppress the effector arm without concurrently restoring the depressed or deleted original suppressor mechanism. Several observations suggest that besides antigen-specific T_s cells, as found under allograft conditions, other types of suppressor cells or mechanisms may prevail in experimental models of ADs. Their respective susceptibility to CS may not necessarily be the same.

It is well documented that rats susceptible to EAE will recover from paralysis and subsequently become resistant to further attempts to induce the disease (981, 419, 63). Spleen or lymph node cells isolated from rats with overt disease and conditioned in vitro are able to passively transfer EAE (419, 806, 896, 418, 64). Spleen, lymph node, and thymic cells obtained from rats that have spontaneously recovered from EAE contain T_s cells which, upon transfer into syngeneic hosts, are able to render them refractory to renewed induction of EAE (981, 66, 896). Interestingly, after recovery from passive EAE, LEW rats were completely susceptible to additional attempts to induce EAE by active or passive means (419) and nude (athymic) rats will also spontaneously recover from adoptively transferred EAE (418).

It was previously reported (IV.D.2.b.) that discontinuation of prophylactic CS therapy usually will be followed by a bout of EAE which indicates that the rats did not build up a state of resistance during treatment. This observation suggests that suppressor cells are not developing under the cover of CS treatment which in turn prevents the stimulation and proliferation of effector cells (108). The EAE experiments in the guinea pig by Fredane et al. (292) and the dramatic relapses in the CREAE in LEW rats after discontinuation of the drug (265) strongly support the contention that CS prevents the formation of T_s cells, at least under certain conditions and in some models of autoimmunity. The experiments of Polman et al. (762), in which they induce a type of CREAE with low-dose CS in rats, can be interpreted in two ways: CS completely abrogates the emergence of suppressor mechanisms, or the low-dose and narrow margin (as it can also occur with very short-term) treatment is too low to prevent the onset of EAE, but high enough to stimulate effector T cells which result in the rebound/relapse effect. However, the experiment of Ellerman et al. (249), in which CS is used in vitro to allow the generation of a T_s cell line, invalidates the concept that CS negatively affects existing T_s cells. Similar experiments were reproduced to generate in vitro T_s cells in rat EAMG (630, 631) and in normal NOD mice (288). It would be important to test the effect of CS on the specifically suppressive and cytolytic CD8 T cells di-

rected against the EAE-inducing CD4 T cell line in the model described by Sun and coworkers (896). The transfer of these in vitro/ex vivo generated T_s cells were shown upon transfer into syngeneic hosts to effectively protect them from EAE (249) or diabetes (288). Some of the T_s cell lines release a soluble suppressor factor which is effective in vitro, but not in vivo (631, 66). The type of suppressor cells may vary since in the EAE model both CD4 (249) and CD8 T_s cells (896) were described. The suppressor cells obtained in vitro in the EAMG are reported as antigen-specific, adherent CD8 T cells (631).

In contrast to the EAE model, prophylactic treatment of rats to prevent development of EAU (296, 657), guinea pigs to prevent onset of EAO (427), and mice to inhibit production of autoantibodies in experimental erythrocyte autoimmunity (185), resulted in each model in the prevention of the disease and in the generation in vivo of suppressor cells which, in adoptive transfer experiments, would effectively protect naive syngeneic recipients from disease induction. In the EAU model these suppressor cells were characterised as antigen-specific, non-adherent CD8 spleen cells (657); control, non-CS-treated rats also produced suppressor cells, but they were not antigen S-specific (657). The suppressor cells found in the CS-treated guinea pigs in the EAO model were isolated from the lymph nodes and appeared to be antigen-specific, whereas non-CS-treated animals did not produce suppressor cells as demonstrated by adoptive transfer experiments (427). The suppressor cells obtaiend from CS-treated mice in the erythrocyte autoimmunity model were of Thy 1.2 phenotype and specifically inhibited autoantibody formation to mice but not rat erythrocytes (185).

The findings reported from the active Heymann nephritis and mercuric chloride-induced autoimmune glomerulonephritis models are at variance from the previous ones. CS prophylactic treatment induced in both models a state of relative unresponsiveness to subsequent nephritogenic challenges (207, 29). However, this form of tolerance could not be transferred by lymphoid cells from CS-treated rats, but could be transferred by lymphoid cells derived from the thymus or spleen of high antigen-dose tolerant rats in active Heymann nephritis (207). The unresponsiveness observed in the other glomerulonephritis model, which was induced by preventive CS plus mercuric chloride treatment, could be broken by reconstitution with naive lymphoid cells, suggesting that this tolerant state to mercuric chloride was not mediated by active suppression, but rather resulted from direct functional deletion of autoreactive T lymphocytes (29).

Clinical data on the effect of CS on suppressor cells in ADs are rare. A study was undertaken to determine the effect of CS on the production of several cytokines as well as on the suppressor function of peripheral mononuclear cells from SLE patients (7). CS strongly inhibited the production of IL-3-like activity by normal cells, an effect noticed only on cells from four out of ten SLE patients, three of whom were in remission. Addition of CS significantly increased suppressor cell function by cells from all SLE patients. CD8 T-cell clones exhibiting strong suppressor activity were obtained from two patients by using CS-conditioned medium containing IL-3.

Routhier et al. (796) have shown that a significant improvement in liver function occurred in patients with primary biliary cirrhosis treated with CS when the pathologically elevated T_H:T_s cell ratio returned to normal values. Cessation of therapy caused a reversal to elevated values. The effects of CS on liver immunohistology and peripheral blood T lymphocyte distribution were studied in five patients with primary biliary cirrhosis receiving CS treatment for 6 months. Several significant immunological changes were seen during the course of treatment: the numbers of infiltrating T cells (CD3) and HLA-DR$^+$ cells (mainly macrophage/dendritic-like) within the lobule parenchyma decreased; the total circulating T-cell numbers increased and the percentage of activated, HLA-DR-expressing CD8 (suppressor/cytotoxic) T cells and CD4 (helper/inducer) T cells decreased. Moroever, a selective decrease of IgM-type (but not IgG-type) antimitochondrial antibody levels was observed in four patients. A reversal towards pretreatment values was observed for all these immunological parameters 3 to 6 months after withdrawal of therapy (491).

6. CS in clinical autoimmune diseases (summary). In recent years, there has been increased interest in studying the potential of CS in ADs. As of June 1988, about 5000 patients suffering from many different autoimmune syndromes have been treated in more than one hundred clinical trials (968, 833, 479). The recent and competent review by von Graffenried and colleagues (968) summarises all the available important information on the effects of CS in clinical autoimmune disorders and discusses the problems of clinical testing and the potential of using this drug in autoimmunity. The review clearly shows that CS is effective in many clinical ADs, for induction as well as maintenance of remission. Efficacy has been demonstrated in randomised controlled studies and in uncontrolled or pilot trials as shown in table 7. The steroid-sparing properties of CS are possibly one of its major contributions to the treatment of ADs. Common features of the clinical effects of CS are the relatively rapid onset of effect, which is dose-dependent, and also that relapses occur when treatment is stopped. These facts are presumably linked to the quick onset and reversibility of the inhibition of lymphokine release by CS. Furthermore, these clinical observations correlate closely with those made in experimental models (table 6). One of the most important issues is safety in long-term therapy. It is evident that more studies are yet needed to define the true benefit of CS in ADs and to position the drug among conventional therapies (968).

TABLE 7
Cyclosporine (SANDIMMUNE) in clinical autoimmune diseases—summary as of June 1988

In the following indications efficacy has been demonstrated in controlled studies:
- Rheumatoid arthritis
- Uveitis posterior
- Behçet's disease
- Psoriasis
- Myasthenia gravis
- Crohn's disease

Convincing evidence of efficacy has been demonstrated in uncontrolled studies in:
- Nephrotic syndrome
- Systemic lupus erythematosus
- Polymyositis/dermatomyositis
- Aplastic anemia, pure red cell anemia
- Endocrine ophthalmopathy

Efficacy of CS is unknown in the following indications:
- Multiple sclerosis (chronic progressive type)
- Amyotrophic lateral sclerosis
- Primary biliary cirrhosis
- Vasculitis syndromes
- Pulmonary sarcoidosis

CS is not effective in:
- Multiple sclerosis (remitting-relapsing)

CS is not recommended (despite evidence of efficacy) in:
- Insulin dependent diabetes mellitus
- Scleroderma (high incidence of hemolytic-uremic syndrome)

E. Interaction of CS with Defence Mechanisms against Infections

1. Modulation of host defences by CS. The effect of CS on the incidence, severity, and outcome of infections remains unclear, although general clinical experience, in spite of some contradictory reports, so far indicates that infection rates in organ transplant recipients are lower today than they were in the pre-cyclosporine era. Several studies clearly demonstrate that the infection rate is no higher, and the severity of infection and the mortality from infection are lower in CS-treated patients (163, 845, 425, 351). A declining incidence of wound infection in transplant patients under CS therapy has also been reported (535). This is not surprising in view of the mechanism of action of CS which does not cause myelodepression and interferes little with the effector functions of phagocytes. Since the drug acts primarily by inhibiting certain T-cell functions, it is obvious that clinical infections, whose eradication depends on an intact cell-mediated immune response, constitute the major risk for CS-treated patients. Such infectious agents are comprised of viruses (e.g., cytomegalovirus (CMV), herpes simplex (HSV), and lymphocytic choriomeningitis (LMCV) virus); bacteria (*Mycobacterium*, *Listeria*, *Salmonella*, *Chlamydia* etc.); fungi (*Cryptococcus* and *Coccidioides*); protozoa (Toxoplasma, *Leishmania*, *Trypanosoma* and Pneumocystis); and worms like *Schistosoma* and others. It has indeed been documented that allograft recipients under CS therapy, and usually with additional prednisone, are immunocompromised, at least during the first year following transplantation (956). More similar trials are needed to assess the immunocompetence in patients on low-dose CS maintenance therapy 2 or more years after organ transplantation; however, it would be anticipated that under those conditions most subjects would have regained near normal immune responsiveness.

Antibody-mediated immunity to Coronavirus (IgA) in small bowel allografted pigs (27) and to varicella-zoster virus in pediatric renal transplant recipients (938) were not inhibited under CS therapy. However, suppression of cell-mediated immunity to varicella virus was observed, but it was difficult to attribute this effect to CS alone, since these patients received concomitant therapy with additional major immunosuppressants (938). The effect of CS on natural killer (NK) cells in immunosuppressed patients remains rather controversial (see IV.B.4.b.).

It is well-known that both primary and reactivated CMV infections occur frequently after organ transplantation. CMV mononucleosis in humans is associated with decreased lymphocyte proliferation responses to mitogens and HSV antigens, decreased IFN production, and reversal of $T_H : T_s$ cell ratio. In a clinical trial of ninety-one organ recipients receiving CS, 42% of these patients were viremic for CMV, and 35% were symptomatic; however, there was no difference in frequency and severity of CMV infections compared with the group receiving conventional (AZA) immunosuppression (422). Other studies have confirmed that the impact of CMV infection in CS-prednisone-treated renal recipients does not differ substantially from those requiring AZA-prednisone therapy (581) or that CS-prednisone immunosuppression leads even to a reduction in CMV infection (356, 621). An in vitro study by Converse et al. (183) on the effect of CS on the response of normal human lymphocytes to CMV indicated that a significant degree of protective immunity to CMV-infected cells was maintained even in the presence of CS. It has recently been observed that transplantation of a CMV-positive organ into a CMV-negative recipient carries a high risk of mortality/morbidity from primary CMV disease (581, 979). Interestingly, it was also shown that among seropositive recipients CMV infection occurred significantly more frequently when kidneys came from seropositive than from seronegative donors, thereby indicating that the excess is probably accounted for by reinfection rather than by reactivation of recipient virus, as proven by restriction enzyme typing of virus isolates (354). Rinaldo et al. (783) have reported that renal transplant patients with primary CMV infection were more likely to have prolonged viremia associated with symptomatic disease than were those with reactivated infection. Similar observations were made in the vanishing bile-duct syndrome after liver transplantation (725).

Furthermore, it has been shown that patients on main-

tenance CS-prednisolone immunosuppression can successfully mount T-cell and antibody responses to HSV infections (739). Other data suggest that CS treatment inhibits the activation of suppressor cells and, thus, depression of cellular immune function that has been associated with HSV infections in renal transplant recipients undergoing conventional immunotherapy (783). Finally, B cells derived from peripheral blood from CS-treated bone marrow transplant patients have been found to be refractory to Epstein-Barr virus (EBV) transformation. The results suggest that this is due to a qualitative deficiency in the patient's monocytes, which cannot support B-cell growth (189).

In view of the paramount importance of infections in immunosuppressed patients (698), it is necessary to briefly review the literature describing some of the effects of CS on the modulation of host defense mechanisms in experimental animals. There is a dire lack of updated and competent reviews in this area.

2. *Effects on viruses.* The reader should be aware that, in this part on viruses, we have not adhered to the systematic classification; instead, we have first reviewed the clinically important organisms and then proceeded to those which were experimentally most thoroughly investigated. Under the heading "other viruses" we have listed all those organisms on which there is only scarce information.

a. HERPES SIMPLEX VIRUS (HSV). Following adsorption to a permissive cell, enveloped animal viruses must transfer their genetic material across at least one membrane barrier before they can infect the cell. These viruses penetrate into cells by two distinct pathways. Viruses such as *Influenzavirus* and vesicular stomatitis virus (VSV) are taken up by coated vesicles and enter endosomes where fusion between the virion envelope and endosomal membrane occurs. Alternatively, viruses such as HSV and Sendai virus fuse directly with the host plasma membrane. The effect of CS on virus-induced cell fusion and virus penetration into cells was analysed in vitro using extremely high drug concentrations (10 to 40 μM). Fusion of cells infected with a syncitial strain of HSV-1 was markedly reduced in the presence of CS, although the compound did not appear to influence viral replication. CS did not inhibit penetration of HSV, a process thought to involve membrane fusion (633). Therefore, CS possibly inhibits cell to cell transmission of virus by inhibiting fusion of infected cells with uninfected cells without activating virus progeny or inhibiting virus penetration.

The modulation by CS of murine natural resistance against HSV infection was investigated by Amerding et al. (25). Adult BALB/c mice, which are medium-high resistant against intraperitoneal infection with HSV-2, manifested a drastic increase in susceptibility to the virus when treated intraperitoneally with CS (2, 10, and 50 mg/kg/d on 5 consecutive days) during infection. Oral administration of the drug had no effect on the natural resistance status. Mice appeared normal 2 weeks after CS treatment with regard to their ability to resist intraperitoneal infections. CS did not interfere with established specific immune protection nor with the induction of immune responses to HSV-2. The same group further showed that CS interfered locally, but not orally, at the site of injection with several resistance functions which are of potential importance in HSV infections in mice. HSV-induced stimulation of macrophage phagocytosis was reduced by CS when the mice were infected 5 days before the assay. The in vivo replication of the virus in macrophages, however, was enhanced. NK cell responses were severely impaired, but IFN levels induced by HSV were not diminished. Inhibitory effects ceased after termination of CS treatment and could be prevented by presensitisation of the mice with attenuated HSV-2 (26). Altmann and Blyth (14) have used the mouse ear model of HSV to investigate the effect of CS on the induction, regulation, and expression of DTH to HSV-1 and on the production of neutralising antibody. Their results clearly demonstrate that CS exhibits a wide range of suppressive or enhancing effects on immunological effector mechanisms depending on mode of priming, timing, dose, and frequency of administration. While paradoxical enhancement of both DTH and antibody production could be demonstrated in some circumstances, only the DTH response was susceptible to suppression.

Schellekens and coworkers (829) have studied the antiviral effect of IFN in a number of experimental virus infections (including HSV, VSV, and pseudorabies virus) in the rat and have shown that the antiviral activity was not inhibited by CS (about 5 mg/kg/d intraperitoneally on days −1, 0, and 1) injected concomitantly with intraperitoneal interferon. The effect of CS on primary lymphotropic herpes virus (HLV) infection was studied in the guinea pig (683). Animals pretreated for 2 days with CS (50 mg/kg/d intramuscularly), were then inoculated intranasally or intraperitoneally with HLV and followed by four additional daily doses of CS. While intranasally-inoculated CS-treated animals expressed higher virus infectivity titres in their lungs compared with controls, this difference was not seen in the intraperitoneally-inoculated groups. At histopathology, lymphoid depletion was seen in all CS-treated animals but lymphocytic interstitial pneumonia was observed only in the lungs of intranasally-inoculated and CS-treated guinea pigs. Thus, CS treatment altered the pathogenesis of primary HLV infection with some notable differences attributed to the route of virus inoculation.

Secondary HSV-induced uveitis was elicited in a rabbit eye that had recovered from primary HSV uveitis. Daily intramuscular injections of CS (25 mg/kg/d) for 7 days prior to the intravitreal HSV challenge significantly suppressed the induction of secondary uveitis, but daily injections of CS after the HSV challenge were ineffective.

CS-treatment of rabbits recovered from primary infection, but not challenged with HSV, resulted in a marked reduction of cell-mediated immunity while leaving the level of circulating HSV specific antibody high. No reactivation of latent HSV was detected in the cervical ganglia of CS-treated rabbits (727). The immunosuppressive effect of CS on the clinical and antiviral immune responses were examined in experimental HSV stromal keratitis in the rabbit. CS (25 mg/kg/d) was administered intramuscularly starting from the time of corneal infection with HSV until day 14 post infection. CS treatment induced more severe and persistent stromal keratitis and a greater incidence and duration of virus recovery from the cornea. Suppression of cellular immune responses to T and B cell mitogens, and HSV antigens were observed in the CS-treated animals (637, 638). These results indicate that CS-sensitive cells are involved in limiting virus replication (638).

b. CYTOMEGALOVIRUS (CMV). The effect of CS (50 mg/kg/d) was tested on the development of T_c cells against murine CMV using infected stimulated macrophages. No significant difference between the CS-treated and untreated BALB/c mice was observed (129). The same group also reported that CS at a dose of 100 mg/kg/d inhibited replication of murine CMV in the lung, liver, and spleen of mice infected intraperitoneally (358). Chatterjee et al. (164) did not observe an increase in CMV infection rates in the CS-treated group (20 mg/kg/d subcutaneously) whether given alone or in combination with prednisone (2 mg/kg/d subcutaneously). On the other hand, Kurtz and Homan (550) have shown that in BALB/c mice CS, in a dose (100 mg/kg/d) that was effective in preventing skin graft rejection, turned a benign CMV infection into a fatal disease. Zhang et al. (1021) fed BALB/c mice a basal diet containing CS (about 50 mg/kg/d orally for 84 days) which achieved in those mice blood levels of 1.4 to 2.2 μg/ml. CS enhanced virus titres and helped maintain chronic persistent infection in certain organs by affecting the development or function of immune cells. Regardless of whether CS was given before or after virus infection, about 2 weeks of CS administration was required before an elevation of virus titre was observed. Furthermore, Selgrade et al. (842) found that the susceptibility to murine CMV, virus-augmented NK cells and IFN induction were not affected in mice treated intraperitoneally with CS (50 mg/kg/d) for 5 days and infected on the 5th day of treatment. In contrast, enhanced susceptibility to CMV and depressed NK cell activity were observed in mice treated by the same exposure regimen on days 1 to 5 postinfection. Susceptibility was not affected by CS given on days 5 to 9 post-infection.

The transfer of CMV infection by latently infected renal allografts was investigated in rats treated with CS (10 mg/kg/d subcutaneously). This immunosuppressive regimen administered in order to prevent renal graft rejection led to a reactivation of rat CMV in 57% of the CS-treated recipients compared with 100% of those treated with TBI or with AZA plus prednisone (132). Results from experiments evaluating cell-mediated immunity to CMV infection in rats receiving RT1 mismatched allogeneic bone marrow transplantation suggested that CS, in a dosage used for GvH prophylaxis (15 mg/kg/d subcutaneously), induced only a transient rise in viral titres during the second week of infection, which resolved in the third week (254).

The effects of CS and cortisone on the pathogenesis of primary infections with CMV were investigated in the guinea pig model. Oral CS (20 mg/kg/d) and/or subcutaneous cortisone (10 mg/kg/d) were administered until the animals were killed on day 14. Guinea pigs treated with CS alone developed weight loss and lymphopenia, showed widespread viral inclusions, and minimal inflammatory response to CMV which was isolated from 53% of their tissues. Animals treated with either cortisone or CS plus cortisone did not develop lymphopenia, and their rates of isolation of CMV were significantly lower than those of CS-treated animals (760). These findings suggest that cortisone protected the guinea pigs from some adverse effects of CS during acute primary infection with CMV. Further experiments demonstrated that CS treatment (20 to 33 mg/kg/d orally) of guinea pigs with chronic CMV infection was associated with a greater frequency of CMV isolation from lymphoid tissues and elevated salivary gland titres of CMV. However, these findings were not accompanied by histopathological evidence of active disease. They additionally indicate that CS itself appears to have a minimal tendency to induce reactivation and dissemination of CMV and corroborate clinical observations (609).

c. HEPATITIS VIRUS. Fully resistant A/J mice were infected intraperitoneally with a high dose of murine hepatitis virus (MHV-3) in the presence of CS (50 mg/kg/d subcutaneously) started 72 hours prior to infection and daily thereafter (mean serum trough levels were 4.8 μg/ml). The CS-treated and infected mice showed similarly to the controls no histologic or biochemical evidence of liver disease, developed a normal humoral response, but had a delayed clearance of the virus from their livers. The results indicate that high CS doses did not alter the resistance to MHV-3 even at a challenge with very high virus doses, in spite of the fact that this resistance is known to be T cell-mediated (1). MHV-3 also induces in C3H mice a persistent CNS infection characterised by meningitis, ependymitis, encephalitis followed by hydrocephalus, and by chronic thrombotic brain stem vasculitis. C3H mice were inoculated intraperitoneally with MHV-3 and treated at different times before and after infection with daily subcutaneous injections of CS (50 mg/kg/d). In mice treated before and at the time of MHV-3 inoculation, a slight increase in acute death was observed. However, ependymitis and hydrocephalus were

markedly decreased and no thrombotic vasculitis was present. This was also found in mice in whom CS treatment was begun 15 days postinfection. Viral persistence in the brain and anti-MHV-3 antibody titres were not affected by treatment (85).

The in vivo influence of CS (20 mg/kg/d) administered once daily by intravenous drip infusion for 4 weeks was assessed in two chimpanzees chronically infected with non-A, non-B hepatitis virus. CS inhibited the proliferation of and possibly killed some hepatitis viruses. After drug withdrawal the histopathological scores increased in number again to the levels seen before CS treatment. The differences in the levels of decrease of histometric scores in the two animals may be related to the differences in serum trough levels of CS (910).

d. INFLUENZAVIRUS. Primary anti-influenza A T_c and B lymphocyte-dependent responses in inbred mice were used as an in vivo model system to study the effects of CS. Five consecutive daily oral doses of CS (200 mg/kg/d), with the first being given 1 or 2 hours before intravenous virus inoculation, resulted in a complete inhibition of anti-influenza T_c cells and a partial reduction of cytotoxic B lymphocyte response. Suppression was of short duration. CS treatment during an ongoing influenza infection suppressed preferentially T_c cells, but did not increase sensitivity to the virus. Mice with no measurable cytolytic anti-influenza T_c cell activity, but significant B cell response, although partially diminished by CS, were completely protected against the lethal effects of influenza infection (24). Direct antiviral effects of CS were not demonstrated, which is supported by the observation that CS treatment did not result in drastic reduction of mortality after intranasal application of pathogenic virus. These experiments disclosed two important findings: i) protective immunity against influenza was as rigid in CS-treated as in untreated mice (intranasal challenge one week after sensitisation), and ii) CS did not interfere with normal resistance of mice against influenza (24). Another study showed that oral administration of CS (200 mg/kg/d) starting 2 or 20 hours after intranasal *influenzavirus* infection for 10 days increased the mortality of BABL/c mice. CS also increased the amount of viruses that could be recovered from the lungs of infected mice and delayed the rate at which it was eliminated. This treatment protocol did not, however, prevent the appearance of haemagglutination-inhibiting antibody in animals infected with a sub-lethal concentration of virus (184).

Schiltknecht and Ada (830, 831, 832) have confirmed and extended these previous results. CS (100 mg/kg/d subcutaneously starting 2 h before virus inoculation) administered to mice substantially affected their immune response to an intranasal influenza A virus infection. If treated for 21 days, lung inflammation was greatly decreased and the lungs contained high titres of virus which cleared more slowly than in controls. The production of haemagglutination-inhibiting antibody was delayed but NK cell activity in the lung was comparable to control levels. In contrast, a DTH response to the virus could not be elicited at 6 or 12 days after infection, but T_c cell activity was present in the lungs of these CS-treated, infected mice, though its appearance was delayed. If administered with a dose of virus lethal for normal mice, CS-treated mice survived, probably due to the greatly reduced level of immunopathological damage in the infected lung (831). Specific effector T cells that mediate DTH to *influenzavirus* were found to be formed in vivo in CS-treated mice. The activity of these cells could only be measured when they were transferred into untreated, naive mice. The T_{DTH} cells were H-2 restricted in the I region of the MHC. Influenza-specific T cells could not be detected in the spleens of CS-treated mice given virus intravenously, even when drug treatment was started 3 days after virus administration. The data suggest that i) class I-restricted responses were more susceptible to CS than the generation of class II responses, and that ii) effector T cells can indeed be formed in the presence of CS but are prevented from acting as long as the drug is present (832). Finally, CS inhibited in vivo the function of transferred influenza-specific H-2-restricted T_c cells, which normally lead to clearance of virus in the lungs of *influenzavirus*-infected mice, but without affecting their migration to the lungs. However, CS had no effect on the in vitro expression of cytotoxicity by the H-2-restricted T_c cells. These findings strongly suggest that the in vivo clearance of *influenzavirus* by H-2-restricted T_c cells involved a lymphokine mechanism (830).

e. LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV). The LCMV causes an infection in which T lymphocytes are responsible for immunopathogenicity. Effector T cells are implicated in the development of the disease which eventually provokes the death of mice 7 to 10 days following intracerebral viral inoculation. T_c and T_{DTH} cells control pathogenesis, while both subpopulations are also implicated in virus clearance. Treatments used to depress the T cell functions in LCMV-infected mice, such as ALS, CP, TBI, and thymectomy, lead to a virus-carrier state. In contrast, CS (50 or 100 mg/kg/d intraperitoneally on days 1, 2, and 3 post infection) spares a majority of lethally infected mice. This remarkable effect is related to the distinct activities exerted by CS on different T-cell subpopulations. CS depresses the T_c cell function, which otherwise would lead to a fatal outcome (821), but does not affect the other T-cell functions responsible for virus clearance, since the surviving animals rapidly eliminate the virus and produce high titres of neutralising IgG antibodies (819). When analysing the role of the dosage and timing of CS administration, it was found that for maximal protection of lethally by the intracerebral route and infected mice, CS should be administered intraperitoneally at a dose of 100 mg/kg/d on days 0 and +1 (820). Evidently, CS treatment did not

affect the activation of T_H and B lymphocytes, because antibodies were present and the spared mice were resistant to a further viral inoculation 20 or 40 days after the first inoculation (940). Mice infected by intracerebral inoculation with LCMV were protected when immunosuppressed with CS (100 mg/kg/d orally from 2 days before until 14 days after infection), although the virus multiplied extensively in all major organs and persisted for as long as the drug was administered. Upon discontinuation of CS, T_c lymphocytes appeared in the spleen and the virus was eliminated from all tested organs (594).

Cole and coworkers (176) have partially confirmed the previous findings. BALB/c mice were given daily CS (75 mg/kg/d subcutaneously), beginning 1 day before and continuing for 12 days after their intracerebral inoculation of LCMV. By day 7 post infection, these animals had developed neither seizures nor the pronounced influx into the cerebrospinal fluid of mononuclear cells that typify unmodified LCMV. However, these mice also failed to develop detectable LCMV-specific antibodies. In a similar experiment performed in C3H/He mice treated with subcutaneous injections of CS (50 mg/kg/d beginning day −1 until day 4, 7, or 10), the mean survival time of intracerebrally infected mice increased in rough proportion to treatment length, indicating that T cell-mediated choriomeningitis was delayed rather than prevented. In intraperitoneally infected mice, however, the magnitude of the T_c cell responses was inversely related to the length of CS treatment. Prolonged treatment maintained viremia and led eventually to lethal disease, presumably because infection had extended to the CNS by the time these mice regained their ability to mount virus-specific T-cell response (176). The results obtained by Hügin et al. (448) agree with the previous ones. CS suppressed primary and secondary antiviral T_c cell responses in vivo (intravenous virus inoculation) and the latter also in vitro in a dose-dependent manner, when given before and up to 12 hours after virus. Optimal doses were 50 to 60 mg/kg/d intraperitoneally starting on day −1 and continued up to day 7; 10 to 20 mg/kg/d induced a small immunostimulatory effect, whereas 180 ng/ml suppressed in vitro responses completely. CS delayed clearance of LCMV (intracerebral inoculation), prevented development of local DTH reaction when LCMV was injected into the footpad, and prevented T cell-mediated immunopathological brain damage during LCM disease.

Junin virus causes Argentine haemorrhagic fever in man and guinea pigs and shows some similarities with LCMV infection. Immunosuppression with CS (25 mg/kg/d intramuscularly from day −1 until 14) or CP had no apparent effect on the disease course of guinea pigs infected with a virulent strain of Junin virus. However, immunosuppression of guinea pigs infected with an attenuated strain of Junin virus led to fulminating Argentine haemorrhagic fever and all treated animals died. Virus distribution patterns in target organs and histopathological lesions in immunosuppressed animals infected with an attenuated virus strain were similar to the nontreated guinea pigs infected with a virulent strain. These animals failed to produce antibodies and virus-specific cytotoxic spleen cell activity, previously shown to be antibody-dependent, also failed to develop in the same animals (502).

f. OTHER VIRUSES. CS treatment, as described for LCMV experiments (448), was reported to inhibit anti-vaccinia virus (VAC) and anti-vesicular stomatitis virus (VSV) T cell-mediated primary and secondary responses in a dose-dependent fashion. CS added to secondary in vitro cultures of VAC and VSV also inhibited the antiviral T_c lymphocyte response dose-dependently (448). When mice were given a lethal inoculum of VAC and treated with seven daily doses of 5, 25, or 50 mg/kg/d CS subcutaneously from day −2 until 4, the primary splenic T_c lymphocyte response correlated inversely with CS dosage and was insignificant in mice treated with 50 mg/kg/d. Interestingly, spleen cells from mice infected with sublethal doses of VAC during CS treatment, if stimulated in vitro with VAC several weeks after treatment was discontinued, yielded normal levels of secondary virus-specific T_c lymphocyte activity, suggesting that, while inhibiting the expression of T_c cell function, CS did not prevent T-cell priming (176). In vitro experiments using mouse embryo cell and human foreskin cell cultures subjected to preinfection treatment with 40 μg/ml CS appeared to facilitate the release of VSV and poliovirus from these cells (357). Whether this effect was due to a cytotoxic concentration of CS remains unknown.

The immunologic and pathologic features of acute CNS infection and inflammation produced by *Alphaviruses*, of which the Sindbis virus (SIN) is the prototype, in man are very similar to those seen during SIN infections of mice. BALB/c mice infected intracerebrally develop a necrotising meningoencephalitis. Treatment of infected mice with CS (50 mg/kg/d subcutaneously from day −1 until 4) suppressed the development of CNS inflammation, which is mediated by SIN-specific T cells, as well as its augmentation by antibody and appeared to hasten the onset of paralysis (176). On the other hand, treatment with CS (50 to 75 mg/kg/d orally), from the time of virus infection, suppressed meningeal inflammation and demyelination in the spinal cord of mice persistently infected with Theiler's murine encephalomyelitis virus. In contrast, no therapeutic response was seen when the drug was begun after the disease process was established. The decrease was independent of serum titres of IgG to purified viral antigen, but did correlate with decreased proliferation of T lymphocytes to virus and myelin antigens (786). Semliki Forest virus (SFV) infection of adult mice results in a demyelinating meningoencephalomyelitis. Demyelination does not result from direct viral damage, but from the activity of T

lymphocytes. CS (50 mg/kg/d orally) had no effect when started 5 days after infection, and little effect when started 4 hours after inoculation. When CS was administered 48 hours before infection and continued thereafter, there was a prolongation of the blood and brain virus titres, and a reduction of serum IgG antiviral antibody synthesis, but an increase in the severity of the CNS inflammatory response and the demyelination. Consideration of these findings along with measurements of CS levels in the serum and cerebrospinal fluid suggested that CS did not cross the blood-brain barrier (262).

The progression from infectious viral myocarditis to congestive cardiomyopathy is thought to be due to a primary viral process inciting an excessive or disordered immunologic response against the myocardium. CS was used in a murine preparation of infectious myocarditis (EMC virus) that has been shown to progress to congestive cardiomyopathy with chronic myocardial failure similar to that seen in man. CS (25 mg/kg/d) was injected subcutaneously for 3 weeks, starting at 1 week after infection during viral replication or at 3 weeks after infection, i.e., after the period of viral replication. The use of CS immunosuppressive therapy in this murine model of acute viral myocarditis was associated with greater mortality when administered early in the illness, and greater myocardial failure when administered during the early recovery period, without evident reduction in pathologic indices of myocardial injury to suggest possible longer term benefit (664).

The effect of CS on the pathogenesis of experimental feline leukemia virus-induced aplastic anaemia was investigated. The failure of CS (15 mg/kg/d orally starting 17 days post infection) and of CP and ATG to alter the pathogenesis of the feline leukemia model suggest that the mechanism of feline retrovirus-induced erythroid aplasia is unlikely to be immune-mediated, but rather mediated by direct viral suppression of erythropoiesis (307).

Young marmosets, all of which lacked antibodies to Epstein-Barr virus (EBV) capsid antigens (VCA), were infected with an EBV-transformed strain. One group received CS (40 mg/kg/d orally for 4 weeks) to investigate the effect of concurrent immunosuppression on EBV infection. CS-treated and EBV-infected marmosets showed no increase in total lymphocyte counts: only two developed heterophile antibody. Four developed persistent antibody to the early antigen R component and all developed antibody to VCA, the mean titres being higher than in animals given EBV alone. Because these responses to EBV resemble those of humans, marmosets may provide a useful model for exploring the potential of cofactors in inducing EBV-associated malignancy (977).

g. SUMMARY. We have reviewed in this chapter the salient findings from a series of experiments in which the effects of CS treatment were compared in several well-studied animal models of viral infections. There was no evidence for a direct antiviral activity of CS. In spite of apparently controversial results due to the widely differing experimental approaches, several assumptions concerning the suppressive effects of CS have been investigated. Since CS primarily inhibits cell-mediated immunity, while sparing the function of phagocytes and NK cells, it follows that animals undergoing prolonged treatment with CS exhibited a drug dose-dependent decrease in resistance to primary infections that normally are controlled or eliminated by T cell-dependent defence mechanisms. Only long-term treatment with relatively high doses of CS was effective in inhibiting antibody-mediated immunity (see also IV.A.3.). CS therapy increased susceptibility to primary CMV infection, but did not stimulate reactivation of the chronic state. Experimental models using latently infected grafts seemed of practical relevance. Since CS-sensitive cells are known to be important for antiviral protection and immunity, it is evident that suppression of lymphokine-dependent effector functions will have deleterious consequences on the course of infection. In such animals, local inflammatory responses caused by the virus were reduced or absent when recruitment of the participating mononuclear cells required the presence of antigen-triggered T cells or their products. On the contrary, the marked antichronic inflammatory effects of CS did alleviate certain pathologic conditions caused by overreactive inflammatory processes of the host. Withdrawal of CS treatment during active infection permitted the reestablishment of specific immunocompetence, as reflected by the development of relatively normal, albeit delayed, cellular or humoral responses to the causative agent.

A great deal of confusion has been engendered by the differing experimental protocols used. Thus, CS-treated, intranasally HSV-infected mice showed higher infectivity than intraperitoneally infected ones. In all those animals which were inoculated by the intracerebral route, interpretation of the results have to take into account that CS does not cross the blood-brain barrier (575). Besides the mode of priming, the timing relative to infection, route, dose, and frequency of CS administration played a key role in the outcome of the experiments (e.g., 842, 14). When all these variables are considered together, the scattered range of results observed with CS therapy in several different models of virally induced meningitis is more easily understood. These general remarks are, of course, also applicable to the next chapter dealing with the effect of CS on bacterial infections.

According to the literature, it is clear that the infection rate, the severity of and the mortality from infection is surprisingly low in CS-treated compared with conventionally treated patients. Given the apparent clear superiority of CS, a prospective, randomised trial at this time does not seem to be ethically justified (163).

3. Effects on bacteria. a. GENERAL CONSIDERATIONS.

The body's defence against potentially pathogenic bacteria consists of a variety of specific and non-specific mechanisms. Since ultimately almost all bacteria are killed by phagocytic cells, the sparing of these cellular functions by CS is an important advantage over non-selective cytostatic drugs. Although the situation is still controversial, CS appears to exert only minimal effects on phagocytic cells as documented by a host of references (672; see also V.). The drug did not modify the responses of guinea pig macrophages (peritoneal exudate cells) to lymphokines in vitro (924) as well as phagocytosis of labelled bacteria by rat pulmonary alveolar macrophages or peritoneal polymorphonuclear leukocytes (230). There is in vitro experimental evidence that the drug did not reduce the levels of several monocyte (isolated from human buffy coats) mRNAs induced by phorbolester plus Con A activation and which include c-myc, IL-$1\alpha/\beta$, TNF/cachectin HLA/DRα, and γ-Ip10 (328). However, CS potently inhibited pulmonary macrophage chemotaxis in vitro and the capacity of macrophages to release superoxide, an index for microbicidal activity (231). In contrast, administration of CS (5 mg/kg/d intraperitoneally) to rats for 30 days had no effect on the number of pulmonary macrophages available for host defence, macrophage oxygen consumption, and superoxide release ex vivo (231). Pulmonary macrophages from these CS-treated rats demonstrated complete inhibition of active migration or chemotaxis in modified Boyden chambers upon incubation with formylmethionyl-leucyl-phenylalanine (FMLP) (231). We have previously also used the Boyden-chamber technique to test the effects of several drugs on the chemotaxis of rabbit peritoneal exudate neutrophils in vitro towards immune complex-activated serum (95), but were unable to show inhibition with CS (J. F. Borel; unpublished data). CS administered orally to rabbits was also ineffective in influencing in vivo granulocyte migration when using a plastic skin collection chamber technique (264, 105) and undiluted, fresh rabbit serum as a chemoattractant (J. F. Borel; unpublished results). A recent study by Pigatto et al. (757), who measured the in vivo chemotactic activity of polymorphonuclear leukocytes toward sterile autologous serum and used a collection chamber placed on the forearm of CS-treated psoriatic patients, indicated a depressed chemotaxis correlating with clinical improvement of psoriasis. (This loss of responsiveness suggests that the granulocytes were chronically activated, as demonstrated in vitro, and which is known to be the case in chronic inflammatory diseases). In a rigorous test system in vitro preventing CS carryover, it was found that CS failed to influence antigen presentation to lysozyme-specific T cell hybridomas (677). Others demonstrated, also in a careful in vitro study avoiding drug carry-over, that CS directly inhibited accessory cell functions of epidermal Langerhans cells (302). However, using a very different murine DTH model in vivo and cell transfer experiments, Knight et al. (529) presented clear evidence that CS (100 mg/kg/d orally on days −1 and 0) prevented acquisition and presentation of antigen by dendritic cells.

Regarding the overall concept of CS's usefulness in massive thermal injury, there seem to be no adverse effects of CS on the ability of either animals or humans to respond to bacterial infections. Topically infected rats (with spray) as well as induced systemic infections by injection of *Pseudomonas aeruginosa* bacteria produced the desired septicemia in CS-treated and skin allografted animals, but without adverse effect on white blood cell responses and animal mortality (80).

The first part on bacterial infections starts with the facultative intracellular bacteria. In these infections there is a preponderant involvement of T-cell components as well as of unspecific macrophage activation which often leads to granuloma formation. The effects of CS in these conditions depend on the balance of immunity between the host and its parasites. The second part deals with the extracellular bacteria. The control of this type of infection and the state of resistance depends mainly on humoral factors (B-cell activation, antibody production, complement etc.). The pathway by which CS may interfere in typical models like pyelonephritis remains unclear, except possibly by interfering with T_H cell activation of B cells.

b. FACULATIVE INTRACELLULAR BACTERIA. ***Chlamydia.*** The guinea pig infected with guinea pig inclusion conjunctivitis (GPIC) agent is a good model for trachoma (chlamydial infection). Following primary ocular infection, animals have microbiological immunity to ocular reinfection. The effects of CS (10 mg/kg/d intraperitoneally) on eye and skin responses to GPIC agent in previously infected animals were studied. CS treatment on days −1 to 6 affected the ocular response to reinfection with GPIC agent by prolonging the duration of GPIC agent in conjunctival cells, the severe ocular inflammatory response, and the polymorphonuclear and mononuclear cell responses. However, the severity of the inflammatory response and the number of inflammatory cells were the same in CS-treated and untreated animals, indicating that cell function was inhibited by CS. This treatment produced therapeutic CS levels (> 200 ng/ml) in the tears of uninfected guinea pigs. CS treatment on days −1 to 3 had no effect on the extent of dermal induration or the increase in skin-thickness, but significantly reduced the extent of erythema in previously infected animals. This confirms that CS did not affect recruitment of inflammatory cells to the site of the reaction, but inhibited their function. These findings suggest that CS impairs the ocular cell-mediated response to GPIC agent which results in prolonged ocular chlamydial infection (663).

Nocardia. *Nocardia asteroides* pneumonia was induced in BALB/c mice by intranasal inoculation and its course studied by histology, bronchoalveolar lavage, and

quantification of colony-forming units in lungs. Mice with intact host defences had an initial inflammatory response, consisting mostly of neutrophils, followed by a mononuclear cell infiltrate, and the *Nocardiae* were eradicated during the 7 days after inoculation. Daily intraperitoneal injections of CS (75 mg/kg/d), beginning 1 day before *Nocardia* infection, markedly reduced the DL50 for *Nocardia* pneumonia: From 6.5×10^7 to 9.3×10^3. Mice in which cell-mediated immunity was impaired with CS or cortisone acetate developed abscesses; neutrophils were abundant, but *Nocardiae* proliferated. CS concentrations ranging from 1 ng/ml to 100 μg/ml did not influence the growth of *N. asteroides* in vitro (270). These observations add evidence that both neutrophils and cell-mediated immunity are necessary for optimal control of nocardiosis.

Mycobacteria. Cell-mediated immunity is largely responsible for host defence to infection with intracellular pathogens like *Mycobacterium* species and *Listeria monocytogenes*. It is obvious that much interest has been focused on the association between this type of infection and CS therapy. The effect of increasing doses of CS (12.5 to 200 mg/kg/d subcutaneously from day −1 to day 20) administered to mice infected intravenously with *Mycobacterium bovis* BCG (Bacille Calmette-Guérin) was investigated. Development of both tuberculin DTH and acquired antituberculous resistance was suppressed in a dose-dependent manner. Daily doses of 100 mg/kg prevented the reduction of the BCG counts within lungs, liver, and spleen. This effect was associated with lowered non-specific resistance to a *Listeria monocytogenes* challenge and a decline in specific protective immunity adoptively transferred to naive recipients. CS treatment had no effect on antilisterial activity by activated macrophages or on the antituberculous immunity expressed by specific memory T cells. CS treatment inhibited the ability of BCG-vaccinated mice to produce γIFN after a secondary stimulation with live BCG or with LPS. Spleen cells from BCG-infected mice, which were exposed to daily CS treatment, showed reduced γIFN production in response to PPD or Con A stimulation, suggesting that the immunosuppressive effect of CS on BCG-infected mice was expressed by inhibiting the development of effector T cells responsible for the production of γIFN (901). Late treatment of chronically infected mice with an immunosuppressive regimen of CS (75 mg/kg/d subcutaneously on days 61 to 89) provided further evidence of the importance of T cells in controlling the growth of *Mycobacterium intracellulare* in the normal host (902).

The effect of CS on the recruitment of monocyte/macrophages to the site of granuloma formation in response to the bacillus Calmette-Guérin (BCG) and the maturation of these cells to epithelioid cells was investigated. The injection of CD5 T lymphocytes intradermally into the ear of guinea pigs leads to the formation in the draining lymph node of granulomata containing epithelioid cells with rough endoplasmic reticulum and an absence of phagocytosed material. CS was given at two doses, 25 mg/kg/d orally or 50 intraperitoneally, starting from the day of immunisation and daily thereafter. BCG granulomata in CS-treated animals contained mononuclear phagocytes with no rough endoplasmic reticulum, but fragments of phagocytosed organisms. The higher dose completely suppressed the DTH to PPD but the lower dose did not, although it had a profound inhibitory effect on epithelioid cell formation. Neither dose of CS had any effect on the recruitment of mononuclear cells to the site of granuloma formation or on the bactericidal action of macrophages (369). It had previously been shown that treatment of rats with CS (20 mg/kg/d orally during the whole experiment) prevented the formation of epithelioid cell granuloma after subcutaneous injection of apathogenic *Mycobacteria* and the development of caseating necrosis. Macrophages accumulated at the local site and in the regional lymph node and contained numerous, mostly well-preserved *Mycobacteria*. The results suggested that epithelioid cell granuloma formation in vivo is i) a T-lymphocyte dependent phenomenon and ii) that inhibition of T-lymphocyte activation by CS prevented harmful effects of mycobacterial infection in this model (677).

Takizawa et al. (905) also induced granulomatous pneumonitis by intravenous injection of BCG in the mouse and observed an increase in CD5 T lymphocytes in bronchoalveolar lavage fluid in non-treated mice. CS treatment (50 mg/kg/d orally from day −5 until day +5 of BCG injection) clearly suppressed development of granuloma. The bronchoalveolar lavage cell count and cell population became almost the same as those in naive animals, which finding suggests the important role of CD5 T cells in the formation of granulomata. The same authors (904) have also induced hypersensitivity pneumonitis with transnasally administered *Thermoactinomyces vulgaris* bacilli in mice. CS (50 mg/kg/d orally) administered throughout the course of bacilli inoculations (3 weeks) markedly suppressed granulomatous pneumonitis and the increase of Thy 1.2^+ lymphocytes present in the bronchoalveolar lavage fluid. When CS was given only during the first half period of the infection treatment, suppression of the disease was minimal, but when CS was administered in the latter half, both the pneumonitis lesions and the increase in bronchoalveolar lavage cell numbers were significantly suppressed.

Erythema nodosum leprosum (ENL) is a reactional state of lepromatous leprosy in which the loss of suppressor cell function, decrease in suppressor cell numbers, and increase of IL-2 production are observed. The possibility that CS might oppose these immune responses was tested in vitro by measuring the effect of CS on *Mycobacterium leprae*-triggered suppressor cells. In twenty-four of twenty-five patients with ENL, suppres-

sor cell activity was restored by CS (50 ng/ml) (949). Preliminary clinical trials indicate a beneficial therapeutic effect associated with increased T_s cells in lesions of chronic ENL patients (649).

Listeria. The effects of CS and cortisone acetate on listeriosis were compared in normal and nude mice. Resistance of mice to an intravenous challenge with *L. monocytogenes* consists of two phases. In the first, *Listeria* multiply in the liver and spleen of nonimmune mice and this multiplication is limited by nonimmune macrophages. In the second phase, beginning 2 days after challenge, T cell-mediated resistance develops, bacterial multiplication stops, and organisms are eliminated by immune-activated macrophages. In contrast to normal mice, nude mice limit listeriosis to a chronic disease, and cortisone, by abolishing early resistance, leads to overwhelming infection and death. Mice treated with cortisone acetate died during the early phase because of inhibition of the antimicrobial activity of nonimmune macrophages. Accordingly its effect was similar in athymic and normal mice. In contrast, immunosuppression with CS (100 to 300 mg/kg/d intramuscularly from 2 days before challenge until sacrifice) did not affect early resistance, but induced overwhelming fatal disease in the later phase when control mice began to acquire resistance. CS did not change the course of listeriosis in nude mice, confirming its specificity for T cell-dependent immunity (825).

The effects of CS on primary and secondary (14 days after primary) intravenous infection of mice with *L. monocytogenes* were studied. When CS was given in an oral dose (100 mg/kg/d starting day −2 until day 5), it was found to inhibit the development of protective immunity after primary infection as well as the expression of acquired immunity to challenge infection. DTH expression was also impaired. When the cellular immune system was functionally intact, the formation of granulomata composed of macrophages and lymphocytes enabled the animals to overcome listeriosis. In CS-treated mice protective granulomatous reaction during secondary infection did not occur. Instead numerous necropurulent lesions developed in the reticuloendothelial organs, such as spleen and liver, of animals unable to control the lethal infection (892).

The effect of CS on immunity to *L. monocytogenes* was further investigated in unprimed and primed mice (intravenous challenge). Different treatment protocols were followed to evaluate the time dependence of CS-mediated immune suppression and the effect of CS on immunological memory to *L. monocytogenes*. The depressing effect of CS (60 mg/kg/d intraperitoneally) was observed only during and after T cell-mediated immunity, whereas early resistance exerted by macrophages 6 and 70 minutes after challenge remained unaffected. CS suppressed efficient elimination of *Listeria* even when given after day 3 of a primary infection. CS suppressed antibacterial resistance in mice primed at various times before challenge; suppression of protection was time-dependent and was virtually complete in livers, whereas CS-resistant memory persisted in spleens for up to 10 months (446).

It has been reported that purified rRNA from *Listeria monocytogenes* or *Pseudomonas aeruginosa* injected in combination with dimethyldioctadecylammonium bromide (DDA), protects mice nonspecifically against a lethal challenge of various extra- and intracellular bacteria. Vaccination of mice with listerial rRNA-DDA resulted in activation of fixed-tissue macrophages, as measured by an enhanced in vivo *L. monocytogenes* killing in spleen and liver. Evidence was found that macrophage activation by vaccination with rRNA-DDA occurred by a T cell-independent mechanism, since i) treatment of mice with CS (20 mg/kg/d intraperitoneally starting 1 day before vaccination) had no effect on the enhanced *Listeria* killing induced with rRNA-DDA, and ii) in vitro exposure of rRNA-DDA to spleen cell cultures did not give rise to any lymphocyte proliferation (955).

The previous data generally support the conclusion that CS acts probably specifically on the T-cell compartment, sparing residual and activated macrophages from direct suppressive effects. CS-induced suppression in this listeriosis model may be due to reduced release of lymphokines, resulting in the inhibition of T-cell proliferation and the release by T cells of macrophage-activating lymphokines. Thus, the significance of IFNs (interferons) induced by *L. monocytogenes* in the anti-listerial defence mechanism was studied in mice. CS (60 to 100 mg/kg/d intraperitoneally on days −1, 0 and 1) had no effect on αIFN production that was induced in the bloodstream after intravenous infection, whereas γIFN that was induced in the bloodstream of control mice 6 hours after stimulation with specific antigen in the late phase of infection was suppressed in CS-treated mice, depending on the CS dose injected. The decrease in γIFN production caused an increase in bacterial growth in spleens and livers of CS-treated animals. Furthermore, administration of CS (80 to 100 mg/kg/d) resulted in fatal listeriosis, even though the dose was not lethal for normal mice. The injection of murine rγIFN on day 0 of infection with *Listeria* prevented CS-treated mice from developing fatal listeriosis and restored their ability to produce γIFN in the bloodstream, in response to specific antigen in the late phase of infection (686). It has, moreover, been confirmed in vitro that CS inhibits the production of γIFN by mouse or human T cells, but appears to have no effect on the production of α/βIFN by virus-infected cells or on the antiviral action of already produced γIFN and α/βIFN (486).

Salmonella. Since cell-mediated immunity contributes a major part to the acquired resistance of mice to infection with *Salmonella typhimurium*, which is able to multiply intracellularly, the influence of CS in this infection model was studied. After a primary infection of

mice with *S. typhimurium*, the initial, non-specific phase of resistance was not affected by oral treatment with CS (100 mg/kg/d on days −2, −1, 0, and 1). Once the untreated mice had survived the primary infection, they were resistant to a challenge infection. Treatment of such animals with CS (100 mg/kg/d orally) beginning 2 days before challenge infection and given up to day 9 reduced protection considerably, but not completely. In contrast, mice which had been treated with CS 14 days before challenge infection had completely restored immune function (426).

DTH can be induced in mice by infection with *Salmonella enteritidis*. The cells which transfer this state of hypersensitivity to untreated recipients are nonadherent CD5 T cells which are H-2 complex restricted. In this system CS prevented the elicitation of DTH by the bacterial antigen and the transfer of hypersensitivity to recipient mice (31).

c. EXTRACELLULAR BACTERIA. ***Propionibacteria.*** The protective effect of CS was demonstrated on experimentally induced acute hepatic injury with heat-killed *Propionibacterium acnes* and a small amount of endotoxin (LPS) injected intravenously in mice at a week's interval. CS (25 mg/kg/d) was given orally on days 5, 6, and 7 after inoculation with bacteria. Most of the control mice died of massive hepatic cell necrosis, whereas drug-treated animals showed both a markedly improved survival rate and rather limited histopathological changes. CS was thought to exert its protective effect by inhibiting the activation of liver adherent cells (macrophages) and suppressing the release of the cytotoxic factor (656).

Streptococcus. Yocum et al. (1005) have investigated the effect of CS on hepatic granulomata in LEW rats with streptococcal cell wall-induced arthritis. Intraperitoneal injection with group A streptococcal cell walls leads over the following 3 to 4 weeks to the concentration of antigens within granulomata in the liver. CS (25 mg/kg/d intramuscularly) administered on days −1 until 12, totally inhibited granuloma formation. There was, however, a low-grade infiltration of mononuclear cells, primarily in the periportal and perivenular areas. A similar drug treatment during the chronic stage (days 7 to 42) also suppressed histological appearance of liver granuloma. In contrast, non-treated nude rats did not develop hepatic granuloma, indicating that this is a T cell-mediated chronic process.

Staphylococcus. A study in mice demonstrated that daily intraperitoneal treatment with CS (15 mg/kg/d) for 1 to 2 weeks delayed the clearance of *Staphylococcus aureus* from the lower respiratory tract. When mice were additionally treated with oral prednisolone, clearance was further inhibited. However, CS did not alter the clearance of *Pseudomonas aeruginosa* or prevent the neutrophil influx observed after challenge with gram-negative bacteria (714).

Pyelonephritis. Pyogenic extracellular organisms are not usually affected by agents that depress only cell-mediated immunity. To determine whether CS additionally affects other defence mechanisms, its effect on renal infection in a rat model of pyelonephritis was investigated. CS was administered intramuscularly with a loading dose of 100 mg/kg, followed by 3, 6, 12.5, 25, or 50 mg/kg/q.o.d. until sacrifice. CS treatment exacerbated acute experimentally induced infection (direct inoculation of *Escherichia coli* into the renal parenchyma) and the higher doses of 12.5 to 50 mg/kg led to a dose-dependent marked increase in bacterial numbers. When renal lesions in untreated controls were resolving, CS-treated animals exhibited purulent abscesses involving large surface areas of the kidneys. However, leukocyte numbers were not reduced (actually increased) in the drug-treated hosts (646). Experiments to assess the relevance of the route of drug administration were carried out using three localised infections in rats: *E. coli*-induced acute pyelonephritis, intradermal injection of yeast cells in the footpad, and *Candida albicans* infection of the hind footpad. The results demonstrated that CS (25 mg/kg/q.o.d.) provoked massive infections of the kidney, subcutaneous tissue, and footpad, irrespective of the route (subcutaneous, intraperitoneal, intramuscular or oral) by which it was administered (271). Rats were depleted of individual cellular components to determine the effects of these manipulations on cellular defence mechanisms in acute and chronic pyelonephritis. T-lymphocyte depletion and macrophage blockade did not affect the course of either acute or chronic infection. Antineutrophil serum led to a 1000-fold increase in bacterial numbers in the acute but not in the chronic phase of pyelonephritis. CS treatment had a dramatic effect on the pathology and bacteriological status of both acute and chronic pyelonephritis (652).

Additional experiments were performed to identify the host defence mechanisms affected by CS which lead to exacerbation of this renal infection model. An athymic rat strain was used to rule out cell-mediated immunity as a relevant host defence component. When CS (3 to 50 mg/kg/q.o.d. intramuscularly) was administered to athymic animals, renal infection was exacerbated. The results do not support an effect on non-cellular defence mechanism; rather they indicate a depression of either cellular defences or of a specific cellular component. The pathway affected by CS has not yet been identified (651).

d. CONCLUSIONS. In conclusion, CS does not appear to directly exert major effects on macrophages and polymorphonuclear leukocytes. Phagocytes from animals treated with CS retain intact phagocytic and bactericidal activity and produce normal levels of αIFN. Despite some controversial findings on chemotactic migration, CS therapy does not impair inflammatory cell recruitment at the reaction site; however, it may affect their effector function. This inhibitory effect may be lymphokine-mediated and result from suppression of effector T cells.

Of particular importance are all those factors inducing enhanced activity in monocyte/macrophages. Since protective immunity or acquired resistance to infections are predominantly cell-mediated phenomena, it is evident that administration of CS shortly before and/or at the time of challenge infection will result in markedly increased susceptibility to infective agents. Finally, it will be interesting to define the cellular component of host defence suppressed by CS in the rat pyelonephritis model induced with *E. coli* (650, 271, 652, 651).

4. Effects on fungi. CS has been reported to exhibit a rather narrow spectrum of antifungal activity which includes a few species among the yeasts and some strains of mucorales, ascomycetes and fungi imperfecti (232). Growth inhibition of these organisms is effected by most cyclosporins, but does not correlate with immunosuppressive activity (see table 4 in reference 969).

The systemic effect of CS in the rat model of *Candida albicans* keratitis resembles that of other fungus-derived antibiotics. In contrast to its preventive efficacy, CS failed to reduce the number of organisms in homogenates of corneal tissue when applied topically in the established disease (737).

Two groups have published the anticoccidioidal activity of CS in vivo as well as in vitro in experimental murine coccidioidomycosis (517, 424). Three antifungal CS derivatives that are equipotent but with negligible immunosuppressive activity, certainly in the doses used in the above model, have been discovered by Fierer and Kirkland (San Diego; personal communication).

Cryptococcus neoformans is a frequent opportunistic infectious agent in patients with impaired T-cell function. Prophylactic treatment of mice with CS (20 mg/kg/d subcutaneously beginning at various times after infection) enhanced survival after inoculation of *C. neoformans* by both the intratracheal and intravenous route (659). In an established, intratracheally induced infection, CS therapy markedly prolonged survival of both normal and athymic nude mice. The drug exerted a potent anti-cryptococcal effect in the lung, liver, spleen, and kidney and appeared to prevent dissemination of the organism to the meninges. However, when mice were inoculated intravenously or in the brain, CS was unable to reduce cryptococcal replication in the central nervous system (660). Likewise, CS (12 mg/kg/d intravenously from day −1 to 7) depressed the highly effective defense mechanisms of normal rabbits against intracerebrally inoculated *C. neoformans* (754). CS treatment did not reduce the number of leukocytes found in the cerebrospinal fluid of rabbits suffering from cryptococcal meningitis, and activation of these macrophages was little affected (755). These findings are not surprising considering that less than 5% of the blood level passes the brain-blood barrier (575). Finally, it has been reported that CS concentrations as low as 5 ng/ml inhibited proliferation of T cells from the peripheral blood of nonimmune control subjects to cryptococcal antigens. Around 30 ng/ml were required to inhibit secondary lymphocyte responses of immune subjects and IL-2 production. CS also depressed IL-2R expression during in vitro primary responses to *Cryptococcus* (647).

5. Effects on parasites. In the beginning CS was used in parasitology as a T-lymphocyte depleting agent in order to find a solution to the problem of the involvement of T cells or antibodies in several experimental systems of parasitic infections (102, 876, 996). Unexpectedly, it was discovered that CS had an intrinsic antiparasitic effect in the rodent models of malaria (921, 696, 619, 679), leishmaniasis (58, 506, 876, 875, 86), schistosomiasis (133, 119, 704, 120, 874, 161, 160, 763), some cestode infections (161a), filariasis (121, 966, 1016), strongyloidiasis (824), and trichinosis (90; A. Perrudet-Badoux, Paris: personal communication) (table 8). There is some evidence suggesting an anti-toxoplasma activity of CS, particularly in vitro (623, 597), but a negative tendency was shown in a rat infection provocation model with *Pneumocystis carinii* (447) and possibly confirmed in a small group of patients (626), and with high doses CS had only marginal effects in *Giardia muris* (61). In contrast, clear exacerbation was demonstrated when *Trypanosoma cruzi* infected mice were treated with CS (440, 507, 624, 787) as well as reactivation of chronic Chagas' disease in dogs immunosuppressed with CS after experimental cardiac transplantation (118). Depending on the model and the parasite used, the compound has preventive and/or therapeutic activity, which may be directed against both immature and adult organisms. CS preferentially affects female worms, causing sterility, and its antiparasitic effects often seem to be host-mediated rather than directed against the parasite itself, as can be demonstrated in vitro. It is not understood how the

TABLE 8

Fungicidal and antiparasitic effects of CS and some derivatives in experimental models[a]

Exp. model	Species	Effect	Derivatives (active)
		Positive	
Cryptococcosis	Mouse	Prev/ther	
Coccidioidomycosis	Mouse	Prev/ther	H7-94
Malaria	Mouse	Prev/ther	B5-49; C5-34; H7-94
Cerebral malaria	Mouse	Prev	H7-94; C5-34
Leishmaniasis	Mouse	Prev	B5-49
Coccidiosis	Chicken	Prev	
Schistosomiasis	Mouse	Prev/ther	B5-49; C5-34
Filariasis	Mastomys	Prev/ther	B5-49; G7-53
Strongyloidosis	Rat (dog)	Prev/ther	
Trichinosis	Mouse	Prev	
		None	
Toxoplasmosis	Mouse	Prev	
Giardiasis	Mouse	Prev/ther	
		Exacerbation	
Pneumocystis	Rat	Provocation	
Leishmaniasis	Mouse	Ther	
Trypanosomiasis	Mouse	Prev/ther	

[a] For Reference, see text.

antiparasitic effects of CS are mediated (927, 923). The available results (published and unpublished) for malaria and schistosomiasis have been summarized in table 9.

Unfortunately, only two experiments were performed in higher species. The protective effect of CS against the fulminant form of strongyloidiasis was confirmed in a canine model (824). Of particular interest is the limited study on CS's potential therapeutic value in the owl monkey model of human *P. falciparum* malaria (176). Treatment was instituted when parasitaemia reached 0.1 to 1.0% between days 10 to 25 post infection. Parasitaemias in all five monkeys began to fall within 2 to 3 days after the first dose of CS (50 mg/kg) was given subcutaneously. In four monkeys circulating parasites became undetectable between 7 to 11 days; one animal died suddenly while its parasitaemia was still falling. All monkeys developed *P. falciparum*-specific antibodies by day 14. Relapses occurred in two monkeys within 3 days of discontinuing CS treatment. All but one owl monkey died from what seemed, at autopsy, to be renal failure due to the combined effects of malaria and CS.

It is difficult to envisage the use of CS proper as an antiparasitic drug. Since there is evidence that its antiparasitic properties are not linked to its immunosuppressive activity, the search for CS derivatives with antiparasitic properties, but without immunosuppressive activity is underway. The majority of our over seven hundred derivatives were screened for antimalarial activity (using the Rane test, 774) and the top dozen compounds were further selected for significantly better toxicological tolerability than CS. Several of these derivatives were sent on request to outside laboratories to be evaluated in their experimental assays and some of the ensuing results have been published.

Scheibel et al. (827) have reported that the in vitro 50% inhibitory concentration for *P. falciparum* of two CS derivatives was at least 3 times lower than that of CS itself. An important finding was that a short course of very low doses of CS and of a CS (H7-94) and a (Thr^2)-CS (C5-34) derivative was preventing the occurrence of cerebral malaria in all mice treated (338). Paradoxically, higher drug doses were parasiticidal but did not afford protection against the neurological symptoms. CS and a dihydro-CS (B5-49) derivative given prophylactically for 7 days were effective in enhancing resistance to *Leishmania major* infected mice but caused some exacerbation when administered to animals with established lesions (58).

The puzzling, long-term protective effect of CS in murine schistosomiasis, first described by Bout et al. (120) and later confirmed by Thompson et al. (927) (see fig. 11), has also been reproduced with the dihydro-CS (B5-49) derivative (same authors; personal communications). Extensive studies comparing the two compounds showed that they exhibited similar efficacy against *S. mansoni* (161, 160). Moreover, a clear synergism of both compounds with amoscanate, using sub-therapeutic doses, has been reported by Bueding (133; E. Bueding, Baltimore, personal communication).

Concerning the antifilarial effect of CS, Litomosoides carinii-infected *Mastomys natalensis* were treated 85 days post infection with CS or one of eight derivatives for 5 days. CS, a dihydro-CS (B5-49) and a dihydro-(Thr^2)-CS (G7-53) derivative reduced the parasitaemia level in a dose-dependent manner, beginning 3 weeks after the first oral drug administration. The other derivatives were less or only marginally effective. None of the drugs affected the number or mobility of adult worms. However, the number of intrauterine stages was pathologically altered (1016). The antiparasitic effects appear independent of the immunosuppressive activity of the derivatives.

Depending on treatment protocol, CS treatment has a differential effect on the course of coccidiosis in the chicken. Daily administration of CS (100 mg/kg/d orally) for 7 days beginning 1 day before primary infection with

TABLE 9

Summary of antiparasitic effects of CS in rodent malaria and schistosomiasis

Antimalarial effect
• Prevention of infection, leading to resistance to subsequent infection
• Cure followed by self-limiting recrudescence (drug-resistant low parasitaemia)
• Cerebral malaria: preventive effect at extremely low oral doses (1 mg/kg/day)
• Drug effective in lethally irradiated mice
• *Plasmodium falciparum* inhibited in vitro: 0.1 to 10 μg/ml/48 h
• Selective reduction of ring-stages (merozoites)
• No cross-resistance with chloroquine
• Synergism with pyrimethamine (schizonticidal)
Antischistosomal effect
• Reproducible longterm protective effect
• Transfer of protection with spleen cells ineffective
• Therapeutic effect in *S. mansoni* infected mice
• Similar therapeutic effect in nude mice
• Host-mediated reduction in globinase activity and protein content in female worms leading to sterility
• No direct in vitro activity
• Synergism with amoscanate

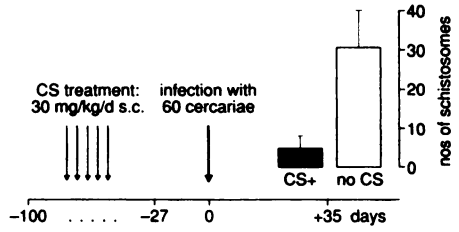

FIG. 11. Long-term protective effect of CS in murine schistosomiasis. Normal adult mice were treated with subcutaneous doses of 30 mg/kg/d subcutaneously or vehicle only on 5 consecutive days. This treatment occurred sometimes between days −100 to −27 with respect to the day 0 of infection, when the mice were infected transcutaneously with 60 cercariae. No further drug was administered and 35 days later the numbers of schistosomes recovered by aortic or portal vein perfusion were determined in both groups.

the intestinal protozoa *Eimeria tenella* enhanced disease resistance, whereas a single dose of CS given before primary infection increased disease susceptibility compared with that of untreated controls. CS treatment of immune chickens at the time of secondary infection abrogated their resistance to reinfection despite high levels of Coccidia-specific secretory IgA and serum IgG antibodies. These findings indicate that cell-mediated immunity plays a major role in host protective immunity to *E. tenella*, since CS was shown to exert no direct toxicity on the parasites (585).

Finally, the insecticidal activity of cyclosporins on mosquito larvae was recently reported (980). The fungal species of Tolypocladium, which contain a larvicidal factor named tolypin, have been observed to infect mosquito larvae in natural habitats and to cause their death by the development of the fungus inside the haemocoel of the insect. Since T. inflatum also produces CS, its insecticidal activity was evaluated after 48 h of incubation with larvae and was determined as having a LC_{50} = 0.6 μg/ml. The general role of these toxic metabolites seems to protect the fungal fructifications against attacks of mycophilic insects and mites. Cyclosporins remain in hyphal cells and are released only when they break and autolyse in late periods of fermentation.

Several laboratories around the world are actively pursuing the study of potentially new antiparasitic effects of CS and some of its derivatives and also investigating their activity in other parasitic models. The study of CS analogues possessing antiparasitic properties but devoid of immunosuppressive activity is perhaps not of immediate practical value. However, both their direct and indirect, i.e., host-mediated antiparasitic effects might uncover new insights into the body's defence mechanism against certain parasites. The host-mediated antiparasitic action and the resistance to later reinfection that they confer on the treated animals will be the effects that deserve the most scientific attention.

Acknowledgments. Many colleagues have contributed stimulating ideas and permitted the use of their partly unpublished results. For this, and for their unfailing support in the realisation of this project, especially when my courage was at a low ebb, I offer them my heartfelt thanks.

I should like to mention in detail the following most valuable contributions: Dr. G. G. B. Klaus (London) to Antibody-mediated immunity; Dr. Z. A. Nagy (Sandoz, Basel) to Cell-mediated immunity; Dr. C. J. Green (London) to Experimental organ and tissue grafting; Dr. A. Gratwohl (Basel) to Bone marrow grafts; Dr. J. R. Batchelor (London) to Induction of unresponsiveness; Dr. K. J. Lafferty (Denver) to Autoimmunity; and Dr. R. M. Zinkernagel (Zurich) to Interactions of CS with defence mechanisms against infections.

Finally, I am particularly grateful to Mrs. S. Burkhardt for maintaining her good humour during the many hours she spent typing the manuscript and her patience with the manyfold corrections she was asked to make.

REFERENCES

1. Abecassis, M., Falk, J., Makowka, L., Dindzans, V., Falk, R., and Levy, G.: Cyclosporine A fails to alter innate host resistance to murine hepatitis virus strain 3 infection in A/J mice. Transplant. Proc. **19:** 1214–1217, 1987.
2. Adachi, H., Rosengard, B. R., Hutchins, G. M., Hall, T. S., Baumgartner, W. A., Borkon, A. M., and Reitz, B. A.: Effects of cyclosporine, aspirin, and cobra venom factor on discordant cardiac xenograft survival in rats. Transplant. Proc. **19:** 1145–1148, 1987.
3. Ades, E. W., Hinson, A., and Butler, L. D.: Natural cytolytic activity in mice with natural or induced cellular defects. I. Differential ability of in vitro interleukin-2 addition to augment natural cytolytic function. Cell. Immunol. **101:** 15–23, 1986.
4. Aeder, M. I., Sutherland, D. E. R., and Najarian, J. S.: Cyclosporine in combination with azathioprine or antilymphocyte globulin for immunosuppression in a canine renal allograft model. Surg. Forum. **34:** 384–387, 1983.
5. Alberti, S., Boraschi, D., Luini, W., and Tagliabue, A.: Effects of in vivo treatments with cyclosporin-A on mouse cell-mediated immune responses. Int. J. Immunopharmacol. **3:** 357–364, 1981.
6. Albrechtsen, D., Flatmark, A., Lundgren, G., Brynger, H., Frödin, L., Groth, C. G., and Gäbel, H.: Retransplantation of renal grafts: prognostic influence of previous transplantation. Transplant. Proc. **19:** 3619–3621, 1987.
7. Alcocer-Varela, J., Vidaller, A., Llorente, L., and Alarcon-Segovia, D.: Presence of an IL-3-producing suppressor T cell resistant to cyclosporin A in the peripheral blood of patients with systematic lupus erythematosus. Clin. Exp. Immunol. **73:** 424–429, 1988.
8. Aldridge, R. D., and Thompson, A. W.: Paradoxical augmentation of tuberculin-like hypersensitivity, but not Jones-Mote or contact hypersensitivity, in cyclosporin A treated guinea pigs. Int. Arch. Allergy Appl. Immunol. **79:** 225–230, 1986.
9. Aldridge, R. D., and Thompson, A. W.: Factors influencing the enhancement of delayed-type hypersensitivity to ovalbumin by cyclosporin A in the guinea pig: possible role of suppressor cells. Int. Arch. Allergy Appl. Immunol. **81:** 17–23, 1986.
10. Aldridge, R. D., Thompson, A. W., Rankin, R., Whiting, P. H., Cunningham, C., and Simpson, J. G.: Inhibition of contact sensitivity reactions to DNFB by topical cyclosporin application in the guinea-pig. Clin. Exp. Immunol. **59:** 23–28, 1985.
11. Alejandro, R., Cutfield, R., Shienvold, F. L., Latif, Z., and Mintz, D. H.: Successful long-term survival of pancreatic islet allografts in spontaneous or pancreatectomy-induced diabetes in dogs: cyclosporine-induced immune unresponsiveness. Diabetes **34:** 825–828, 1985.
12. Alejandro, R., Latif, Z., Noel, J., Shienvold, F. L., and Mintz, D. H.: Effect of anti-Ia antibodies, culture and cyclosporine on prolongation of canine islet allograft survival. Diabetes **36:** 269–273, 1987.
13. Alexander, P.: Cyclosporin A and the growth, dissemination and induction of tumours. *In* Cyclosporin A, ed. by D. J. G. White, pp. 299–305, Elsevier Biomed. Press, Amsterdam, 1982.
14. Altmann, D. M., and Blyth, W. A.: The effects of cyclosporin A on the induction expression and regulation of the immune response to herpes simplex virus. Clin. Exp. Immunol. **59:** 17–22, 1985.
15. Amemiya, H., Suzuchi, S., Niiya, S., Watanabe, H., and Kotake, T.: Synergistic effect of cyclosporine and mizoribine on survival of dog renal allografts. Transplantation **46:** 768–771, 1988.
16. Amlot, P. L., Hayes, A. E., Gray, D., Gordon-Smith, E. C., and Humphrey, J. H.: Human immune responses in vivo to protein (KLH) and polysaccharide (DNP/Ficoll) neoantigens: normal subjects compared with bone marrow transplant patients on cyclosporine. Clin. Exp. Immunol. **64:** 125–135, 1986.
17. Amor, B., and Dougados, M.: Cyclosporine: therapeutic effects in rheumatic diseases. Transplant. Proc. **20** (suppl. 4): 218–223, 1988.
18. Anderson, C.: The effect of some immunomodulating agents on the sensitisation phase of experimental contact allergic reactions. Int. Arch. Allergy Appl. Immunol. **79:** 127–131, 1986.
19. Anderson, C., and Groth, O.: Suppression of the allergic contact reaction in the guinea pig by cyclosporin A. Int. Arch. Allergy Appl. Immunol. **78:** 396–400, 1985.
20. Aoki, I., Ishii, N., Minami, M., Misugi, K., Dorf, M. E., and Okuda, K.: Analysis of alloantigen-specific suppressor T cells. Transplantation **43:** 888–892, 1987.
21. Appelbaum, F. R., Deeg, H. J., Storb, R., Self, S., Graham, T. C., Sale, G. E., and Weiden, P. L.: Marrow transplant studies in dogs with malignant lymphoma. Transplantation **39:** 499–504, 1985.
22. Aramant, R., and Turner, J. E.: Cross-species grafting of embryonic mouse and grafting of older rat retinas into the lesioned adult rat eye: the importance of cyclosporin A for survival. Dev. Brain Res. **41:** 303–307, 1988.
23. Araujo, J. L., Kupiec-Weglinski, J. W., Araneda, D., Towpik, E., Heidecke, C. D., Williams, J. M. and Tilney, N. L.: Phenotype, activation status, and suppressor activity of host lymphocytes during acute rejection and after cyclosporine-induced unresponsiveness of rat cardiac allografts. Transplantation **40:** 278–284, 1985.
24. Armerding, D.: Selective induction of immunological tolerance in antiviral T killer cells of inbred mice after treatment with cyclosporin A. Infect. Immun. **32:** 1164–1175, 1981.
25. Armerding, D., Scriba, M., Hren, A., and Rossiter, H.: Modulation by cyclosporin A of murine natural resistance against herpes simplex virus

infection. I. Interference with the susceptibility to herpes simplex virus infection. Antiviral Res. **2:** 3–11, 1982.
26. ARMERDING, D., SCRIBA, M., KIRCHNER, H., HREN, A., AND ROSSITER, H.: Modulation by cyclosporin A of murine natural resistance against herpes simplex virus infection. II. Influence on the HSV-induced natural killer cell responses, macrophage activities and interferon levels. Antiviral Res. **2:** 13–26, 1982.
27. ARNAUD-BATTANDIER, F., AYNAUD, J. M., BERNARD, S., LABONNARDIÈRE, C., REVILLON, Y., AND RICOUR, C.: Allotransplantation du grêle chez le porc sous cyclosporine: étude in vivo de la barrière immune muqueuse. Gastroenterol. Clin. Biol. **9:** 95A, (abstract) 1985.
28. ARNAUD-BATTANDIER, F., SALMON, H., VAIMAN, M., REVILLON, Y., GALLIX, P., OLIVIER, M., AND RICOUR, C.: Small intestinal allotransplantation in swine with cyclosporine treatment: studies of the intestinal lymphoid populations. Transplant. Proc. **17:** 1440–1441, 1985.
29. ATEN, J., BOSMAN, C. B., DE HEER, E., HEODEMAEKER, P. J., AND WEENING, J. J.: Cyclosporin A induces long-term unresponsiveness in mercuric chloride-induced autoimmune glomerulonephritis. Clin. Exp. Immunol. **73:** 307–311, 1988.
30. ATKINSON, K.: Cyclosporin in bone marrow transplantation. Bone Marrow Transplant. **1:** 265–270, 1987.
31. ATTRIDGE, S. R., AND KOTLARSKI, I.: Local transfer of delayed-type hypersensitivity after Salmonella enteritis infection in mice. Infect. Immun. **50:** 807–812, 1985.
32. AUCHINCLOSS, H.: Xenogeneic transplantation. Transplantation **46:** 1–20, 1988.
33. AUCHINCLOSS, H., AND WINN, H. J.: Murine CD8$^+$ T cell helper function is particularly sensitive to cyclosporine suppression in vivo. J. Immunol. **143:** 3940–3943, 1989.
34. AZZAM, N. A., ZALEWSKI, A. A., FAHY, G. M., AND KADOTA, Y.: Survival of cycropreserved nerve allografts in rats immunosuppressed with cyclosporin A. Anat. Rec. **218:** 11A–12A (abstract), 1987.
35. BABANY, G., MORRIS, R. E., BABANY, I., AND KATES, R. E.: Evaluation of the in vivo dose-response relationship of immunosuppressive drugs using a mouse heart transplant model: application to cyclosporine. J. Pharmacol. Exp. Ther. **244:** 259–262, 1988.
36. BACH, J. F.: The risk/benefit ratio in immunointervention for autoimmune diseases. J. Autoimmun. **1:** 711–720, 1988.
37. BACH, J. F.: Cyclosporine in autoimmunity (summation). Transplant. Proc. **20** (suppl. 4): 379–381, 1988.
38. BACH, J. F., FEUTREN, G., AND BOITARD, C.: The prospects of immunosuppression in type I diabetes. Adv. Nephrol. **17:** 321–340, 1988.
39. BACH, J. F., AND STROM, T. B.: The mode of action of immunosuppressive agents. *In* Res. Monographs Immunol. vol. 9, Elsevier, Amsterdam, 1985.
40. BACIGALUPO, A., HOWS, J., GORDON-SMITH, E. C., GLUCKMAN, E., VAN LINT, M. T., CONGIU, M., JAMES, D. C. O., BARRETT, A. J., GMUR, J., DE PLANQUE, M. M., SIIMES, M. A., TOIVANEN, A., RINGDÉN, O., AND MARMONT, A. M. FOR THE SAA WORKING PARTY OF THE EBMTG: Bone marrow transplantation for severe aplastic anemia from donors other than HLA identical siblings: a report of the BMT Working Party. Bone Marrow Transplant. **3:** 531–535, 1988.
41. BAILEY, L. L., JANG, J., JOHNSON, W., AND JOLLEY, W. B.: Orthotopic cardiac xenografting in the newborn goat. J. Thorac. Cardiovasc. Surg. **89:** 242–247, 1985.
42. BAILEY, L. L., LI, Z. J., ROOST, H., AND JOLLEY, W.: Host maturation after orthotopic cardiac transplantation during neonatal life. Heart Transplant. **3:** 265–267, 1984.
43. BAIN, J. R., MACKINNON, S. E., HUDSON, A. R., FALK, R. E., FALK, J. A., AND HUNTER, D. A.: The peripheral nerve allograft: a dose-response curve in the rat immunosuppressed with cyclosporin A. Plast. Reconstr. Surg. **82:** 447–457, 1988.
44. BAIN, J. R., MACKINNON, S. E., HUDSON, A. R., FALK, R. E., FALK, J. A., AND HUNTER, D. A.: The peripheral nerve allograft: an assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. Plast. Reconstr. Surg. **82:** 1052–1064, 1988.
45. BAKER, B. S., GRIFFITHS, C. E. M., LAMBERT, S., POWLES, A. W., LEONARD, J. N., VALDIMARSSON, H., AND FRY, L.: The effects of cyclosporine A on T lymphocyte and dendritic cell subpopulations in psoriasis. Transplant. Proc. **20** (suppl. 4): 72–77, 1988.
46. BALOW, J. E. (moderator): Lupus nephritis; NIH Conference. Ann. Inter. Med. **106:** 79–94, 1987.
47. BANDLIEN, K. O., TOLEDO-PEREYRA, L. H., BARNHART, M. I., CHOUDBURY, S. P., DIAZ-VELEZ, A., MACKENZIE, G. H., AND CORTEZ, J. A.: Improved survival of venous allograft in dogs following graft pretreatment with cyclosporine. Transplant. Proc. **15** (suppl. 1): 3084–3091, 1983.
48. BANIA, M. B., ANTEL, J. P., REDER, A. T., NICHOLAS, M. K., AND ARNASON, B. G. W.: Suppressor and cytolytic cell function in multiple sclerosis. Effects of cyclosproine and interleukin 2. J. Clin. Invest. **78:** 582–586, 1986.
49. BANNER, B., DEAN, P., AND WILLIAMS, J.: Morphologic features of rejection in long-surviving canine small bowel transplants. Transplantation **46:** 665–669, 1988.
50. BARAN, D., VENDEVILLE, B., VIAL, M. C., COSSON, C., BASCOU, C., TEYCHENNE, P., AND DRUET, P.: Effect of cyclosporine A on mercury-induced autoimmune glomerulonephritis in the Brown Norway rat. Clin. Nephrol. **25** (suppl. 1): 175–180, 1986.
51. BARBER, W. H., HUTCHINSON, I. V., AND MORRIS, P. J.: Mechanisms of kidney allograft maintenance in rats treated with cyclosporine. Transplant. Proc. **17:** 1391–1393, 1985.
52. BARBERÁ-GUILLEM, E., CAÑAVATE, M. L., LOPEZ DE TEJADA, I., AND VIDAL-VANACLOCHA, F.: Influence of host defenses on the hepatic colonisation of B16F10 melanoma cells. Clin. & Exp. Metastasis **6:** 153–169, 1988.
53. BASADONNA, G., KAKIZAKI, K., AND MERRELL, R. C.: Effect of cyclosporine on established islet autografts. J. Surg. Res. **40:** 450–454, 1986.
54. BASADONNA, G., MONTORSI, F., KAKIZAKI, K., AND MERRELL, R. C.: Cyclosporin A and islet function. Am. J. Surg. **156:** 191–193, 1988.
55. BATCHELOR, J. R.: Genetic role in autoimmunity. *In* Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 16–23, Springer-Verlag, Berlin, 1985.
56. BATCHELOR, J. R., LOMBARDI, G., AND LECHLER, R. I.: Speculations on the specificity of suppression. Immunol. Today **10:** 37–40, 1989.
57. BEATTY, P. G., CLIFFT, A., MICKELSON, E. M., NISPEROS, B. B., FLOURNOY, N., MARTIN, P. J., SANDERS, J. E., STEWART, P., BUCKNER, C. D., STORB, R., THOMAS, E. D., AND HANSEN, J. A.: Marrow transplantation from related donors other than HLA-identical siblings. N. Engl. J. Med. **313:** 765–771, 1985.
58. BEHFOROUZ, N. C., WENGER, C. D., AND MATHISON, B. A.: Prophylactic treatment of BALB/c mice with cyclosporine A and its analog B-5-49 enhances resistance to Leishmania major. J. Immunol. **136:** 3067–3075, 1986.
59. BELENDIUK, G., AND SOLCH, S.: Cyclosporine in neurological autoimmune disease. Clin. Neuropharmacol. **11:** 291–302, 1988.
60. BELL, T. A. G., EASTY, D. L., AND MCCULLAGH, K. G.: A placebo-controlled blind trial of cyclosporin A in prevention of corneal graft rejection in rabbits. Br. J. Ophthalmol. **66:** 303–308, 1982.
61. BELOSEVIC, M., FAUBERT, G., AND MACLEAN, J. D.: The effects of cyclosporin A on the course of infection with Giardia muris in mice. Am. J. Trop. Med. Exp. Hyg. **35:** 496–500, 1986.
62. BENNETT, J. A., PILON, V. A., BRIGGS, D. B., AND MCKNEALLY, M. F.: Evaluation of cyclosporin-treated mice as hosts for growing and testing the chemosensitivity of first-transplant-generation human tumor xenografts implanted under the kidney capsule. J. Nat. Cancer Inst. **75:** 925–936, 1985.
63. BEN-NUN, A., AND COHEN, I. R.: Spontaneous remission and acquired resistance to autoimmune encephalomyelitis (EAE) are associated with suppression of T cell reactivity: suppressed EAE effector T cells recovered as T cell lines: J. Immunol. **128:** 1450–1457, 1982.
64. BEN-NUN, A., AND COHEN, I. R.: Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterisation of the cells. J. Immunol. **129:** 303–308, 1982.
65. BENTLEY, F. R., SUTHERLAND, D. E. R., RYNASIEWICZ, J. J., AND NAJARIAN, J. S.: Synergistic effect of posttransplant total lymphoid irradiation and pharmacologic immunosuppression with low-dose anti-lymphocyte globulin or cyclosporine on prolongation of rat heart allograft survival. Transplant. Proc. **15:** 671–673, 1983.
66. BERAUD, E., VARRIALE, S., FARNARIER, C., AND BERNARD, D.: Suppressor cells in Lewis rats with experimental allergic encephalomyelitis: prevention of the disease and inhibition of lymphocyte proliferation by the suppressor cells or their products. Eur. J. Immunol. **12:** 926–930, 1982.
67. BERDEN, J. H. M., FAABER, P., ASSMANN, K. J. M., AND RIJKE, T. P. M.: Effects of cyclosporin A on autoimmune disease in MRL/lpr and BXSB mice. Scand. J. Immunol. **24:** 405–411, 1986.
68. BERTSCHMANN, M., SCHÄREN, B., AND LÜSCHER, E. F.: Correlation of in vivo immune reactions against the intradermally developing P-815 mastocytoma in the syngeneic mouse. Immunobiology **156:** 382–399, 1979.
69. BESCHORNER, W. E., HESS, A. D., SHINN, C. A., AND SANTOS, G. W.: Transfer of cyclosporine-associated syngeneic graft-versus-host disease by thymocytes. Transplantation **45:** 209–215, 1988.
70. BESCHORNER, W. E., NAMNOUM, J. D., HESS, A. D., SHINN, C. A., AND SANTOS, G. W.: Cyclosporin A and the thymus. Immunopathology. Am. J. Pathol. **126:** 487–496, 1987.
71. BESCHORNER, W. E., SHINN, C. A., FISCHER, A. C., SANTOS, G. W., AND HESS, A. D.: Cyclosporine-induced pseudo-graft-versus-host disease in the early post-cyclosporine period. Transplantation **46** (suppl.): 112S–117S, 1988.
72. BEVERIDGE, T.: Clinical transplantation—overview. Prog. Allergy **38:** 269–292, 1986.
73. BIGELOW, C. L., ADLER, L. T., AND APPELBAUM, F. R.: Allogeneic bone marrow transplantation in irradiated adult rabbits. Transplantation **44:** 351–354, 1987.
74. BIGGS, J. C., ATKINSON, K., GILLETT, E., DOWNS, K., CONCANNON, A., AND DODDS, A.: A randomized prospective trial comparing cyclosporine and methotrexate given for prophylaxis of graft-versus-host disease after bone marrow transplantation. Transplant. Proc. **18:** 253–255, 1986.
75. BIRD, A. G.: Cyclosporin A, lymphomata and Epstein-Barr virus. *In* Cyclosporin A, ed. by D. J. G. White, pp. 307–315, Elsevier Biomed. Press, Amsterdam, 1982.
76. BIREN, C. A., BARR, R. J., MCCULLOUGH, J. L., BLACK, K. S., AND HEWITT,

C. W.: Prolonged viability of human skin xenografts in rats by cyclosporin. J. Invest. Dermatol. **86:** 611–614, 1986.
77. Biren, C. A., Ganderup, G., Lemus, L., McCullough, J., and Barr, R.: Topical cyclosporine: effect on contact dermatitis in guinea pigs. (abstract). Clin. Res. **32:** 136A, 1984.
78. Black, C. D. V., Kroczek, R. A., Barbet, J., Weinstein, J. N., and Shevach, E. M.: Induction of IL-2 receptor expression in vivo: response to concanavalin A. Cell Immunol. **111:** 420–432, 1988.
79. Black, K. S., Hewitt, C. W., Aniel, M., Grisham, G. R., Caiozzo, V. J., and Achauer, B. M.: Neuromuscular capabilities in long-term composite tissue allografts. Transplant. Proc. **20** (suppl. 2): 269–271, 1988.
80. Black, K. S., Hewitt, C. W., Fraser, L. A., Howard, E. B., Martin, D. C., Achauer, B. M., and Furnas, D. W.: Composite tissue (limb) allografts in rats. II. Indefinite survival using low-dose cyclosporine. Transplantation **39:** 365–368, 1985.
81. Black, K. S., Hewitt, C. W., Henson, L. E., Chau, L. C., Pizzo, L., and Achauer, B. M.: Cyclosporine-induced long-term allograft survival and its potential in posttrauma tissue replacement. J. Burn Care Rehab. **8:** 531–535, 1987.
82. Black, K. S., Hewitt, C. W., Smelser, S., Yearsley, S., Bazzo, D. E., and Achauer, B. M.: Cyclosporine and skin allografts for the treatment of thermal injury. II. Development of an experimental massive third-degree burn model demonstrating extensive graft survival. Transplantation **45:** 13–16, 1988.
83. Black, K. S., Nguyen, D. K., Proctor, C. M., Patel, M. P., and Hewitt, C. W.: Site specific T cell-mediated immunomodulation in vivo with cyclosporine. (in submission)
83a. Blank, M., Palestine, A., Nussenblatt, R., and Shoenfeld, Y.: Down-regulation of autoantibody levels of cyclosporine and bromocriptine treatment in patients with uveitis. Clin. Immunol. Immunopathol. **54:** 87–97, 1990.
84. Bloom, A. D., Economou, S. G., and Gebel, H. M.: Extension of survival of rat parathyroid allografts by depletion of Ia donor cells plus preoperative cyclosporine. Transplantation **44:** 171–174, 1987.
85. Boespflug, O., Godfraind, C., and Tardieu, M.: Effect of cyclosporin A on an experimental chronic viral infection of the central nervous system. J. Neuroimmunol. **21:** 49–57, 1989.
86. Bogdan, C., Streck, H., Röllinghoff, M., and Solbach, W.: Cyclosporin A enhances elimination of intracellular L major parasites by murine macrophages. Clin. Exp. Immunol. **75:** 141–146, 1989.
87. Bogman, M. J. J. T., de Waal, R. M. W., and Koene, R. A. P.: Persistent expression of donor-antigens in endothelium of long standing skin xenografts. Transplant. Proc. **19:** 205–207, 1987.
88. Böhm, D., Krombach, F., Hammer, C., Gebhard, F., and Brendel, W.: Fine needle aspiration cytology in cyclosporine-treated xenogeneic kidney rejection. Transplant. Proc. **17:** 2128–2129, 1985.
89. Boitard, C., Feutren, C., Castano, L., Debray-Sachs, M., Assan, R., Hors, J., and Bach, J. F.: Effect of cyclosporin A treatment on the production of antibody in insulin-dependent (type I) diabetic patients. J. Clin. Invest. **80:** 1607–1612, 1987.
90. Bolas-Fernandez, F., Grencis, R. K., and Wakelin, D.: Cyclosporin A and Trichinella spiralis: antihelminthic effects of immunosuppressed mice. Parasite Immunol. **10:** 111–116, 1988.
91. Bolton, C., Allsopp, G., and Cuzner, M. L.: The effect of cyclosporin A on the adoptive transfer of experimental allergic encephalomyelitis in the Lewis rat. Clin. Exp. Immunol. **47:** 127–132, 1982.
92. Bolton, C., Borel, J. F., Cuzner, M. L., Davison, A. N., and Turner, A. M.: Immunosuppression by cyclosporin A of experimental allergic encephalomyelitis: J. Neurol. Sci. **56:** 147–153, 1982.
93. Bom-van Noorloos, A. A., Visser, J. J., Drexhage, H. A., Meijer, S., and Hoitsma, H. F. W.: Cyclosporin A in orthotopic porcine liver transplantation. Long-term survival after short-term treatment. Eur. Surg. Res. **16:** 329–335, 1984.
94. Bordes-Aznar, J., Kupiec-Weglinski, J. W., Duarte, A. J. S., Milford, E. L., Strom, T. B., and Tilney, N. L.: Function and migration of suppressor lymphocytes from cyclosporine-treated heart graft recipients. Transplantation **35:** 185–190, 1983.
95. Borel, J. F.: Effects of some drugs on the chemotaxis of rabbit neutrophils in vitro. Experientia **29:** 676–678, 1973.
96. Borel, J. F.: Comparative study of in vitro and in vivo drug effects on cell-mediated cytotoxicity. Immunology **31:** 631–641, 1976.
97. Borel, J. F.: Effect of ketotifen in some immunological animal models. Respiration **39** (suppl. 1): 38–43, 1980.
98. Borel, J. F.: Pharmacology and pharmacokinetics of cyclosporin A. *In* Transplantation and Clinical Immunology, vol. 13, pp. 3–6, Excerpta Medica, Amsterdam, 1981.
99. Borel, J. F.: Cyclosporin A—present experimental status. Transplant. Proc. **13:** 344–348, 1981.
100. Borel, J. F.: From our laboratories: cyclosporin A. Triangle **20:** 97–105, 1981.
101. Borel, J. F.: The history of cyclosporin A and its significance. *In* Cyclosporin A, ed. by D. J. G. White, pp. 5–17, Elsevier Biomed. Press, Amsterdam, 1982.
102. Borel, J. F.: Cyclosporine: historical perspectives. Transplant. Proc. **15** (suppl. 1): 2219–2229, 1983.
103. Borel, J. F.: Basic science summary. Transplant. Proc. **20** (suppl. 2): 722–730, 1988.
104. Borel, J. F.: The cyclosporins. Transplant. Proc. **21:** 810–815, 1989.
105. Borel, J. F., and Feurer, C.: In-vivo effects of anti-inflammatory and other drugs on granulocyte emigration in the rabbit skin collection chamber. J. Pathol. **124:** 85–93, 1978.
106. Borel, J. F., Feurer, C., Gubler, H. U., and Stähelin, H.: Biological effect of cyclosporin A: a new antilymphocytic agent. Agents Actions **6:** 468–475, 1976.
107. Borel, J. F., Feurer, C., Magnée, C., and Stähelin, H.: Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology **32:** 1017–1025, 1977.
108. Borel, J. F., Gubler, H. U., Hiestand, P. C., and Wenger, R. M.: Immunopharmacological properties of cyclosporine (Sandimmune®) and Val²)-dihydrocyclosporine and their prospect in chronic inflammation. Adv. Inflamm. Res. **11:** 277–291, 1986.
109. Borel, J. F., and Gunn, H. C.: Cyclosporine as a new approach to therapy of autoimmune diseases. Ann. NY Acad. Sci. **475:** 307–319, 1986.
110. Borel, J. F., and Meszaros, J.: Skin transplantation in mice and dogs. Effect of cyclosporin A and dihydrocyclosporin C. Transplantation **29:** 161–162, 1980.
111. Borel, J. F., and Wiesinger, D.: Effects of cyclosporin A on murine lymphoid cells. *In* Regulatory Mechanisms in Lymphocyte Activation, ed. by D. O. Lucas, pp. 716–718, Academic Press, New York, 1977.
112. Borel, J. F., Wiesinger, D., and Gubler, H. U.: Effects of the antilymphocytic agent cyclosporin A in chronic inflammation. Eur. J. Rheumatol. Inflamm. **1:** 237–241, 1987.
113. Borleffs, J. C. C., Neuhaus, P., and Balner, H.: Cyclosporin A as optimal immunosuppressant after kidney allografting in rhesus monkeys. Heart Transplant. **2:** 111–117, 1983.
114. Borleffs, J. C. C., Neuhaus, P., Marquet, R. L., Zurcher, C., and Balner, H.: Cyclosporin A and kidney transplantation in rhesus monkeys. *In* Cyclosporin A, ed. by D. J. G. White, pp. 329–342, Elsevier Biomed., Amsterdam, 1982.
115. Bos, J. D.: The pathomechanisms of psoriasis; the skin immune system and cyclosporin. Br. J. Dermatol. **118:** 141–155, 1988.
116. Bos, G. M. J., Majoor, G. D., and van Breda-Vriesman, P. J. C.: Cyclosporin-A induces a selective, reversible suppression of T-helper lymphocyte regeneration after syngeneic bone marrow transplantation: association with syngeneic graft-versus-host disease in rats. Clin. Exp. Immunol. **74:** 443–448, 1988.
117. Bottazzo, G. F., Todd, I., Mirakian, R., Belfiore, A., and Pujol-Borrell, R.: Organ-specific autoimmunity: a 1986 overview. Immunol. Rev. **94:** 137–165, 1986.
118. Boullon, F., Sinagra, A., Riarte, A., Lauricella, M., Barra, J., Besanson, M., Lejour, C., Lopez-Blanco, O., Favoloro, R., and Segura, E. L.: Experimental cardiac transplantation and chronic Chagas' disease in dogs. Transplant. Proc. **20** (suppl. 1): 432–437, 1988.
119. Bout, D. T., Deslée, D., and Capron, A. R.: Protection against schistosomiasis produced by cyclosporin A. Am. J. Trop. Med. Hyg. **33:** 185–186, 1984.
120. Bout, D. I., Deslée, D., and Capron, A. R.: Antischistosomal effect of cyclosporin A: cure and prevention of mouse and rat schistosomiasis mansoni. Infect. Immun. **52:** 823–287, 1986.
121. Bout, D., Haque, A., and Capron, A.: Filaricidal effects of cyclosporin-A against Dipetalonema viteae in Mastomys natalensis. Trans. R. Soc. Trop. Med. Hyg. **78:** 670–671, 1984.
122. Bowen, K. M., Andrus, L., and Lafferty, K. J.: Successful allotransplantation of mouse pancreatic islets to nonimmunosuppressed recipients. Diabetes **29** (suppl. 1): 98–104, 1980.
123. Bowen, K. M., Thompson, S., and Burton, R. C.: Rat fetal pancreas allograft survival is enhanced following donor tissue organ culture and host immunosuppression. Transplant. Proc. **19:** 2926–2929, 1987.
124. Bradley, J. A., Mason, D. W., and Morris, P. J.: Evidence that rat renal allografts are rejected by cytotoxic T cells and not by nonspecific effectors. Transplantation **39:** 169–175, 1985.
125. Braida, M., and Knop, J.: Effect of cyclosporin A on the T-effector and T-suppressor cell response in contact sensitivity. Immunology **59:** 503–507, 1986.
126. Brayman, K. L., Armstrong, J., Shaw, L. M., Rosano, T. G., Tomaszewski, J. E., Barker, C. F., and Naji, A.: Prevention of diabetes in BB rats by intermittent administration of cyclosporine. Surgery **102:** 235–241, 1987.
127. Brayman, K. L., Hickey, W. F., Barker, C. F., and Naji, A.: Thymic MHC antigen expression and suppressor cell sparing by cyclosporine. Transplant. Proc. **19:** 1181–1183, 1987.
128. Brayman, K. L., Markmann, J. F., Roza, A., Barker, C. F., Shaw, L., and Naji, A.: The effect of carrier vehicle on cyclosporine-induced unresponsiveness to rat cardiac allografts. Transplant. Proc. **20** (suppl. 1): 197–199, 1988.
129. Breinig, M. K., Camp, P., Dummer, S., and Ho, M.: Human and murine MHC-restricted cytotoxic lymphocyte responses to CMV infection. Birth Defects Orig. Artic. Ser. **20:** 375–379, 1984.
130. Broekhuyse, R. M., Kuhlmann, E. D., van Vugt, A. H. M., and Winkens, H. J.: Immunological and immunopathological aspects of op-

sin-induced uveoretinits. Graefe's Arch. Clin. Exp. Ophthalmol. **225:** 45–49, 1987.
131. Brundin, P., Nilsson, O. G., Gage, F. H., and Björklund, A.: Cyclosporin A increases survival of cross-species intrastriatal grafts of embryonic dopamine-containing neurons. Exp. Brain Res. **60:** 204–208, 1985.
132. Bruning, J. H., Bruggeman, C. A., and van Breda-Vriesman, P. J. C.: The transfer of cytomegalovirus infection in rats by latently infected renal allografts, and the role of various immunosuppressive regimens in virus reactivation. Transplantation **46:** 623–624, 1988.
132a. Bryson, J. S., Jennings, C. D., Caywood, B. E. and Kaplan, A. M.: Induction of a syngeneic graft-versus-host disease-like syndrome in DBA/2 mice. Transplantation **48:** 1042–1047, 1989.
133. Bueding, E., Hawkins, J., and Cha, Y. N.: Antischistosomal effects of cyclosporin A. Agents Actions **11:** 380–383, 1981.
134. Bunjes, D., Heit, W., Arnold, R., Schmeiser, T., and Heimpel, H.: Cyclosporine as an alternative to cyclophosphamide in the treatment of chronic graft-versus-host disease. Transplantation **41:** 170–172, 1986.
135. Burke, G., Esquenazi, V., Milgrom, M., Fuller, L., Roth, D., Olson, L., and Miller, J.: A brief course of cyclosporine A pretransplant reduces posttransplant cyclosporine A requirements in beagles and humans. Transplant. Proc. **20** (suppl. 2): 478–480, 1988.
136. Butler, L. D., DeRiso, P. E., Marder, P., and Scheetz, M. E.: In vivo expression and regulation of murine IL 2 receptors after antigen sensitization. J. Immunol. **138:** 470–477, 1987.
137. Cain, G. R., Cain, J. L., Turrel, J. M., Theilen, G., and Jain, N. C.: Immune-mediated hemolytic anemia and thrombocytopenia in a cat after bone marrow transplantation. Vet. Pathol. **25:** 161–162, 1988.
138. Cain, G. R., Champlin, R. E., and Gale, R. P.: Survival and immune recovery in dogs following fetal liver transplantation. Transplant. Proc. **19:** 2695–2697, 1987.
139. Cairo, M., VandeVen, C., Toy, C., Litke, T., Talesnik, D., and Treichel, R.: Membrane perturbation: a possible mechanism for cyclosporin enhancement of intracellular chemotherapeutic cytotoxicity of VP-16 using PMN membrane degranulation as a model. Blood **68** (suppl. 1): 80a (abstract no. 200), 1986.
140. Calder, V. L., Bellamy, A. S., Owen, S., Lewis, C., Rudge, P., Davison, A. N., and Feldman, M.: Effects of cyclosporin A on expression of IL-2 and IL-2 receptors in normal and multiple sclerosis patients. Clin. Exp. Immunol. **70:** 570–577, 1987.
141. Calder, V. L., Owen, S., Watson, C., Feldman, M., and Davison, A. N.: MS: a localized immune disease of the central nervous system. Immunol. Today **10:** 99–107, 1989.
142. Calne, R. Y.: Organ transplantation between widely disparate species. Transplant. Proc. **2:** 550–556, 1970.
143. Calne, R. Y.: Pancreas transplantation. Prog. Allergy **38:** 395–403, 1986.
144. Calne, R. Y., Collier, D. St. J., and Evans, D. B.: Alternative day cyclosporin and azathioprine plus steroids. Lancet **I:** 1448 (June 22), 1985.
145. Calne, R. Y., Rolles, K., White, D. J. G., Thiru, S., Evans, D. B., Henderson, R. G., Hamilton, D. L., Boone, N., McMaster, P., Gibby, O., and Williams, R.: Cyclosporin-A in clinical organ grafting. Transplant. Proc. **13:** 349–358, 1981.
146. Calne, R. Y., Rolles, K., White, D. J. G., Thiru, S., Evans, D. B., McMaster, P., Dunn, D. C., Craddock, G. N., Henderson, R. G., Aziz, S., and Lewis, P.: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases and 2 livers. Lancet **II:** 1033–1036, 1979.
147. Calne, R. Y., and White, D. J. G.: Cyclosporin A: a powerful immunosuppressant in dogs with renal allografts. IRCS Med. Sci. **5:** 595, 1977.
148. Calne, R. Y., White, D. J. G., Evans, D. B., Thiru, S., Henderson, R. G., Hamilton, D. V., Rolles, K., McMaster, P., Duffy, T. J., MacDougall, B. R. D., and Williams, R.: Cyclosporin A in cadaveric organ transplantation. Br. Med. J. **282:** 934–936, 1981.
149. Calne, R. Y., White, D. J. G., Evans, D. B., and Wight, C.: Three years' experience with cyclosporin A in clinical cadaveric kidney transplantation. *In* Cyclosporin A, ed. by D. J. G. White, pp. 347–353, Elsevier Biomed. Press, Amsterdam, 1982.
150. Calne, R. Y., White, D. J. G., Rolles, K., Smith, D. P., and Herbertson, B. M.: Prolonged survival of pig orthotopic heart grafts treated with cyclosporin A. Lancet **I:** 1183–1185, 1987.
151. Calne, R. Y., White, H. J. D., Herbertson, B. M., Millard, P. R., Davis, D. R., Salaman, J. R. and Samuel, J. R.: Pig-to-baboon liver xenografts. Lancet **I:** 1176–1178, 1968.
152. Cammisuli, S., and Feurer, C.: The effect of cyclosporin-A and dihydrocyclosporin-D on the therapy and prophylaxis of experimental allergic encephalomyelitis. Prog. Clin. Biol. Res. **146:** 415–421, 1984.
153. Carrière, V., Auriault, C., Dessaint, J. P., and Capron, A.: Differential effect of cyclosporin A on IgE response: role of cyclosporin A-induced suppressor cells. Immunol. Lett. **16:** 145–149, 1987.
154. Caspi, R. R., McAllister, C. G., Gery, I., and Nussenblatt, R. B.: Differential effects of cyclosporins A and G on functional activation of a T-helper-lymphocyte line mediating experimental autoimmune uveoretinitis. Cell. Immunol. **113:** 350–360, 1988.
155. Cattran, D. C.: Effect of ciclosporin on active Heymann nephritis. Nephron **48:** 142–148, 1988.
156. Cavanak, T., and Sucker, H.: Formulation of dosage forms. Prog. Allergy **38:** 65–72, 1986.
157. Chan, C. C., Caspi, R., Mochizuki, M., Diamantstein, T., Gery, I., and Nussenblatt, R. B.: Cyclosporine and dexamethasone inhibit T-lymphocyte MHC class II antigens and IL-2 receptor expression in experimental autoimmune uveitis. Immunol. Invest. **16:** 319–331, 1987.
158. Chan, C. C., Mochizuki, M., Palestine, A. G., BenEzra, D., Gery, I., and Nussenblatt, R. B.: Kinetics of T-lymphocyte subsets in the eyes of Lewis rats with experimental autoimmune uveitis. Cell. Immunol. **96:** 430–434, 1985.
159. Chan, C. C., Palestine, A. G., and Nussenblatt, R. D.: Cyclosporine-induced alterations of humoral response in experimental autoimmune uveitis. Invest. Ophthalmol. & Visual Sci. **25:** 867–870, 1984.
160. Chappell, L. H., and Thompson, A. W.: Studies on the action of cyclosporin A against Schistosoma mansoni and other parasitic infections. Transplant. Proc. **20** (suppl. 2): 291–297, 1988.
161. Chappell, L. H., Thomson, A. W., Barker, G. C., and Smith, S. W. G.: Dosage, timing, and route of administration of cyclosporin A and non-immunosuppressive derivatives of dihydrocyclosporin A and cyclosporin C against Schistosoma mansoni in vivo and in vitro. Antimicrob. Agents Chemother. **31:** 1567–1571, 1987.
161a. Chappell, L. H., Wastling, J. M., and Hurd, H.: Action of cyclosporin A on the tapeworms Hymenolepis microstoma, H., diminuta and Mesocestoides corti in vivo. Parasitology **98:** 291–299, 1989.
162. Charan, S., Hügin, A. W., Cerny, A., Hengartner, H., and Zinkernagel, R. M.: Effects of cyclosporin A on humoral immune response and resistance against vesicular stomatis virus in mice. J. Virol. **57:** 1139–1144, 1986.
163. Chatterjee, S. N.: Does cyclosporine reduce the risk of post-transplant infection? Infect. Surg. **7:** 95–96, 1988.
164. Chatterjee, S. N., Faulken, L., Gardner, M., and Chatterjee, S.: Murine cytomegalovirus infection and cyclosporine. Transplant. Proc. **17:** 2732–2733, 1985.
165. Cheney, R. T., and Sprent, J.: Capacity of cyclosporine to induce autograft-versus-host disease and impair intrathymic T cell differentiation. Transplant. Proc. **17:** 528–530, 1985.
166. Chisholm, P. M., and Bevan, D. J.: T cell activation in the presence of cyclosporine in three in vivo allograft models. Transplantation **46** (suppl.): 80S–85S, 1988.
167. Chisholm, P. M., Drayson, M. T., Cox, J. H., and Ford, W. L.: The effects of cyclosporin on lymphocyte activation in a systemic graft-vs.-host reaction. Eur. J. Immunol. **15:** 1054–1059, 1985.
168. Chow, L. H., Feurer, C., and Borel, J. F.: Chronic relapsing experimental allergic encephalomyelitis in the Lewis rat: studies on immune regulation. J. Neuroimmunol. **19:** 329–339, 1988.
169. Chow, L. H., Mosbach-Ozmen, L., Ryffel, B., and Borel, J. F.: Syngeneic graft-versus-host disease induced by cyclosporine-a reappraisal. Transplantation **46** (suppl.): 107S–112S, 1988.
170. Chui, Y. L., and Batchelor, J. R.: Mechanisms underlying continued survival of rat kidney allograft after a short period of chemical immunosuppression. Transplantation **40:** 150–153, 1985.
171. Chused, T. M., McCoy, K. L., Lal, R. B., Brown, E. M., and Baker, P. J.: Multigenic basis of autoimmune disease in New Zealand mice. Concepts Immunopathol. **4:** 129–143, 1987.
172. Cockburn, I.: Assessment of the risks of malignancy and lymphomas developing in patients using Sandimmune. Transplant. Proc. **19:** 1804–1807, 1987.
173. Cohen, I. R.: Regulation of autoimmune disease: physiological and therapeutic. Immunol. Rev. **94:** 5–21, 1986.
174. Cohen, I. R.: The self, the world and autoimmunity. Sci. Am. **258** (no 4): 52–60, 1988.
175. Cohen, S., Nordgren, S. D., Mackenzie, R. D., Lossing, A. G., Stiller, C. R., and Langer, B.: Pharmacokinetics of cyclosporine in a canine intestinal transplantation model. Transplant. Proc. **15:** 3013–3018, 1983.
176. Cole, G. A., Nickell, S. P., Mokhtarian, F., and Scheibel, L. W.: Effects of cyclosporine on experimental infections. Transplant. Proc. **15** (suppl. 1): 2271–2277, 1983.
177. Cole, G. W., Shimomaye, S., and Goodman, M.: The effect of topical cyclosporin A on the elicitation phase of allergic contact dermatitis. Contact Dermatitis **19:** 129–132, 1988.
178. Collier, D. St. J., Calne, R. Y., De Curtins, M., Thiru, S., White, D. J. G., Jamieson, N. V., Thick, M., and Barroso, E.: Alternate-day cyclosporine and azathioprine in experimental dog renal allografts. Transplant. Proc. **19:** 1279–1280, 1987.
179. Collier, D. St. J., Calne, R. Y., Thick, M., Jamieson, N. V., De Curtins, M., Barroso, E., White, D. J. G., and Thiru, S.: Alternative day immunosuppression. Lancet **I:** 267–268, 1986.
180. Collin, J., Dennison, A. R., Watkins, R. M., Millard, P. R., and Morris, P. J.: Segmental small intestinal allografts. II. Inadequate function with cyclosporine immunosuppression: evidence of a protein-losing enteropathy. Transplantation **44:** 479–483, 1987.
181. Confino, E., Vermesh, M., Thomas, W., and Gleicher, N.: Non-vascular transplantation of the rabbit uterus. Int. J. Gynaecol. Obstet. **24:** 321–325, 1986.
182. Connolly, K. M., Stecher, V. J., Danis, E., Pruden, D. J., and La

BRIE, T.: Alteration of interleukin-1 production and the acute phase response following medication of adjuvant arthritic rats with cyclosporin-A or methotrexate. Int. J. Immunopharmacol. **10:** 717–728, 1988.
183. CONVERSE, P. J., HESS, A. D., TUTSCHKA, P. J., AND SANTOS, G. W.: Effect of cyclosporine on the response of normal human lymphocytes to cytomegalovirus in vitro. Infect. Immun. **41:** 1226–1233, 1983.
184. COOK, R. M.: Activity of cyclosporin A in experimental influenza virus infection in mice. Agents Actions **13:** 98–100, 1983.
185. COX, K. O., ALLISON, A. C., AND SAMCEWICZ, B.: Experimental erythrocyte autoimmunity prevented by suppressor T cells in mice treated with cyclosporin-A. Clin. Immunol. Immunopathol. **28:** 90–95, 1983.
186. COX, J. H., AND CHISHOLM, P.: Mechanism of action of cyclosporine in preventing cardiac allograft rejection: I. Rate of entry of lymphocytes from the blood, fibrin deposition, and expression of Ia antigens on infiltrating cells. Transplantation **43:** 338–342, 1987.
187. CRABTREE, G. R.: Contingent genetic regulatory events in T lymphocyte activation. Science **243:** 355–361, 1989.
188. CRADDOCK, G. N., NORDGREN, S. R., REZNICK, R. K., GILAS, T., LOSSING, A. G., COHEN, Z., STILLER, C. R., CULLEN, J. B., AND LANGER, B.: Small bowel transplantation in the dog using cyclosporine. Transplantation **35:** 284–288, 1983.
189. CRAWFORD, D. H., POWLES, R., ILIESEN, V., HAWKINS, R. E., AND EDWARDS, J. M. B.: Epstein Barr virus infection of B cells from bone marrow transplant patients. Exp. Hematol. **11** (suppl. 13): 113–116, 1983.
190. CROOP, J. M., GROS, P., AND HOUSMAN, D. E.: Genetics of multi-drug resistance. J. Clin. Invest. **81:** 1303–1309, 1988.
191. CROY, B. A., ROSSANT, J., AND CLARK, D. A.: Effects of alterations in the immunocompetent status of Mus musculus females on the survival of transferred Mus caroli embryos. J. Reprod. Fertil. **74:** 479–489, 1985.
192. CUNNINGHAM, C., POWER, D. A., STEWART, K. N., AND CATTO, G. R. D.: The influence of cyclosporin A on alloantibody responses in inbred rats: provisional evidence for a serum factor with antiidiotypic activity. Clin. Exp. Immunol. **72:** 130–135, 1988.
193. DAMJANOVICH, S., ASZALOS, A., MULHERN, S., BALAZS, M., AND MATYUS, L.: Cytoplasmic membrane potential of mouse lymphocytes is decreased by cyclosporins. Mol. Immunol. **23:** 175–180, 1986.
194. DANIEL, R. K., EGERSZEGI, E. P., SAMULACK, D. D., SKANES, S. E., DYKES, R. W., AND RENNIE, W. R. J.: Tissue transplants in primates for upper extremity reconstruction: a preliminary report. J. Hand Surg. **11A:** 1–8, 1986.
195. DAVIES, H. F. S., COLLIER, D. S. J., THIRU, S., AND CALNE, R. Y.: Analysis of tolerance induced in dogs with renal allografts after alternate-day therapy with cyclosporine and azathioprine. Transplant. Proc. **20:** 142–143, 1988.
196. DAVISON, A. N., WATSON, C. M., OWEN, S. J., AND CALDER, V. L.: Nervous and immune system disorders in multiple sclerosis. Brain Behaviour Immun. (submitted).
197. DEBRAY-SACHS, M., SAI, P., FEUTREN, G., LANG, F., MAUGENDRE, D., BOITARD, C., HORS, J., AND BACH, J. F.: Inhibition of insulin release in vitro mediated by mononuclear cells from diabetic patients treated with cyclosporin A or placebo. Diabetes **37:** 873–877, 1988.
198. DEEG, H. J., HACKMAN, R. C., WEIDEN, P. L., AND STORB, R.: Growth of canine tumors transplanted into normal adult dogs immunosuppressed by cyclosporin A. Cancer Immunol. Immunother. **12:** 147–152, 1982.
199. DEEG, H. J., LOUGHRAN, T. P., STORB, R., KENNEDY, M. S., SULLIVAN, K. M., DONEY, K., APPELBAUM, F. R., AND THOMAS, E. D.: Treatment of human acute graft-versus-host disease with antithymocyte globulin and cyclosporine with or without methylprednisolone. Transplantation **40:** 162–166, 1985.
200. DEEG, H. J., RAFF, R. F., SEVERNS, E., APPELBAUM, F. R., THOMAS, E. D., AND STORB, R.: Suppressor and cytotoxic cells in DLA nonidentical canine radiation chimeras given cyclosporine and methotrexate as prophylaxis for graft-versus-host disease. Transplant. Proc. **15** (suppl. 1): 3042–3045, 1983.
201. DEEG, H. J., SEVERNS, E., RAFF, R. F., SALE, G. E., AND STORB, R.: Specific tolerance and immunocompetence in haptoidentical, but not in competely allogeneic, canine chimeras treated with methotrexate and cyclosporine. Transplantation **44:** 621–632, 1987.
202. DEEG, H. J., AND STORB, R.: Experimental marrow transplantation. *In*: Cyclosporin A, ed. by D. J. G. White, pp. 121–134, Elsevier Biomed. Press, Amsterdam, 1982.
203. DEEG, H. J., STORB, R., APPELBAUM, F. R., KENNEDY, M. S., GRAHAM, T. C., AND THOMAS, E. D.: Combined immunosuppression with cyclosporine and methotrexate in dogs given bone marrow grafts from DLA-haploidentical littermates. Transplantation **37:** 62–65, 1984.
204. DEEG, H. J., STORB, R., GERHARD-MILLER, L., SHULMAN, H. M., WEIDEN, P. L., AND THOMAS, E. D.: Cyclosporin A, a powerful immunosuppressant in vivo and in vitro in the dog, fails to induce tolerance. Transplantation **29:** 230–235, 1980.
205. DEEG, H. J., STORB, R., THOMAS, E. D., FLUORNOY, N., KENNEDY, M. S., BANAJI, M., APPELBAUM, F. R., BENSINGER, W. I., BUCKNER, C. D., CLIFT, R. A., DONEY, K., FEFER, A., MCGUFFIN, R., SANDERS, J. E., SINGER, J., STEWART, P., SULLIVAN, K. M., AND WITHERSPOON, R. P.: Cyclosporine as prophylaxis for graft-versus-host disease: a randomized study in patients undergoing marrow transplantation for acute nonlymphoblastic leukemia. Blood **65:** 1325–1334, 1985.
206. DEEG, H. J., STORB, R., WEIDEN, P. L., RAFF, R. F., SALE, G. E., ATKINSON, K., GRAHAM, T. C., AND THOMAS, E. D.: Cyclosporin A and methotrexate in canine marrow transplantation: engraftment, graft-versus-host-disease, and induction of tolerance. Transplantation **34:** 30–35, 1982.
207. DE HEER, E., DAHA, M. R., AND VAN ES, L. A.: The autoimmune response in active Heymann's nephritis in Lewis rats is regulated by T-lymphocyte subsets. Cell. Immunol. **92:** 254–264, 1985.
208. DE LA HERA, A., MARCOS, M. A. R., TORIBIO, M. L., MARQUEZ, C., GASPAR, M. L., AND MARTINEZ-A, C.: Development of Ly-1$^+$ B cells in immunodeficient CBA/N mice. J. Exp. Med. **166:** 804–809, 1987.
209. DE LA MOUTE, S. M., BOUR, C., RADHAKRISHNAN, V. V., JUPITER, B. B., SMITH, R. J., AND HEDLEY-WHYTE, E. T.: Effects of cyclosporin A and predegeneration on survival and regeneration of peripheral nerve allografts in rabbits. Surg. Neurol. **29:** 95–100, 1988.
210. DEL POZO, E., GRAEBER, M., ELFORD, P., AND PAYNE, T.: Regression of bone and cartilage loss in adjuvant arthritic rats after treatment with Sandimmune® (cyclosporin A). Arthritis Rheum. **33:** 247–252, 1990.
211. DELTZ, E., ULRICHS, K., ENGEMANN, R., SCHACK, T., FRIEDRICHS, B., MÜLLER-RUCHHOLZ, W., MÜLLER-HERMELINK, H. K., AND THIEDE, A.: Prevention of graft-versus-host reaction following small bowel transplantation by temporary cyclosporine treatment. Transplant. Proc. **15:** 3027–3031, 1983.
212. DENHAM, S., ATTRIDGE, S., AND BARFOOT, R. K.: The effect of limited courses of cyclosporine on survival and immunocompetence of allogeneic bone marrow chimeras. Transplantation **40:** 477–482, 1985.
213. DENHAM, S., ATTRIDGE, S., BARFOOT, R. K., AND ALEXANDER, P.: Effect of cyclosporin A on the anti-leukaemia action associated with graft-versus-host disease. Br. J. Cancer **47:** 791–795, 1983.
214. DENHAM, S., STYTES, J. M., BARFOOT, R. K., AND DEAN, C. J.: Reversible suppression of allo-antibody production by cyclosporin A. Int. Arch. Allergy Appl. Immunol. **62:** 453–458, 1980.
215. DE WAAL, R. M. W., BOGMAN, M. J. J. T., CORNELISSEN, I. M. H. A., VERMEULEN, A. N., AND KOENE, R. A. P.: Expression of donor class I major histocompatibility antigens on the vascular endothelium of mouse skin allografts. Transplantation **42:** 178–183, 1986.
216. DIBELIUS, A., KÖNIGSBERGER, H., WALTER, P., PERMANETTER, W., BRENDEL, W., AND VON SPECHT, B. V.: Prolonged reversal of diabetes in the rat by transplantation of allogeneic islets from a single donor and cyclosporine treatment. Transplantation **41:** 426–431, 1986.
217. DIDLAKE, R., KIM, E. K., AND KAHAN, B. D.: Ability of 3M KCl-extracted histocompatibility antigen to potentiate the immunosuppressive effect of cyclosporine to prolong the survival of heterotopic rat cardiac allografts. Transplantation **46:** 743–747, 1988.
218. DIENST, S. G., AMPRIM, F. L., AND SCHERVISH, E.: Donor-specific blood transfusion reduces cyclosporine effect on the survival of canine renal allografts. Transplant. Proc. **20** (suppl. 3): 1102–1104, 1988.
219. DIEPERINK, H., STEINBRUCHEL, D., STARKLINT, H., LARSEN, S., AND KEMP, E.: Improvement in hare-to-rabbit kidney transplant survival. Transplant. Proc. **19:** 1140–1142, 1987.
220. DILIZ-PEREZ, H. S., HONG, H. Q., DE SANTIBANES, E., BEDETTI, C., IWATSUKI, S., SHAW, B. W., AND STARZL, T. E.: Total pancreaticoduodenal homotransplantation in dogs immunosuppressed with cyclosporine and steroids. Am. J. Surg. **147:** 677–680, 1984.
221. DILIZ-PEREZ, H. S., MCCLURE, J., BEDETTI, C., HONG, H. Q., DE SANTIBANES, E., SHAW, B. W., VAN THIEL, D., IWATSUKI, S., AND STARZL, T. E.: Successful small bowel allotransplantation in dogs with cyclosporine and prednisone. Transplantation **37:** 126–129, 1984.
222. DOMERGUE, J., BOUHADDIOUI, N., BARNEON, G., AND MARCHAL, G.: Survie indéfinie d'allogreffes pancréatiques sous cyclosporine A chez le rat. J. Chir. **121:** 195–201, 1984.
223. DONOHOE, J. A., ANDRUS, L., BOWEN, K. M., SIMEONOVIC, C., PROWSE, S. J., AND LAFFERTY, K. J.: Cultured thyroid allografts induce a state of partial tolerance in adult recipient mice. Transplantation **35:** 62–67, 1983.
224. DÖRRLER, J., GÖRING, H., GOSSMANN, R., HOLZMANN, T., RÜSSE, I., HAMMER, C., BLÜMEL, G., MAURER, P. C., GEBHARDT, U., GMEINWIESER, J., AND LEHMANN-HORN, F.: Limb allograft survival under cyclosporine treatment. Transplant. Proc. **18:** 1431–1433, 1986.
225. DORSCH, S. E., AND ROSER, B.: A quantitative lymph node weight assay for allogeneic interactions in the rat. Anst. J. Exp. Biol. Med. Sci. **52:** 253–264, 1974.
226. DOUGADOS, M., AWADA, H., AND AMOR, B.: Cyclosporin in rheumatoid arthritis: a double blind, placebo controlled study in 52 patients. Ann. Rheum. Dis. **47:** 127–133, 1988.
227. DOUGADOS, M., DUCHESNE, L., AND AMOS, B.: Bromocriptine and cyclosporin A combination therapy in rheumatoid arthritis. Arthritis Rheum. **31:** 1333–1334, 1988.
228. DOWNING, TP ., SADEGHI, A. M., REITZ, B. A., AND SHUMWAY, N. E.: Cardiac allotransplantation in rats supported with preoperative total lympoid irradiation, low-dose cyclosporine, and splenectomy. Transplantation **37:** 636–638, 1984.
229. DRACHMAN, D. B., ADAMS, R. N., MCINTOSH, K., AND PESTRONCK, A.: Treatment of experimental myasthenia gravis with cyclosporin A. Clin. Immunol. Immunopathol. **34:** 174–188, 1985.

230. Drath, D. B., and Kahan, B. D.: Pulmonary macrophage and polymorphonuclear leukocyte function in response to immunosuppressive therapy. Transplant. Proc. **15** (suppl. 1): 2367–2372, 1983.
231. Drath, D. B., and Kahan, B. D.: Alterations in rat pulmonary macrophage function by the immunosuppressive agents cyclosporine, azathioprine, and prednisone. Transplantation **35:** 588–592, 1983.
232. Dreyfuss, M., Härri, E., Hofmann, H., Kobel, H., Pache, W., and Tscherter, H.: Cyclosporin A and C. New metabolites from Trichoderma polysporum (Link ex Pers.) Rifai. Eur. J. Appl. Microbiol. **3:** 125–133, 1976.
233. Drugge, R. J., and Handschumacher, R. E.: Cyclosporine—mechanism of action. Transplant. Proc. **20** (suppl. 2): 301–309, 1988.
234. D'Souza, M. J., Solomon, H. M., Fowler, L. C., and Pollock, S. H.: Pharmacokinetics of cyclosporine in streptozotocin-induced diabetic rats. Drug Metab. Dispos. **16:** 778–780, 1988.
235. Dunn, C. J., and Miller, S. K.: The effects of cyclosporin A on leucocyte infiltration and procoagulant activity in the mouse delayed hypersensitivity response in vivo. Int. J. Immunopharmacol. **8:** 635–643, 1986.
236. Dunn, D. C.: The specificity of post-cyclosporin 'tolerance'. Transplant. Proc. **13:** 383–385, 1981.
237. Dunn, D. C., White, D. J. G., and Herbertson, B. M.: Persistent nonspecific immunosuppression after a courese of cyclosporin A. Transplantation **29:** 349–351, 1980.
238. Dunn, D. C., White, D. J. G., Herbertson, B. M., and Wade, J.: Prolongation of kidney survival during and after cyclosporin A therapy. Transplantation **27:** 359–361, 1979.
239. Dunn, D. C., White, D. J. G., and Wade, J.: Survival of first and second kidney allografts after withdrawal of cyclosporin A therapy. IRCS Med. Sci. **6:** 464, 1987.
240. DuToit, D. ., Heydenrych, J. J., Louw, G., Zuurmond, T., Laker, L., Els, D., Weideman, A., Wolfe-Coote, S., and Davids, H.: Prolongation of intraperitoneal segmental pancreatic allografts in primates receiving cyclosporin A. Surgery **96:** 14–22, 1984.
241. DuToit, D. F., Heydenrych, J. J., Smit, B., Louw, G., Zuurmond, T., Els, D., TuToit, L. B., Weideman, A., Davids, H., Van der Merve, E., and Wolfe-Coote, S.: Prolongation of segmental and pancreaticoduodenal allografts in the primate with total-lymphoid irradiation and cyclosporine. Transplant. Proc. **44:** 346–350, 1987.
242. DuToit, D. F., Heydenrych, J. J., Smit, B., Louw, G., Zuurmond, T., Laker, L., Els, D., Weideman, A., Wolfe-Coote, S., Van der Merve, E. A., and Groenewald, W. A.: Experimental vascularized segmental pancreatic and islet transplantation in the baboon. World J. Surg. **8:** 236–243, 1984.
243. DuToit, D. F., Heydenrych, J. J., Smit, B., Zuurmond, T., Louw, G., Els, D., Baker, L., Weideman, A., Wolfe-Coote, S., DuToit, L., Davids, H., Groenewald, W., Van der Merve, E., and Pistorius, S.: Effect of cyclosporine and irradiation on experimental pancreatic allografts in the primate. J. Surg. Oncol. **37:** 215–219, 1988.
244. DuToit, D. F., Homan, W. P., and Morris, P. J.: The effect of cyclosporin A on experimental renal and pancreatic allografts in the dog. *In* Cyclosporin A, ed. by D. J. G. White, pp. 101–120, Elsevier Biomed. Press, Amsterdam, 1982.
245. Eccles, S. A., Heckford, S. E., and Alexander, P.: Effects of cyclosporin A on the growth and spontaneous metastasis of syngeneic animal tumours. Br. J. Cancer. **42:** 252–259, 1980.
246. Eckes, D. W., Perrotta, N. J., Kaneko, M., Saigenji, H., Russell, D. H., and Copeland, J. G.: Synergistic effect of bromocriptine mesylate on cyclosporin immunosuppression in the rat heart/lung allograft. Clin. Res. **36:** 142A, 1988.
247. Edelman, G., Duke, D., Renn, E., and Jonasson, O.: Fetal islet allotransplantation in rabbits. Transplantation **46:** 660–664, 1988.
248. Elder, J. T., Gupta, A. K., Fisher, G. J., and Voorhees, J. J.: Cyclosporine inhibits ornithine decarboxylase gene expression and acute inflammation in response to phorbol ester treatment of hairless mouse skin. Transplant. Proc. **20** (suppl. 4): 95–104, 1988.
249. Ellerman, K. E., Powers, J. M., and Brostoff, S. W.: A suppressor T-lymphocyte cell line for autoimmune encephalomyelitis. Nature **331:** 265–267, 1988.
250. Engemann, R., Ulrichs, K., Thiede, A., and Hamelmann, H.: Suppressorzellmechanismen bei cyclosporin A induzierter Toleranz nach orthotoper Rattenlebertransplantation. In vivo und in vitro Daten zur Toleranzkinetik. *In* Chirurgisches Forum 1985 f. exp. klin. Forsch., F. Stelzner, Hrsg., pp. 193–197, Springer-Verlag, Berlin, 1985.
251. Engemann, R., Ulrichs, K., Thiede, A., Müller-Ruchholtz, W., and Hamelmann, H.: Induction of liver graft tolerance in a primarily nontolerant rat strain combination with temporary treatment of cyclosporine. Transplant. Proc. **15** (suppl. 1): 2986–2991, 1983.
252. Ertel, W., Reichenspurner, H., Hammer, C., Welz, A., Ueberfuhr, P., Hemmer, W., Reichart, B., Gokel, M., and Brendel, W.: Heart transplantation in closely related species: a model for humoral rejection. Transplant. Proc. **16:** 1259–1261, 1984.
253. Ertel, W., Ueberfuhr, P., Reichenspurner, H., Hemmer, W., Hammer, C., Reichart, B., Welz, A., and Gokel, M.: Immunologic monitoring in dogs after allogenic heterotopic heart transplantation. Heart Transplant. **3:** 268–273, 1984.
254. Esa, A. H., Vogelsang, G. B., Sandford, G. R., Saral, R., Hess, A. D., and Burns, W. H.: Cell-mediated immune responses in rat cytomegalovirus infection. Transplant. Proc. **19:** 2726–2729, 1987.
255. Estrin, M., Herzum, M., Buie, C., and Huber, S. A.: Immunosuppressives in myocarditis. Eur. Heart J. **8** (suppl. J): 259–262, 1987.
256. Estrin, M., and Huber, S.: Coxsackievirus B3-induced myocarditis. Autoimmunity is L3T4$^+$ T helper cell and IL-2 independent in BALB/c mice. Am. J. Pathol. **127:** 337–341, 1987.
257. Estrin, M., Smith, C., and Huber, S.: Coxsackievirus B-3 myocarditis. T-cell autoimmunity to heart antigens is resistant to cyclosporin-A treatment. Am. J. Pathol. **125:** 244–251, 1986.
258. Ethridge, C. P., Mitchell, G. M., Barton, R. M., Morrison, W. A., and O'Brien, B. McC.: Long microvenous allografts in rabbit femoral arteries and veins. Br. J. Plast. Surg. **41:** 52–61, 1988.
259. Fan, T. P. D., Cox, J. H., and Chisholm, P. M.: Mechanism of action of cyclosporine in preventing cardiac allograft rejection. Transplantation **43:** 343–345, 1987.
260. Fässler, R., Schauenstein, K., Krömer, G., Schwarz, S., and Wick, G.: Elevation of corticosteroid-binding globulin in obese strain (OS) chickens: possible implications for the disturbed immunoregulation and the development of spontaneous autoimmune thyroiditis. J. Immunol. **136:** 3657–3661, 1986.
261. Faustman, D. L., Steinman, R. M., Gebel, H. M., Hauptfeld, V., Davie, J. M., and Lacy, P. E.: Prevention of rejection of murine islet allografts by pretreatment with anti-dendritic cell antibody. Proc. Natl. Acad. Sci. USA **81:** 3864–3868, 1984.
262. Fazakerley, J. K., and Webb, H. E.: Cyclosporine enhances virally induced T cell-mediated demyelination. The effect of cyclosporine on a demyelinating virus infection. J. Neurol. Sci. **78:** 35–50, 1987.
263. Ferrero, M. E., Marni, A., Corbetta, G., and Gaja, G.: Survival of pancreas allografts in rats treated with cyclosporine and bromocriptine. Transplant. Proc. **19:** 3923–3926, 1987.
264. Feurer, C., and Borel, J. F.: Localised leukocyte mobilisation in the rabbit ear. An in vivo migration technique using plastic collection chambers. Antibiot. Chemother. (Basel) **19:** 161–178, 1974.
265. Feurer, C., Chow, L. H., and Borel, J. F.: Preventive and therapeutic effects of cyclosporine and valine^2-dihydro-cyclosporine in chronic relapsing experimental allergic encephalomyelitis in the Lewis rat. Immunoloty **63:** 219–223, 1988.
266. Feurer, C., Prentice, D. E., and Cammisuli, S.: Chronic relapsing experimental allergic encephalomyelitis in the Lewis Rat. J. Neuroimmunol. **10:** 159–166, 1985.
267. Feutren, G., Boitard, C., Assan, R., and Bach, J. F.: Therapeutic immunosuppression in type I (insulin-dependent) diabetes. J. Autoimmun. **1:** 603–614, 1988.
268. Feutren, G., Quérin, S. Chatenoud, L., Noël, L. H., Beaurain, G., Tron, F., Lesavre, P., and Bach, J. F.: The effects of ciclosporin in twelve patients with severe systemic lupus. *In* Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 366–372, Springer, Berlin, 1985.
269. Feutren, G., Quérin, S., Noël, L. H., Chatenoud, L., Beaurain, G., Tron, F., Lesavre, P., and Bach, J. F.: Effects of cyclosporine in severe systemic lupus erythematosus. J. Pediatr. **111:** 1063–1068, 1987.
270. Filice, G. A., and Niewoehner, D. E.: Contribution of neutrophils and cell-mediated immunity to control of Nocardia asteroides in murine lungs. J. Infect. Dis. **156:** 113–121, 1987.
271. Findon, G., and Miller, T. E.: Modulation of host defenses by cyclosporine. Influence of the route of administration on the course of infection. Transplantation **45:** 815–817, 1988.
272. Fingert, H. J., Treiman, A., and Pardee, A. B.: Transplantation of human or rodent tumors into cyclosporine treated mice: a feasible model for studies of tumor biology and chemotherapy. Proc. Nat. Acad. Sci. (Wash. DC) **81:** 7927–7931, 1984.
273. Finsen, B., Poulsen, P. H., and Zimmer, J.: Xenografting of fetal mouse hippocampal tissue to the brain of adult rats: effects of cyclosporin A treatment. Exp. Brain Res. **70:** 117–133, 1988.
274. Fischer, A., Durandy, A., de Villartay, J. F., Le Deist, F., Gérota, I., and Griscelli, C.: HLA-haploidentical bone marrow transplantation for severe combined immunodeficiency using E rosette fractionation and cyclosporine. Blood **67:** 444–449, 1986.
275. Fischer, A. C., Beschorner, W. E., and Hess, A. D.: Syngeneic graft-versus-host disease: failure of autoregulation in self/non-self discrimination. Transplant. Proc. **20** (suppl. 3): 493–500, 1988.
276. Fisher, G. J., Duell, E. A., Nickoloff, B. J., Annesley, T. M., Kowalke, J. K., Ellis, C. N., and Voorhees, J. J.: Levels of cyclosporin in epidermis of treated patients differentially inhibit growth of keratinocytes cultured in serum free versus serum containing media. J. Invest. Dermatol. **91:** 142–146, 1988.
277. Fite, K. V., Pardue, S., Bengston, L., Hayden, D., and Smyth, J. R.: Effects of cyclosporine in spontaneous, posterior uveitis. Curr. Eye Res. **5:** 787–796, 1986.
278. Flake, A. W., Laberge, J. M., Adzick, N. S., Hill, A. C., Clausen, G. A., and Harrison, M. R.: Auxiliary transplantation of the fetal liver. I. Development of a sheep model. J. Pediatr. Surg. **21:** 515–520, 1986.
279. Florack, G., Sutherland, D. E. R., Sibley, R. K., Najarian, J. S. and

SQUIFFLET, J. P.: Combined kidney and segmental pancreas allotransplantation in dogs. Transplant. Proc. **17:** 374–377, 1985.
280. FLYE, M. W.: Histologic evidence of modification of liver allograft rejection in inbred miniature swine by cyclosporine. Transplant. Proc. **15:** 2983–2985, 1983.
281. FLYE, M. W., RODGERS, G., KACY, S., MAYSCHAK, D. M., AND THORPE, L.: Prevention of fatal rejection of SLA-mismatched orthotopic liver allografts in inbred miniature swine by cyclosporin-A. Transplant. Proc. **15:** 1269–1271, 1983.
282. FOETS, B., MISSOTTEN, L., VANDERVEEREN, P., AND GOOSSENS, W.: Prolonged survival of allogeneic corneal grafts in rabbits treated with topically applied cyclosporin A: systemic absorption and local immunosuppressive effect. Br. J. Ophthalmol. **69:** 600–603, 1985.
283. FORBES, R. D. C., PARFREY, N. A., GOMERSALL, M., DARDEN, A. G., AND GUTTMANN, R. D.: Dendritic cell-lymphoid cell aggregation and major histocompatibility antigen expression during rat cardiac allograft rejection. J. Exp. Med. **164:** 1239–1258, 1986.
284. FORD, W. L., BURR, W., AND SIMONSEN, M.: A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. Transplantation **10:** 258–266, 1970.
285. FORMAN, S. J., BLUME, K. G., KRANCE, R. A., MINER, P. J., METTER, G. E., HILL, L. R., O'DONNELL, M. R., NADEMANEE, A. P., AND SNYDER, D. S.: A prospective randomized study of acute graft-v-host disease in 107 patients with leukemia: methotrexate/prednisone *v* cyclosporine/prednisone. Transplant. Proc. **19:** 2605–2607, 1987.
286. FORMBY, B., MILLER, N., GARRET, R., AND PETERSON, C. M.: Effects of low-dose cyclosporine prophylaxis in non-obese diabetic mide. J. Pharmacol. Exp. Ther. **241:** 1106–1111, 1987.
387. FORMBY, B., MILLER, N., AND PETERSON, C. M.: Adoptive immunotherapy of diabetes in autologous nonobese diabetic mice with lymphoid cells ex vivo exposed to cyclosporin plus interleukin 2. Diabetes **37:** 1305–1309, 1988.
288. FORMBY, B., WALKER, L., AND PETERSON, C. M.: ^{3}H-cyclosporine internalization and secretion by human fetal pancreatic islets. Proc. Soc. Exp. Biol. Med. **189:** 72–78, 1988.
289. FÖRRE, O., WAALAN, K., RUGSTAD, H. E., BERG, K. J., SOLBU, D., AND KÄSS, E.: Cyclosporine and rheumatoid arthritis. Springer Semin. Immunopathol. **10:** 263–277, 1988.
290. FOXWELL, B. M. J., AND RYFFEL, B.: The mechanisms of action of cyclosporine. Immunol. Allergy Clinics North Am. **9:** 79–93, 1989.
291. FRANCIS, D. M. A., MASENDYCZ, P. J., DUMBLE, L. J., AND CLUNIE, G. J. A.: Enhancement of renal allografts by simultaneous cyclosporine and donor-specific blood transfusion. Transplant. Proc. **19:** 1464–1466, 1987.
292. FREDANE, L. M., HASHIM, G. A., AND MCCABE, R. E.: The effect of cyclosporine on lymphocyte subsets in experimental allergic encephalomyelitis: functional loss of disease-suppressing cells in vivo. Transplant. Proc. **15** (suppl. 1): 2909–2913, 1983.
293. FRIEDMAN, A. L., BEYER, M. M., JOSEPHSON, A. S., AND SCHIFFMANN, G.: Alternate day cyclosporine. Allograft and antibody immuno-suppression. Transplantation **43:** 457–458, 1987.
294. FRIEDMAN, A. L., SCHIFFMAN, G., JOSEPHSON, A. S., COBURGER, L. E., AND FRIEDMAN, E. A.: Low-dose azathioprine is synergistic with low-dose cyclosporine in rabbit allograft survival. Transplant. Proc. **19:** 1270–1271, 1987.
295. FUDEM, G. M., DAVIES, C. T., POWELL, A., WONG, D., AND FURNAS, D. W.: Growth of vascularized somatic tissue allografts in young rabbit given cyclosporine. Surg. Forum **36:** 604–606, 1985.
296. FUJINO, Y., OKUMURA, A., NUSSENBLATT, R. B., GERY, I., AND MOCHIZUKI, M.: Cyclosporine-induced specific unresponsiveness to retinal soluble antigen in experimental autoimmune uveoretinitis. Clin. Immunol. Immunopathol. **46:** 234–248, 1988.
297. FUJIWARA, H., GROGAN, J. B., AND RAJU, S.: Total orthotopic small bowel transplantation with cyclosporine. Transplantation **44:** 469–474, 1987.
298. FUJIWARA, H., RAJU, S., GROGAN, J. B., LEWIN, J. R., AND JOHNSON, W. W.: Total orthotopic small bowel allotransplantation in the dog. Features of atypical rejection and graft-versus-host reaction. Transplantation **44:** 747–753, 1987.
299. FUKUZAWA, M., AND SHEARER, G. M.: Effect of cyclosporin A on T cell immunity. I. Dose-dependent suppression of different murine T helper cell pathways. Eur. J. Immunol. **19:** 49–56, 1989.
300. FURNAS, D. W., RANDZIO, J., KNIHA, H., FUDEM, G. M., CRUZ, H. G., AND GOLD, M. E.: Growth of craniofacial and forelimb allotransplants in young rabbits. Transplant. Proc. **20:** (suppl. 1): 332–334, 1988.
301. FURUE, M., GASPARI, A. A., AND KATZ, S. I.: The effect of cyclosporin A on epidermal cells. II. Cyclosporin A inhibits proliferation of normal and transformed keratinocytes. J. Invest. Dermatol. **90:** 796–800, 1988.
302. FURUE, M., AND KATZ, S. I.: The effect of cyclosporine on epidermal cells. I. Cyclosporine inhibits accessory cell functions of epidermal Langerhans cells in vitro. J. Immunol. **140:** 4139–4143, 1988.
303. GAETA, A., CIPRIANI, P., BRENCIAGLIA, M. I., RIVANERA, D., MANCINI, C., ALFANI, D., PRETAGOSTINI, R., MOLAJONI, E. R., AND CORTESINI, R.: Effect of cyclosporin A on natural killer cell response during viral infections. Microbiologica **8:** 225–232, 1985.
304. GAO, E. K., LO, D., CHENEY, R., KANAGAWA, O., AND SPRENT, J.: Abnormal differentiation of thymocytes in mice treated with cyclosporin A. Nature (Lond.) **336:** 176–179, 1988.
305. GARIN, E. H., ORAK, J. K., HIOTT, K. L., AND SUTHERLAND, S. E.: Cyclosporine therapy for steroid-resistant nephrotic syndrome. Am. J. Dis. Child. **142:** 985–988, 1988.
306. GARVIN, P. J., NICHOFF, M., AND STAGGENBORG, J.: Cyclosporine's effect on canine pancreatic endocrine function. Transplantation **45:** 1027–1031, 1988.
307. GASPER, P. W., HOOVER, K. A., DORNSIFE, R. E., AND WEISER, M. G.: The effect of cyclosporine, cyclophosphamide, or antithymocyte globulin on the feline leukemia virus-induced aplastic anemia. (abstract). 4th Int. FeLV meeting, St. Thomas U.S. Virgin Islands, December 11–16, 1983.
308. GASPER, P. W., THRALL, M. A., WENGER, D. A., MACY, D. W., HAM, L., DORNSIFE, R. E., MCBILES, K., QUACKENBUSH, S. L., KESEL, M. L., GILLETTE, E. L., AND HOOVER, E. A.: Correction of feline arylsulphatase B deficiency (mucopolysaccharidosis VI) by bone marrow transplantation. Nature (Lond.) **312:** 467–469, 1984.
309. GASSEL, H. J., TELLIDES, G., ENGEMANN, R., AND MORRIS, P. J.: Cyclosporine in orthotopic rat liver transplantation: influence of major histocompatibility complex antigen expression and graft adaptation. Transplant. Proc. **20** (suppl. 3): 1081–1090, 1988.
309a. GAVENÉNAUX, C., BOESCH, D., BÖLSTERLI, J. J. BOLLINGER, P., EBERLE, M. K., THIESTAND, P., PAYNE, T., TRABER, R., WENGER, R., AND LOOR, F.: Overcoming multidrug resistance in Chinese hamster ovary cells in vitro by cyclosporin A (Saudimmune) and non-immunosuppressive derivatives. Br. J. Cancer **60:** 867–871, 1989.
310. GEORGIOU, H. M., LAGARDE, A. C., AND BELLGRAU, D.: T cell dysfunction in the diabetes-prone BB rat. A role for thymic migrants that are not T cell precursors. J. Exp. Med. **167:** 132–148, 1988.
311. GILHAR, A., WOJCIECHOWSKI, Z. J., PIEPKORN, M. W., SPANGRUDE, G. J., ROBERTS, L. K., AND KRUEGER, G. G.: Description of and treatment to inhibit the rejection of human split-thickness skin grafts by congenitally athymic (nude) rats. Exp. Cell. Biol. **54:** 263–274, 1986.
312. GILLOU, G. P., RAMSDEN, C., AND HEGARTY, J. S.: In vitro interferon stimulation of NK and ADCC effector cells from immunosuppressed patients. Transplant. Proc. **15:** 1742, 1983.
313. GIMÉNEZ, A., LEYVA-COBIÁN, F., FIERRO, C., RIO, M., BRICIO, T., AND MAMPASO, F.: Effect of cyclosporin A on autoimmune tubulointerstitial nephritis in the Brown Norway rat. Clin. Exp. Immunol. **69:** 550–556, 1987.
314. GLAZIER, A., TUTSCHKA, P. J., FARMER, E. R., AND SANTOS, G. W.: Graft-versus-host disease in cyclosporin A-treated rats after syngeneic and autologous bone marrow reconstitution. J. Exp. Med. **158:** 1–8, 1983.
315. GOICHOT-BONNAT, L., CHEMLA, P., AND POULIQUEN, Y.: Cyclosporine A collyre dans la prévention du rejet de greffe cornée à haut risque. J Fr. Ophthalmol. **10:** 213–217, 1987.
316. GOLDBERG, H., LING, V., WONG, P. Y., AND SKORECKI, K.: Reduced cyclosporin accumulation in multidrug-resistant cells. Biochem. Biophys. Res. Commun. **152:** 552–558, 1988.
317. GOLDBERG, M., LIMA, O., MORGAN, E., AYABE, H. A., LUK, S., FERDMAN, A., PETERS, W. J., AND COOPER, J. D.: A comparison between cyclosporin A and methylprednisolone plus azathioprine on bronchial healing following canine lung autotransplantation. J. Thorac. Cardiovasc. Surg. **85:** 821–826, 1983.
318. GOODMAN, M. M., AND MCCULLOUGH, J. L.: Human skin xenografts in cyclosporine-immunosuppressed rats. Models Dermatol. **3:** 141–147, 1987.
319. GOODMAN, M. M., MCCULLOUGH, J. L., BIREN, C. A., AND BARR, R. J.: A model of human melanoma in cyclosporine-immunosuppressed rats. J. Invest. Dermatol. **88:** 141–144, 1987.
320. GORCZYNSKI, R. M., BOULANGER, M., AND LAU, C.: T cell-derived factor alone or in combination with immunosuppressive drugs augments prolongation of allogeneic skin graft survival in mice receiving donor-specific transfusion. J. Immunol. **138:** 3197–3202, 1987.
321. GORCZYNSKI, R. M., AND HOLMES, W.: Cyclosporin alters the induction of allospecific tolerance in vivo. Immunol. Lett. **15:** 193–197, 1987.
322. GOTOH, M., MAKI, T., SATOMI, S., PORTER, J., AND MONACO, A. P.: Immunological characteristics of purified pancreatic islet grafts. Transplantation **42:** 387–390, 1986.
323. GOTTESMAN, M. M., AND PASTAN, I.: Resistance to multiple chemotherapeutic agents in human cancer cells. TIPS **9:** 54–58, 1988.
324. GOULD, C. L., MCMANNAMA, K. G., BIGLEY, N. J., GIRON, D. J.: Virus-induced murine diabetes. Enhancement by immunosuppression. Diabetes **34:** 1217–1221, 1985.
325. GOULET, O. J., RÉVILLON, Y., CERF-BENSUSSAN, N., NEZELOF, C., FISCHER, A., BUISSON, C., HUBERT, P., LOKIEC, F., MARTELLI, H., NIAUDET, P., JAN, D., PELLERIN, D., AND RICOUR, C.: Small intestinal transplantation in a child using cyclosporine. Transplant. Proc. **20** (suppl 3): 288–296, 1988.
326. GOZES, Y., THEOFILOPOULOS, A. N., PARK, C. H., SLACK, J. H., BALDERAS, R. S., AND DIXON, F. J.: Effects of cyclosporin-A on in vitro and in vivo Ig and anti-DNA autoantibody production by splenocytes of autoimmune and normal mice. (abstract 1619). Fed. Proc. **41:** 547, 1982.
327. GRACE, D. M., FEINDEL, C. M. S., HARRIS, K. A., INCULET, R. I., WALLACE, A. C., AND STILLER, C. R.: Immune response to transplanted pancreas in pigs. Transplant. Proc. **14:** 649–655, 1982.

328. GRANELLI-PIPERNO, A., KEANE, M., AND STEINMAN, R. M.: Evidence that cyclosporine inhibits cell-mediated immunity primarily at the level of the T lymphocyte rather than the accessory cell. Transplantation **46** (suppl.): 53S–60S, 1988.
329. GRANT, D., DUFF, J., ZHONG, R., GARCIA, B., LIPOHAR, C., KEOWN, P., AND STILLER, C.: Successful intestinal transplantation in pigs treated with cyclosporine. Transplantation **45**: 279–284, 1988.
330. GRANT, D., ZHONG, R., STILLER, C., WALLACE, C., KEOWN, P., AND DUFF, J.: A comparison of cyclosporine A and Nva^2-cyclosporine (cyclosporine G) in a rat renal allograft model. Transplantation **44**: 9–12, 1987.
331. GRATWOHL, A., BALDOMERO, H., NISSEN, C., AND SPECK, B.: Engraftment of T-cell-depleted rabbit bone marrow. Acta Haematol. (Basel) **77**: 208–214, 1987.
332. GRATWOHL, A., FORSTER, I., AND SPECK, B.,: Skin grafts in rabbits with cyclosporin A. Absence of induction of tolerance and untoward side effects. Transplantation **31**: 136–138, 1981.
333. GRATWOHL, A., FORSTER, I., AND SPECK, B.: Histoincompatible skin and marrow grafts in rabbits on cyclosporin A. Transplantation **33**: 361–364, 1982.
334. GRATWOHL, A., RIEDERER, I., GRAF, E., AND SPECK, B.: Cyclosporin toxicity in rabbits. Lab. Anim. **20**: 213–220, 1986.
335. GRATWOHL, A., RIEDERER, I., WALSTRA, K., BALDOMERO, H., AND SPECK, B.: Cyclosporin resistant effector cells in rabbit skin allografts. Experientia (Basel) **43**: 910–912, 1987.
336. GRATWOHL, A., AND SPECK, B.: Bone marrow transplantation with ciclosporin. Prog. Allergy **38**: 404–431, 1986.
337. GRATWOHL, A., TICHELLI, A., WÜRSCH, A., DIETERLE, A., LORI, A., THOMSSEN, C., BALDOMERO, H., DE WITTE, T., NISSEN, C., AND SPECK, B.: Irradiated donor buffy coat following T cell-depleted bone marrow transplants. Bone Marrow Transplant. **3**: 577–582, 1988.
338. GRAU, G. E., GRETLER, D., AND LAMBERT, P. H.: Prevention of murine cerebral malaria by low-dose cyclosporin A. Immunology **61**: 521–525, 1987.
339. GRAY, D. W. R.: Pancreatic islet transplantation: is purity always a virtue? Cyclosporine Quarterly **3** (no. 2): June 1988.
340. GRAY, D. W. R., MCSHANE, P., FAIRBROTHER, B., MORRIS, P. J.: The use of spleen transplantation and cyclosporine treatment to induce unresponsiveness to pancreatic islet transplantation in rats. J. Surg. Res. **40**: 77–84, 1986.
341. GRAY, D. W. R., REECE-SMITH, H., FAIRBORTHER, B., MCSHANE, P., AND MORRIS, P. J.: Isolated pancreatic islet allografts in rats rendered immunologically unresponsive to renal allografts. Transplantation **37**: 434–437, 1984.
342. GREEN, C. J.: Immunosuppression with cyclosporin A: a review. Diagn. Histopathol. **4**: 157–174, 1981.
343. GREEN, C. J.: Experimental transplantation. Prog. Allergy **38**: 123–158, 1986.
344. GREEN, C. J.: Experimental transplantation and cyclosporine. Transplantation **46** (suppl.): 3S–10S, 1988.
345. GREEN, C. J., AND ALLISON, A. C.: Extensive prolongation of rabbit kidney allograft survival after short-term cyclosporin A treatment. Lancet **I**: 1182–1183 (June 3), 1978.
346. GREEN, C. J., ALLISON, A. C., AND PRECIOUS, S.: Induction of specific tolerance in rabbits by kidney allografting and short periods of cyclosporin A treatment. Lancet **II**: 123–125 (July 21), 1979.
347. GREEN, C. J., ALLISON, A. C., AND PRECIOUS, S.: Immunosuppression with cyclosporin A in rabbits. Transplant. Clin. Immunol. **11**: 54–58, 1980.
348. GREEN, C. J., GRIMALDI, G., SIMPKIN, S., AND JOHNSON, A.: Immunosupression of rabbit ovarian and adnexal allografts with cyclosporin A. *In* Cyclosporin A, ed. by D. J. G. White, pp. 165–171, Elsevier Biomed. Press, Amsterdam, 1982.
349. GREEN, C. J., KEMP, E., AND KEMP, G.: The effects of cyclosporin A, ticlopidine hydrochloride and cobra venom factor on the hyperacute rejection of discordant renal xenografts. Invest. & Cell Pathol. **3**: 415–416, 1980.
350. GREEN, C. J., SIMPKIN, S., AND GRIMALDI, G.: Pregnancy after autografting and allografting vascularized ovaries and en bloc vascularised ovaries with adnexa in rabbits. Br. J. Obstet. Gynaecol. **89**: 645–651, 1982.
351. GREGER, B., SCHARECK, W. D., BÜSING, M., MELLERT, J., MÜLLER, G. H., HOPT, V. T., AND BOCKBORN, H.: Are the risks of viral infections increased in kidney transplant patients receiving triple-drug therapy? Transplant. Proc. **20**: 466–468, 1988.
352. GREGORY, C. R., GOURLEY, I. M., AND CAIN, G. R.: Effects of mizoribine and combination mizoribine/cyclosporine immunosuppression on canine renal allograft recipients. Transplant. Proc. **20** (suppl. 2): 223–225, 1988.
353. GROENEWEGEN, G., BUURMAN, W. A., JEUNEHOMME, G. M. A. A., AND VAN DER LINDEN, C. J.: Effects of cyclosporine on MHC class II antigen expression or arterial and venous endothelium in vitro. Transplantation **40**: 21–25, 1985.
354. GRUNDY, J. E., LUI, S. F., SUPER, M., BERRY, N. J., SWENY, P., FERNANDO, O. N., MOORHEAD, J., AND GRIFFITHS, P. D.: Symptomatic cytomegalovirus infection in seropositive kidney recipients: reinfection with donor virus rather than reactivation of recipient virus. Lancet **II**: 132–135 (July 16), 1988.
355. GSCHWENDT, M., KITTSTEIN, W., AND MARKS, F.: The weak immunosuppressant cyclosporine D as well as the immunologically inactive cyclosporine H are potent inhibitors in vivo of phorbol ester TPA-induced biological effects in mouse skin and of Ca^{2+}/calmodulin dependent EF-2 phosphorylation in vitro. Biochem. Biophys. Res. Commun. **150**: 545–551, 1988.
356. GUERIN, C., POZZETTO, B., GENIN, C., BERTHOUX, F. C., AND GAUDIN, O. G.: Incidence of cytomegalovirus infections in renal transplant patients treated with conventional or cyclosporin therapy. Nephrol. Dial. Transplant. **3**: 77–80, 1988.
357. GUI, X. E., ATCHISON, R. W., AND HO, M.: The effects of cyclosporine on viruses. Transplant. Proc. **15** (suppl. 1): 2917–2922, 1983.
358. GUI, X. E., HO, M., AND CAMP, P. E.: Effect of cyclosporin A on murine natural killer cells. Infect. Immun. **36**: 1123–1127, 1982.
359. GUI, X. E., RINALDO, C. R., AND HO, M.: Natural killer cell activity in renal transplant recipients receiving cyclosporine. Infect. Immun. **41**: 965–970, 1983.
360. GUILLÉN, F. J., HANCOCK, W. W., TOWPIK, E., KUPIEC-WEGLINSKI, J. W., RICKLES, F. R. TILNEY, N. L., AND MURPHY, G. F.: Inhibition of rat skin allograft rejection by cyclosporine. In situ characterization of the impaired local immune response. Transplantation **41**: 734–739, 1986.
361. GULATI, A. K., AND ZALEWSKI, A. A.: Muscle allograft survival after cyclosporin A immunosuppression. Exp. Neurol. **77**; 378–385, 1982.
362. GUNN, H. C.: Successful treatment of autoimmunity in (NZB × NZW) F_1 mice with cyclosporin and (Nva^2)-cyclosporin: I. Reduction of autoantibodies. Clin. Exp. Immunol. **64**: 225–233, 1986.
363. GUNN, H. C.: Successful treatment of autoimmunity in (NZB × NZW) F_1 mice with cyclosporin and (Nva$^+$)-cyclosporin: I. Reduction of autoantibodies. Clin. Exp. Immunol. **64**: 225–233, 1986.
364. GUNN, H. C., AND HIESTAND, P. C.: Cyclosporin A and G enhance IgG rheumatoid factor production in MRL/lpr mice. Transplant. Proc. **20** (suppl. 4): 238–242, 1988.
365. GUNN, H. C., HIESTAND, P. C., AND HANGLOW, A. C.: Cyclosporin treatment in experimental autoimmune myasthenia gravis. Monogr. Allergy **25**: 96–107, 1988.
366. GUNN, H. C., AND RYFFEL, B.: Glomerulonephritis in NZB/W mice: therapeutic effect of cyclosporine. Clin. Nephrol. **25** (suppl. 1): 189S–192S, 1986.
367. GUNN, H. C., AND RYFFEL, B.: Successful treatment of autoimmunity in (NZB × NZW) F_1 mice with cyclosporine and (Nva^2)-cyclosporin: II. Reduction of glomerulonephritis. Clin. Exp. Immunol. **64**: 234–242, 1986.
368. GUNN, H. C., RYFFEL, B., HIESTAND, P. C., AND BOREL, J. F.: The effects of (Nva^2)-cyclosporine on autoimmunity in the NZB/W mice. Transplant. Proc. **18**: 667–668, 1986.
369. GUPTA, S. K., CURTIS, J., AND TURK, J. L.: The effect of hydrocortisone and cyclosporin A on bacillus Calmette-Guérin epithelioid cell granulomas. Cell. Immunol. **93**: 189–198, 1985.
370. HAAS, G. S., DELMONICO, F. L., HALPERIN, E., SUIT, M., DOSERTZ, D., DAGGETT, W. M., BARRETT, L., RUSSELL, P. S., JAFFERS, G., AND COSIMI. A. B.: The effects of cyclosporine and lymphoid irradiation on allograft survival and peripheral blood T-cells. Heart Transplant. **3**: 152–159, 1984.
371. HAHN, H. J., DUNGER, A., LAUBE, F., BESCH, W., RADLOFF, E., KAUERT, C., AND KOTZKE, G.: Reversibility of the acute toxic effect of cyclosporin A on pancreatic B cells of Wistar rats. Diabetologia **29**: 489–494, 1986.
372. HAHN, H. J., LAUBE, F., LUCKE, S., KLÖTING, I., KOHNERT, K. D., AND WARZOCK, R.: Toxic effects of cyclosporine on the endocrine pancreas of Wistar rats. Transplantation **41**: 44–47, 1986.
373. HAHN, H. J., LUCKE, S., KLÖTING, I., VOLK, H. D., BAEHR, R. V., AND DIAMANTSTEIN, T.: Curing BB rats of freshly manifested diabetes by short-term treatment with a combination of a monoclonal anti-interleukin 2 receptor antibody and a subtherapeutic dose of cyclosporin A. Eur. J. Immunol. **17**: 1075–1078, 1987.
374. HALL, B. M., GURLEY, K., AND DORSCH, S. E.: Specific unresponsiveness in rats with prolonged allograft survival is dependent upon the graft and suppressor T cells. Transplant. Proc. **19**: 495–496, 1987.
375. HALL, B. M., GURLEY, K. E., AND DORSCH, S. E.: Tempo of induction of W3/25$^+$ suppressor cells in cyclosporine-treated rat cardiac allograft recipients. Transplant. Proc. **19**: 504, 1987.
376. HALL, B. M., JELBART, M. E., AND DORSCH, S. E.: Suppressor T cells in rats with prolonged cardiac allograft survival after treatment with cyclosporine. Transplantation **37**: 595–600, 1984.
377. HALL, B. M., JELBART, M. E., GURLEY, K. E., AND DORSCH, S. E.: Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. J. Exp. Med. **162**: 1683–1694, 1985.
378. HALLORAN, P. F., BUSHUK, M., AND STEWART, J. A.: Effects of cyclosporine on the healing of vascularized and nonvascularized bone allografts in rodents. Transplant. Proc. **15**: 3053–3056, 1983.
379. HALLORAN, P. F., COLE, E. H., BOOKMAN, A. A., UROWITZ, M. B., AND CLARKE, W. T. W.: Possible beneficial effect of ciclosporin in some cases of severe systemic lupus erythematosus. *In* Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 356–360, Springer-Verlag, Berlin, 1985.
380. HALLORAN, P. F., URMSON, J., FARKAS, S., PHILLIPS, R. A., FULOP, G., COCKFIELD, S., AND AUTENRIED, P.: Effects of cyclosporine on systemic MHC expression. Evidence that non-T cells produce interferon-gamma

in vivo and are inhibitable by cyclosporine. Transplantation **46** (suppl.): 68S–72S, 1988.

381. HALLORAN, P. F., URMSON, J., RAMASSAR, V., LASKIN, C., AND AUTENRIED, P.: Increased class I and class II MHC products and mRNA in kidneys of MRL-lpr/lpr mice during autoimmune nephritis and inhibition by cyclosporine. J. Immunol. **141:** 2303–2312, 1988.
382. HALLORAN, P. F., WADGYMAR, A., AND AUTENRIED, P.: Inhibition of MHC product induction may contribute to the immunosuppressive action of ciclosporin. Prog. Allergy **38:** 258–268, 1986.
383. HAMAGUCHI, K., NAKAMURA, M., ONO, J., AND TAKAKI, R.: Ultrastructural and functional studies of pancreatic B cells in Wistar rats treated with immunotherapeutic doses of cyclosporin. Diabetes Res. Clin. Pract. **5:** 135–143, 1988.
384. HANCOCK, W. W., LORD, R. H., COLBY, A. J., DIAMANTSTEIN, T., RICKLES, F. R., DIJKSTRA, C., HOGG, N., AND TILNEY, N. L.: Identification of IL-2$^+$ T cells and macrophages within rejecting rat cardiac allografts, and comparison of the effects of treatment with anti-IL 2R monoclonal antibody or cyclosporin. J. Immunol. **138:** 164–170, 1987.
385. HARDY, M. A., LAU, H., WEBER, C., AND REEMTSMA, K.: Induction of graft acceptance by ultraviolet irradiation of donor tissue. Ann. Surg. **200:** 441–450, 1984.
386. HARJULA, A., BALDWIN, J. C., TAZELAAR, H. D., JAMIESON, S. W., REITZ, B. A., AND SHUMWAY, N.: Minimal lung pathology in long-term primate survivors of heart-lung transplantation. Transplantation **44:** 852–854, 1987.
387. HARMEL, R. P., AND STANELY, M.: Improved survival after small intestinal transplantation in the rat using cyclosporine immunosuppression. J. Pediatr. Surg. **21:** 214–217, 1986.
388. HARTUNG, H. P., SCHÄFER, B., FIERZ, W., HEININGER, K., AND TOYKA, K. V.: Ciclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat. Neurosci. Lett. **83:** 195–200, 1987.
389. HASKINS, M. E., WORTMAN, J. A., WILSON, S., AND WOLFE, J. H.: Bone marrow transplantation in the cat. Transplantation **37:** 634–636, 1984.
390. HASSMAN, R. A., DIEGUEZ, C., RENNIE, D. P., WEETMAN, A. P., HALL, R., AND MCGREGORY, A. M.: The influence of cyclosporin A on the induction of experimental autoimmune thyroid disease in the PVG/c rat. Clin. Exp. Immunol. **59:** 10–16, 1985.
391. HATTORI, A., KUNZ, H. W., GILL, T. J., PAN, S. F., AND SHINOZUKA, H.: Diversity of the promoting action of cyclosporine on the induction of murine lymphoid tumors. Carcinogenesis (Lond.) **9:** 1091–1094, 1988.
391a. HATTORI, A., PERERA, M. I. R., WITKOWSKI, L. A., KUNZ, H. W., GILL, T. J., AND SHINUZOKA, H.: Accelerated development of spontaneous thymic lymphomas in male AKR mice receiving cyclosporine. Transplantation **44:** 784–787, 1986.
392. HAUG, C. E., GILL, R. G., BABCOCK, S. K., LAFFERTY, K. J., BELLGRAU, D., AND WEIL, R.: Cyclosporine-induced tolerance requires antigens capable of initiating an immune response. J. Immunol. **139:** 2947–2949, 1987.
393. HAVERICH, A., BILLINGHAM, M. E., SCOTT, W. C., JAMIESON, S. W., AND DAWKINS, K. D.: Asymmetric pattern of rejection following orthotopic cardiac transplantation in primates. Heart Transplant. **3:** 280–285, 1984.
394. HAVERICH, A., DAWKINS, K. D., BALDWIN, J. C., REITZ, B. A., BILLINGHAM, M. E., AND JAMIESON, S. W.: Long-term cardiac and pulmonary histology in primates following combined heart and lung transplantation. Transplantation **39:** 356–360, 1985.
395. HEERING, P., BACH, D., AND GRABENSEE, B.: Cyclosporin A in der Therapie der Glomerulonephritis. Med. Welt **39:** 673–677, 1988.
396. HELIN, H. J., AND EDGINGTON, T. S.: Cyclosporin A regulates monocyte/macrophage effector functions by affecting instructor T cells: inhibition of monocyte procoagulant response to allogeneic stimulation. J. Immunol. **132:** 1074–1076, 1984.
397. HELMCHEN, U., SCHMIDT, W. E., SIEGEL, E. G., AND CREUTZFELD, W.: Morphological and functional changes of pancreatic B cells in cyclosporin A-treated rats. Diabetologia **27:** 416–418, 1984.
398. HENDERSON, B., STAINES, N. A., BURRAI, I., AND COX, J. H.: The anti-arthritic and immunosuppressive effects of cyclosporine on arthritis induced in the rat by type II collagen. Clin. Exp. Immunol. **57:** 51–56, 1984.
399. HENNY, F. C., VAN ES., L. A. AND PAUL, L. C.: Effect of cyclosporin A on the non-MHC endothelial antibody response in the rat. Clin. Immunol. Immunopathol. **36:** 249–253, 1985.
400. HERBERMAN, R. B.: Natural killer cells and their possible relevance to transplantation biology. Transplantation **34:** 1–7, 1982.
401. HERBERT, J., AND ROSER, B.: Strategies of monoclonal antibody therapy that induce permanent tolerance of organ transplants. Transplantation **46** (suppl): 128S–134S, 1988.
402. HERRMANN, P.: Untersuchungen über die Wirkung von Ciclosporin in der verzögerten Ueberempflindlichkeit an der Maus. Inaugural-dissertation, Universität Basel, Hausdruckerei Sandoz AG, 1987.
403. HERRMANN, P., SCHREIER, M. H., AND BOREL, J. F.: Suppression of DTH mediated by cloned helper T cells with ciclosporin and dexamethasone. Agents Actions **19:** 280–281, 1986.
404. HERRMANN, P., SCHREIER, M. H., BOREL, J. F., AND FEURER, C.: Mast cell degranulation as a major event in the effector phase of delayed-type hypersensitivity induced by cloned helper T cells. Int. Arch. Allergy Appl. Immunol. **86:** 102–105, 1988.
405. HESS, A. D., COLOMBANI, P. M., AND ESA, A.: Cyclosporine. Immunobiologic aspects in transplantation. *In* Kidney Transplant Rejection: Diagnosis and Treatment. vol. 7, ed. by G. M. Williams, J. F. Burdick, and K. Solez, pp. 353–382, M. Dekker Inc., New York, 1986.
406. HESS, A. D., TUTSCHKA, P. J., AND SANTOS, G. W.: The effect of cyclosporin A on T lymphocyte subpopulations. *In* Cyclosporin A, ed. by D. J. G. White, pp. 209–231, Elsevier Blomed. Press, Amsterdam, 1982.
407. HEWITT, C. W., BLACK, K. S., AGUINALDO, A. M. A., ACHAUER, B. M. AND HOWARD, E. B.: Cyclosporine and skin allografts for the treatment of thermal injury. I. Extensive graft survival with low-level long-term administration and prolongation in a rat burn model. Transplantation **45:** 8–12, 1988.
408. HEWITT, C. W., BLACK, K. S., DOWDY, S. F., GONZALEZ, G. A., ACHAUER, B. M., MARTIN, D. C., FURNAS, D. W., AND HOWARD, E. B.: Composite tissue (limb) allografts in rats. III. Development of donor-host lymphoid chimeras in long-term survivors. Transplantation **41:** 39–43, 1986.
409. HEWITT, C. W., BLACK, K. S., FRASER, L. A., HOWARD, E. B., MARTIN, D. C., ACHAUER, B. M., AND FURNAS, D. W.: Composite tissue (limb) allografts in rats. I. Dose-dependent increase in survival with cyclosporine. Transplantation **39:** 360–364, 1985.
410. HEWITT, C. W., BLACK, K. S., GONZALEZ, G. A., DOWDY, S. F., REYES, M., MARTIN, D. C., AND ACHAUER, B. M.: Long-term residual cyclosporine levels following short-term administration in various allograft models demonstrating extensive survival prolongation. Transplant. Proc. **19:** 1244–1245, 1987.
411. HIESTAND, P. C., GALE, J. M., AND MEKLER, P.: Soft immunosuppression by inhibition of prolactin release: synergism with cyclosporine in kidney allograft survival and in the localized graft-versus-host reaction. Transplant. Proc. **18:** 870–872, 1986.
412. HIESTAND, P. C., AND GUBLER, H. U.: Cyclosporins: immunopharmacologic properties of natural cyclosporins. *In* Handbook Exp. Pharmacol. vol. 85, pp. 487–502, Springer-Verlag, Berlin, 1988.
413. HIESTAND, P. C., GUNN, H. C., GALE, J. M., RYFFEL, B., AND BOREL, J. F.: Comparison of the pharmacological profiles of cyclosporine, (Nva^2)-cyclosporine and (Val^2) dihydro-cyclosporine. Immunology **55:** 249–255, 1985.
414. HIESTAND, P. C., AND MEKLER, P.: Mechanism of action: Ciclosporin- and prolactin-mediated control of immunity. Prog. Allergy **38:** 239–246, 1986.
415. HIESTAND, P. C., MEKLER, P., NORDMANN, R., GRIEDER, A., AND PERMMONGKOL, C.: Prolactin as a modulator of lymphocyte responsiveness provides a possible mechanism of action for cyclosporine. Proc. Natl. Acad. Sci. USA **83:** 2599–2603, 1986.
416. HIGHAM, A. D., SELLS, R. A., AND MARSHALL-CLARKE, S.: Cyclosporin A has differential effects on the responses of murine B cells to TI antigens and B-cell mitogens. Immunology **59:** 203–207, 1986.
417. HILL, J. C., AND MASKE, R.: An animal model for corneal graft rejection in high-risk keratoplasty. Transplantation **46:** 26–30, 1988.
418. HINRICHS, D. J., AND HUMPHRES, R. C.: The response of the nude (athymic) rat to actively induced and adoptively transferred experimental allergic encephalomyelitis. J. Immunol. **131:** 4–5, 1983.
419. HINRICHS, D. J., ROBERTS, C. M., AND WAXMAN, F. J.: Regulation of paralytic allergic encephalomyelitis in rats: susceptibility to active and passive disease reinduction. J. Immunol. **126:** 1857–1862, 1981.
420. HINRICHS, D. J., WEGMANN, K. W., AND PETERS, B. A.: The influence of cyclosporin A on the development of actively induced and passively transferred experimental allergic encephalomyelitis. Cell. Immunol. **77:** 202–209, 1983.
421. HIRAMINE, C., HOJO, K., AND MATSUMOTO, H.: Abnormal distribution of T cell subsets in the thymus of cyclosporin A-treated mice. Thymus **11:** 243–252, 1988.
422. HO, M.: Immunology of cytomegalovirus: immunosuppressive effects during infections. Birth Defects Orig. Artic. Ser. **20:** 131–147, 1984.
423. HODGKIN, P. D., AGOSTINO, M., SELLINS, K., PROWSE, S. J., BELLGRAU, D., AND LAFFERTY, K. J.: T lymphocyte function in vivo. Ambivalence of the class I MHC antigen-reactive subset. Transplantation **40:** 288–292, 1985.
424. HOEPRICH, P. D., AND MERRY, J. M.: Comparative efficacy of forphenicinol cyclosporine, and amphotericin B in experimental murine coccidioidomycosis. Diagn. Microbiol. Infect. Dis. **6:** 287–292, 1987.
425. HOF, H.: Infektrisiko bei Behandlung mit Ciclosporin A. Dtsch. Med. Wochenschr. **111:** 1770–1775, 1986.
426. HOF, H., AND STRAUSS, R.: Suppression of acquired immunity to infection with Salmonella typhimurium by cyclosporin A. Curr. Microbiol. **13:** 177–178, 1986.
427. HOJO, K., AND HIRAMINE, C.: In vivo effects of cyclosporin A: abrogation of the induction of experimental allergic orchitis and sparing of the generation of suppressor cells. Int. Arch Allergy Appl. Immunol. **78:** 63–70, 1985.
428. HOM, J. T., BUTLER, L. D., RIEDL, P. E., AND BENDELE, A. M.: The progression of the inflammation in established collagen-induced arthritis can be altered by treatments with immunological or pharmacological agents which inhibit T cell activities. Eur. J. Immunol. **18:** 881–888, 1988.
429. HOMAN, W. P., FABRE, J. W., FRENCH, M. E., MILLARD, P. R., DENTON, T. G., AND MORRIS, P. J.: Reversal of acute rejection episodes by cyclo-

sporin A in dogs receiving renal allografts. Transplantation **29:** 262–263, 1980.
430. HOMAN, W. P., FABRE, J. W., MILLARD, P. R., AND MORRIS, P. J.: Interaction of cyclosporin A with antilymphocyte serum and with enhancing serum for the suppression of renal allograft rejection in the rat. Transplantation **29:** 219–222, 1980.
431. HOMAN, W. P., FABRE, J. W., MILLARD, P. R., AND MORRIS, P. J.: Effects of cyclosporin A upon second-set rejection of rat renal allografts. Transplantation **30:** 354–357, 1980.
432. HOMAN, W. P., FABRE, J. W., AND MORRIS, P. J.: Nature of the unresponsiveness induced by cyclosporin A in rats bearing reno-allografts. Transplantation **28:** 439–441, 1979.
433. HOMAN, W. P., FABRE, J. W., WILLIAMS, K. A., MILLARD, R. P., AND MORRIS, P. J.: Studies on the immunosuppressive properties of cyclosporin A in rats receiving renal allografts. Transplantation **29:** 361–366, 1980.
434. HOMAN, W. P., FRENCH, M. E., MILLARD, P., DENTON, T. G., FABRE, J. W., AND MORRIS, P. J.: Studies on the effects of cyclosporin A upon renal allograft rejection in the dog. Surgery **88:** 168–173, 1980.
435. HOMAN, W. P., FRENCH, M. E., MILLARD, P. R., AND MORRIS, P. J.: A study of eleven drug regimens using cyclosporin-A to suppress renal allograft rejection in the dog. Transplant. Proc. **13:** 397–401, 1981.
436. HOMAN, W. P., WILLIAMS, K. A., FABRE, J. W., MILLARD, P. R., AND MORRIS, P. J.: Prolongation of cardiac xenograft survival in rats receiving cyclosporin A. Transplantation **31:** 164–166, 1981.
437. HOMAN, W. P., WILLIAMS, K. A., MILLARD, P. R., AND MORRIS, P. J.: Prolongation of renal allograft survival in the rat by pretreatment with donor antigen and cyclosporin A. Transplantation **31:** 423–427, 1981.
438. HOOGENHOUT, J., KAZEM, I., JERUSALEM, C. R., BAKKEREN, J. A. J., DE JONG, J., KAL, H. B., AND VAN MUNSTER, P. J. J.: Growth pattern of tumor xenografts in Wistar rats after treatment with cyclophosphamide, total lymphoid irradiation and/or cyclosporin A. Int. J. Radiat. Oncol. Biol. Phys. **9:** 871–879, 1983.
439. HOSHINO, T., TUTTLE, T. M., MALEY, W. R., SMITH, W. J., AND WILLIAMS, G. M.: Attempts to induce tolerance with cyclophosphamide after renal transplantation in swine. Transplant. Proc. **20** (suppl. 1)**:** 144–148, 1988.
440. HOUSE, R. V., AND DEAN, J. H.: Trypanosoma musculi: characterisation of the T-lymphocyte dependency of immunity by selective immunomodulation of the mouse, Mus musculus. Exp. Parisitol. **67:** 104–115, 1988.
441. HOWARD, M. A., DACEY, R. C., AND WINN, H. R.: Brain xenografts: the effect of cyclosporin A on graft survival. J. Neurosurg. **69:** 121–126, 1988.
442. HOWS, J. M., PALMER, S., AND GORDON-SMITH, E. C.: Use of cyclosporin A in allogeneic bone marrow transplantation for severe aplastic anemia. Transplantation **33:** 382–386, 1982.
443. HOWS, J. M., YIN, J. L., MARSH, J., SWIRSKY, D., JONES, L., APPERLEY, J. F., JAMES, D. C. O., SMITHERS, S., BATCHELOR, J. R., GOLDMAN, J. M., AND GORDON-SMITH, E. C.: Histocompatible unrelated volunteer donors compared with HLA nonidentical family donors in marrow transplantation for aplastic anemia and leukemia. Blood **68:** 1322–1328, 1986.
444. HUANG, K. L., ARMSTRONG, J. A., AND HO, M.: Antibody response after influenza immunisation in renal transplant patients receiving cyclosporin A or azathioprine. Infect. Immunity **40:** 421–424, 1983.
445. HUETZ, F., JACQUEMART, F., ROSSI, C. P., VARELA, F., AND COUTINHO, A.: Autoimmunity: the moving boundaries between physiology and pathology. J. Autoimmun. **1:** 507–518, 1988.
446. HUGH-JONES, K., HOBBS, J. R., VELLODI, A., HANCOCK, M., WHITE, T., STARRER, F., AND STODDART, Y.: Treatment of Gaucher's disease by bone marrow transplantation. Bone Marrow Transplant. **2** (suppl. 1)**:** 123, 1987.
447. HUGHES, W. T., AND SMITH, B.: Provocation of infection due to Pneumocystis carinii by cyclosporin A. J. Infect. Dis. **145:** 767, 1982.
448. HÜGIN, A. W., CERNY, A., HENGARTNER, H., AND ZINKERNAGEL, R. M.: Suppression by cyclosporin A of murine T-cell-mediated immunity against viruses in vivo and in vitro. Cell. Immunol. **90:** 464–473, 1985.
449. HÜGIN, A. W., CERNY, A., WRANN, M., HENGARTNER, H., ADN ZINKERNAGEL, R. M.: Effect of cyclosporin A on immunity to Listeria monocytogenes. Infect. Immun. **52:** 12–17, 1986.
450. HUNT, B. J., YACOUB, M., AMIN, S., DEVENISH, A., AND CONTRERAS, M.: Induction of red blood cell destruction by graft-derived antibodies after minor ABO-mismatched heart and lung transplantation. Transplantation **46:** 246–249, 1988.
451. HUNTER, P. A., GARNER, A., WILHELMUS, K. R., RICE, N. S. C., AND JONES, B. R.: Corneal rejection: a new rabbit model and cyclosporin A. Br. J. Ophthalmol. **66:** 292–302, 1982.
452. HURTENBACH, U., AND MAURER, C.: Type I diabetes in NOD mice is not associated with insulin-specific, autoreactive T cells. J. Autoimmun. **2:** 151–161, 1989.
453. HUTCHINSON, I. V., AND MORRIS, P. J.: Allogreactive suppressor T cells. Transplant. Proc. **19:** 528–530, 1987.
454. HUTCHINSON, I. V., AND MORRIS, P. J.: Two distinct populations of suppressor T cells in rats bearing long-term surviving kidney allografts. Transplant. Proc. **19:** 3070–3071, 1987.
455. HUTCHINSON, I. V., RODRIGUES, M. A. F., AND MORRIS, P. J.: Suppressor-inducer T cells in a rat renal allograft model. Transplant. Proc. **19:** 3089–3090, 1987.
456. INABA, K., AND STEINMAN, R. M.: Accessory cell-T lymphocyte interactions. Antigen-dependent and -independent clustering. J. Exp. Med. **163:** 247–261, 1986.
457. INOUE, S., KAWANO, N., AND MORIOKA, Y.: Cyclosporine and partial liver allotransplants in a simplified rat model. Jpn. J. Surg. **15:** 299–311, 1985.
458. INOUE, H., KOHSAKA, S., YOSHIDA, K., OTANI, M., TOYA, S., AND TSUKADA, Y.: Immunohistochemical studies on mouse cerebral cortex grafted into the third ventricle of rats treated with cyclosporin A. Neurosci. Lett. **57:** 289–294, 1985.
459. INOUE, H., KOHSAKA, S., YOSHIDA, K., OHTANI, M., TOYA, S., AND TSUKADA, Y.: Cyclosporin A enhances the survivability of mouse cerebral cortex grafted into the third ventricle of rat brain. Neurosci. Lett. **54:** 85–90, 1985.
460. ISRAËL-BIET, D., NOËL, L. H., BACH, M. A., DARDENNE, M., AND BACH, J. F.: Marked reduction of DNA antibody production and glomerulopathy in thymulin (FTS-Zn) or cyclosporin A treated (NZB×NZW) F_1 mice. Clin. Exp. Immunol. **54:** 359–365, 1983.
461. ITO, T., STEPKOWSKI, S., AND KAHAN, B. D.: Frequency of T cytotoxic cells after perioperative treatment with extracted antigen and cyclosporine in rat cardiac transplantation. Transplant. Proc. **20** (suppl. 3)**:** 1045–1052, 1988.
462. IWAKIRI, R., NAGAFUCHI, S., KOUNOUE, E., NAKANO, S., KOGA, T., NAKAYAMA, M., NAKAMURA, M., AND NIHO, Y.: Cyclosporin A enhances streptozotocin-induced diabetes in CD-1 mice. Experientia (Basel) **43:** 324–327, 1987.
463. JACOBS, P.: Effect of cyclosporin A on the incidence of graft-versus-host disease (GvHD) and survival of rabbits following allogenic bone marrow transplantation. *In* Experimental Hematology Today, ed. by S. J. Baum, G. D. Ledney, and A. Khan, pp. 78–97, Karger, Basel, 1981.
464. JAMIESON, S. W., BURTON, N. A., BIEBER, C. P., REITZ, B. A., OYER, P. E., STINSON, E. B., AND SHUMWAY, N. E.: Cardiac-allograft survival in primates treated with cyclosporin A. Lancet **I:** 545, (March 10), 1979.
465. JANSSON, L., AND SANDLER, S.: The influence of cyclosporin A on the vascular permeability of the pancreatic islets and on diabetes induced by multiple low doses of streptozotocin in the mouse. Virchows Arch. (A) Pathol. Anat. Histopathol. **412:** 225–230, 1988.
466. JAWORSKI, M. A., HONORE, L., JEWELL, L. D., MEHTA, J. G., MCGUIRE-CLARK, P., SCHOULS, J. J., AND YAP, W. Y.: Cyclosporin prophylaxis induces long-term prevention of diabetes, and inhibits lymphocytic infiltration in multiple target tissues in the high-risk BB rat. Diabetes Res. **3:** 1–6, 1986.
467. JAWORSKI, M. A., JEWELL, L. D., HONORE, L., MEHTA, J. G., HAYENS-SIMMONDS, J., MCGUIRE-CLARK, P., SCHOULS, J. J., AND YAP, W. Y.: Immunosuppression in autoimmune disease: the double-edge sword. Clin. Invest. Med. **10:** 488–495, 1987.
468. JENG, L. B. B., SUTHERLAND, D. E. R., HESSE, U. J., AND NAJARIAN, J. S.: The course of cyclosporine-pretreated renal allografts in dogs. Transplantation **41:** 395–396, 1986.
469. JENKINS, M. K., SCHWARTZ, R. H., AND PARDOLL, D. M.: Effects of cyclosporine A on T cell development and clonal deletion. Science **241:** 1655–1658, 1988.
470. JEPHTHAH-OCHOLA, J., URMSON, J., FARKAS, S., AND HALLORAN, P. F.: Regulation of MHC expression in vivo. Bacterial lipopolysaccharide induces class I and II MHC products in mouse tissues by a T-cell independent, cyclosporine-sensitive mechanism. J. Immunol. **141:** 792–800, 1988.
471. JOLLEY, W. B., KNIERIM, K., HAM, J., AND LONGERBEAM, J. K.: The effect of cyclosporine on simultaneous skin and pancreatic islet allografts in the rabbit. Transplant. Proc. **15:** 3011–3012, 1983.
472. JONES, M. G., AND HARRIS, G.: Prolongation of life in female NZB/NZW (F_1) hybrid mice by cyclosporin A. CLin. Exp. Immunol. **59:** 1–9, 1985.
473. JONES, M. G., HARRIS, G., AND COWING, G.: Response of murine autoimmune disease to cyclosporine and thiols. Transplant. Proc. **15** (suppl. 1)**:** 2904–2908, 1983.
474. JONES, M. C., POWER, D. A., CUNNINGHAM, C., AND CATTO, G. R. D.: The influence of repeated transfusions and cyclosporine on secondary alloantibody responses in inbred rats. Transplantation **45:** 1094–1099, 1988.
475. JONES, M. C., POWER, D. A., CUNNINGHAM, C., STEWART, K. N., AND CATTO, G. R. D.: Alloantibody and transferable suppressor activity induced by cyclosporine and blood transfusions in the rat. Transplantation **46:** 645–649, 1988.
476. JONES, M. C., POWER, D. A., STEWART, K. N., AND CATTO, G. R. D.: Cyclosporin A prevents sensitization after blood transfusion in multiparous rats. Clin. Sci. (Lond.) **74:** 389–392, 1988.
477. JURADO, A., IMM, A., AND HEUSSER, C.: Evidence for a B-cell memory-forming factor (BMFF) able to reconstitute cyclosporin-induced suppression of the secondary response. Ann. Inst. Pasteur Immunol. **138:** 612–617, 1987.
478. KAHALY, G., SCHREZENMEIER, J., SCHWEIKERT, B., KRAUSE, U., DENNEBAUM, R., MÜLLER, W., UND BEYER, J.: Untersuchungen zum Einfluss von Cyclosporin A auf die endokrine Ophthalmopathie. Therapiewoche **37:** 523–528, 1987.
479. KAHAN, B. D. (ed.): Cyclosporine. Applications in autoimmune diseases. Transplant. Proc. **20** (suppl. 4), 1988.
480. KAHAN, B. D., DIDLAKE, R., KIM, E. E., YOSHIMURA, N., KONDO, E., AND STEPKOWSKI, S.: Important role of cyclosporine for the induction of

immunologic tolerance in adult hosts. Transplant. Proc. **20** (suppl. 3): 23–35, 1988.

481. KAHN, D., LAI, H. S., ROMOVACEK, H., MAKOWKA, L., VAN THIEL, D., AND STARZL, T. E.: Cyclosporine A augments the regenerative response after partial hepatectomy in the rat. Transplant. Proc. **20** (suppl. 3): 850–852, 1988.
482. KAIBARA, N., HOTOKEBUCHI, T., TAKAGISHI, K., AND KATSUKI, I.: Paradoxical effects of cyclosporin A on collagen arthritis in rats. J. Exp. Med. **158**: 2007–2015, 1983.
483. KAIBARA, N., HOTOKEBUCHI, T., TAKAGISHI, K., KATSUKI, I., MORINAGA, M., ARITA, C., AND JINGUSHI, S.: Pathogenetic difference between collagen arthritis and adjuvant arthritis. J. Exp. Med. **158**: 1388–1396, 1984.
484. KAIBARA, N., MORINAGA, M., ARITA, C., HOTOKEBUCHI, T., AND TAKAGISHI, K.: Serum transfer of collagen arthritis to cyclosporin-treated type, type II collagen-tolerants rats. Clin. Immunol. Immunopathol. **35**: 252–260, 1985.
485. KAKIZAKI, K., BASADONA, G., AND MERRELL, R. C.: Allotransplantation of islet endocrine aggregates. Diabetes **36**: 315–319, 1987.
486. KALMAN, V. K., AND KLIMPEL, G. R.: Cyclosporin A inhibits the production of gamma interferon (IFNγ), but does not inhibit production of virus-induced IFN α/β. Cell. Immunol. **78**: 122–129, 1983.
487. KANA, J. S., HOFFMAN, F., AND BUCHEN, R.: Rabbit corneal allograft survival following topical administration of cyclosporin A. Invest. Ophthalmol. & Visual Sci. **22**: 686–690, 1982.
488. KANAI, T., GOTOH, M., PORTER, J., MONACO, A. P., AND MAKI, T.: Multiple donor composite and sequential allografts: a new approach to pancreatic islet transplantation. Transplant. Proc. **20** (suppl. 1): 891–893, 1988.
489. KAPLAN, E., DRESDALE, A. R., DIEHL, J. T., ARONOVITZ, M. J., KONSTAM, M. A., KATZEN, N. A., GOULD, K. E., ISNER, J. M., CONNOLLY, R. J., PAYNE, D. D., AND CLEVELAND, R. J.: Donor-specific transfusion and cardiac xenografts. Transplantation **46**: 605–607, 1988.
489a. KAPPLER, J. W., ROEHM, N., AND MARRACK, P.: T cell tolerance by clonal elimination in the thymus. Cell **49**: 273–280, 1987.
489b. KAPPLER, J. W., STAERZ, U., WHITE, J., AND MARRACK, P. C.: Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature **332**: 35–40, 1988.
490. KAPPOS, L., PATZOLD, U., DOMMASCH, D., POSER, S., HAAS, J., KRAUSENECK, P., MALIN, J. P., FIERZ, W., GRAFFENRIED, B. U., AND GUGGERLI, U. S.: Cyclosporine versus azathioprine in the long-term treatment of multiple sclerosis—results of the German multicenter study. Ann. Neurol. **23**: 56–63, 1988.
491. KARLSSON-PARRA, A., TÖTTERMAN, T. H., NYBERG, A., MENDEL-HARTVIG, I., LÖÖF, L., AND FORSUM, U.: Immunological effects of ciclosporin in primary biliary cirrhosis: suppression of activated T cells and autoantibody levels. Int. Arch. Allergy Appl. Immunol. **83**: 256–264, 1987.
492. KASAHARA, K., WHITE, D. J. G., AND CALNE, R. Y.: Antigen dependence of cyclosporin A-induced allograft acceptance. Transplantation **34**: 216–218, 1982.
493. KASWAN, R. L., AND KAPLAN, H. J.: Comparison of the efficacy of unilateral, bilateral, and oral cyclosporine in experimental immunogenic uveitis in rabbits. Transplant. Proc. **20** (suppl. 4): 149–157, 1988.
494. KASWAN, R. L., KAPLAN, H. J., AND MARTIN, C. L.: Topically applied cyclosporin for modulation of induced immunogenic uveitis in rabbits. Am. J. Vet. Res. **49**: 1757–1759, 1988.
495. KATO, N., HALPRIN, K. M., AND TAYLOR, J. R.: Cyclosporin A does not inhibit epidermal cell growth at therapeutic levels. J. Invest. Dermatol. **88**: 52–54, 1987.
496. KAUFMAN, D. B., RABE, F. L., STOCK, P. G., VAN DER VLIET, J. A., PLATT, J., AND SUTHERLAND, D. E. R.: Cyclosporine suppresses immune-mediated pancreatic islet allograft primary nonfunction and enhances long-term functional survival. Transplant. Proc. **20** (suppl. 3): 426–430, 1988.
497. KAWASHIMA, H., FUJINO, Y., AND MOCHIZUKI, M.: Effects of a new immunosuppressive agent, FK 506, on experimental autoimmune uveoretinitis in rats. Invest. Ophthalmol. & Visual Sci. **29**: 1265–1271, 1988.
498. KELLY, G. E., SHEIL, A. G. R., WASS, J., AND ZBROJA, R. A.: Effects of ultraviolet radiation and immunosuppressive therapy on mouse epidermal cell kinetics. Br. J. Dermatol. **114**: 197–208, 1986.
499. KEMP, E., KEMP, G., STARKLINT, H., AND LARSEN, S.: Immunosuppression with cobra venom factor, anti-platelet aggregator, and cyclosporin A in renal xenotransplantation. Transplant. Proc. **14**: 116–118, 1982.
500. KENNEDY, M. S., DEEG, H. J., STORB, R., DONEY, K., SULLIVAN, K. M., WITHERSPOON, R. P., APPELBAUM, F. R., STEWART, P., SANDERS, J., BUCKNER, C. D., MARTIN, P., WEIDEN, P., AND THOMAS, E. D.: Treatment of acute graft-versus-host disease after allogeneic marrow transplantation. Randomised study comparing corticosteroids and cyclosporine. Am. J. Med. **78**: 978–983, 1985.
501. KENNEDY, M., WASSMER, P., AND ERB, P.: A novel role for interleukin 2 in antibody responses: down-regulation of T helper cell activation. J. Immunol. **139**: 110–113, 1987.
502. KENYON, R. H., GREEN, D. E., AND PETERS, C. J.: Effect of immunosuppression on experimental Argentine hemorrhagic fever in guinea pigs. J. Virol. **53**: 75–80, 1985.
503. KERMAN, R. H., FLECHNER, S. M., VAN BUREN, C. T., LORBER, M. I., AND KAHAN, B. D.: Immunoregulatory mechanisms in cyclosporine-treated renal allograft recipients. Transplantation. **43**: 205–210, 1987.
504. KERMAN, R. H., WOLINSKY, J. S., NATH, A., AND SEARS, E. S.: Serial immune evaluation of cyclosporine- and placebo-treated multiple sclerosis patients. J. Neuroimmunol. **18**: 325–331, 1988.
505. KIDA, K., KAINO, Y., MIYAGAWA, T., GOTOH, Y., AND MATSUDA, H.: Effect of cyclosporin on insulitis and ICSA in NOD mice. In Insulitis and Type I Diabetes: Lessons from the NOD Mouse, ed. by S. Tarui, Y. Tochino, and K. Nonaka, pp. 137–142, Academic Press, New York, 1986.
506. KIDERLEN, A. F., KIDERLEN, M., AND LOHMANN-MATTHES, M. L.: Cyclosporin A (CyA) and CyA-derivatives protect C57BL/6 mice from infection with Leishmania donovani. (abstract G 18). Immunobiology **173**: 248, 1986.
507. KIERSZENBAUM, F., GOTTLIEB, C. A., AND BUDZKO, D. B.: Exacerbation of Trypanosoma cruzi infection in mice treated with the immunoregulatory agent cyclosporin A. Tropenmed. Parasitol. **34**: 4–6, 1983.
508. KIM, D. H.: The effect of pertussis vaccine and cyclosporin on streptozotocin induced diabetic rats. Yonsei Med. J. **28**: 143–151, 1987.
509. KIM, M. K., CHAN, C. C., NUSSENBLATT, R. B., AND PALESTINE, A. G.: Pharmacologic effects on the expression of class II histocompatibility antigen in experimental endotoxin-induced uveitis. Clin. Immunol. Immunopathol. **45**: 70–77, 1987.
510. KIM, Y. I., AND CALNE, R. Y.: Cyclosporin A stimulates proliferation of the liver cells after partial hepatectomy in rats. Surg. Gynecol. Obstet. **166**: 317–322, 1988.
511. KIM, Y. I., SALVINI, P., AUXILIA, F., AND CALNE, R. Y.: Effect of cyclosporin A on hepatocyte proliferation after partial hepatectomy in rats: comparison with standard immunosuppressive agents. Am. J. Surg. **155**: 245–249, 1988.
512. KIMURA, K., MONEY, S. R., AND JAFFE, B. M.: The effects of cyclosporine on varying segments of small-bowel grafts in the rat. Surgery **104**: 64–69, 1988.
513. KING, P., CLUNIE, G. J. A., AND DUMBLE, L. J.: Pretransplant transfusion and cyclosporine induced enhancement of rabbit skin allografts. Donor-specific versus third-party blood. Transplantation **37**: 418–419, 1984.
514. KING, R. H. M., CRAGGS, R. I., GROSS, M. L. P., TOMPKINS, C., AND THOMAS, P. K.: Suppression of experimental allergic neuritis by cyclosporin-A. Acta Neuropathol. **59**: 262–268, 1983.
515. KIRBY, J. A., PARFETT, G. J., READER, J. A., AND PEPPER, J. R.: Lung transplantation in the rat: a model for study of the cellular mechanisms of allograft rejection. Immunology **63**: 369–372, 1988.
516. KIRBY, J. A., READER, J. A., CORBISHLEY, C. M., PEPPER, J. R., AND HUDSON, L.: Canine unilateral lung transplantation: effect of ciclosporin A on the frequency of donor-lytic lymphocytes. Int. Arch. Allergy Appl. Immunol. **84**: 62–68, 1987.
517. KIRKLAND, T. N., AND FIERER, J.: Cyclosporin A inhibits coccidioides immitis in vitro and in vivo. Antimicrob. Agents Chemother. **24**: 921–924, 1983.
517a. KISIELOW, P., BLÜTHMANN, H., STAERZ, U. D., STEINMETZ, M., AND VON BOEHMER, H.: Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. Nature (Lond.) **333**: 742–746, 1988.
518. KITAGAWA, T., AND LUDVIGSSON, J.: No cyclosporin A to diabetic children. Diabetes Care **11**: 447, 1988.
519. KLAUS, G. G. B.: Cyclosporin as a probe for different modes of lymphocyte activation. Ann. Inst. Pasteur Immunol. **138**: 626–628, 1987.
520. KLAUS, G. G. B.: Cyclosporine-sensitive and cyclosporine-insensitive modes of B cell stimulation. Transplantation **46**: (suppl.): 11S–14S, 1988.
521. KLAUS, G. G. B., AND CHISHOLM, P. M.: Does cyclosporine act in vivo as it does in vitro? Immunol. Today **7**: 101–103, 1986.
522. KLAUS, G. G. B., AND DONGWORTH, D. W.: Effect of cyclosporin A on B cell functions in the mouse. *In* Cyclosporin A, ed. by D. J. G. White, pp. 233–241, Elsevier Biomed. Press, Amsterdam, 1982.
523. KLAUS, G. G. B., AND KUNKL, A.: Effects of cyclosporine on the immune system of the mouse. II. Cyclosporine inhibits the effector function of primary T helper cells, but not helper cell priming. Transplantation **36**: 80–84, 1983.
524. KLEIN, J.: Immunology. The Science of Self-nonself Discrimination. pp. 463–471, John Wiley & Sons, New York, 1982.
525. KLEMPNAUER, J., WAGNER, E., STEINIGER, B., WONIGEIT, K., AND PICHLMAYR, R.: Pancreas and kidney allograft rejection responds differently to cyclosporine immunosuppression. Transplant. Proc. **15**: 3001–3003, 1983.
526. KNECHTLE, S. J., HALPERIN, E. C., AND BOLLINGER, R. R.: Xenograft survival in two species combinations using total-lymphoid irradiation and cyclosporine. Transplantation **43**: 173–175, 1987.
527. KNECHTLE, S. J., HALPERIN, E. C., SAAD, T., AND BOLLINGER, P. R.: Prolonged heart xenograft survival using combined total lymphoid irradiation and cyclosporine. J. Heart Transplant. **5**: 254–261, 1986.
528. KNETEMAN, N. M., ALDERSON, D., AND SCHARP, D. W.: Long-term normoglycemia in pancreatectomized dogs following pancreatic islet allotransplantation and cyclosporine immunosuppression. Transplantation **44**: 595–599, 1987.
529. KNIGHT, S. C., ROBERTS, M., MACATONIA, S. E., AND EDWARDS, A. J.:

Blocking of acquisition and presentation of antigen by dendritic cells with cyclosporine. Studies with fluorescein isothiocyanate. Transplantation **46** (suppl.): 48S–53S, 1988.

530. KOLB, H., OSCHILEWSKI, M., SCHWAB, E., OSCHILEWSKI, U., AND KIESEL, U.: Effect of cyclosporine A on low-dose streptozotocin diabetes in mice. Diabetes Res. **2:** 191–193, 1985.
531. KOPP, W. C., DIERKS, S. E., BUTLER, J. E., UPADRASHTA, B. S., AND RICHERSON, H. L.: Cyclosporine immunomodulation in a rabbit model of chronic hypersensitivity pneumonitis. Am. Rev. Respir. Dis. **132:** 1027–1033, 1985.
532. KOSTAKIS, A. J., WHITE, D. J. G., AND CALNE, R. Y.: Prolongation of rat heart allograft survival by cyclosporin A. IRCS Med. Sci. **5:** 280, 1977.
533. KOSUGI, A., SHARROW, S. O., AND SHEARER, G. M.: Effect of cyclosporin A on lymphopoesis. I. Absence of mature T cells in thymus and periphery of bone marrow transplanted mice treated with cyclosporin A. J. Immunol. **142:** 3036–3032, 1989.
534. KOYAMA, I., WILLIAMS, M., CAMERON, J. L., AND ZUIDEMA, G. D.: Experimental pancreatic allotransplantation in large animals. The role of donor kidney and cyclosporin in modifying rejection. Transplantation **42:** 333–336, 1986.
535. KOYLE, M. A., GLASSOCK, R. J., WARD, H. J., RAJFER, J., AND TWOMEY, P. A.: Declining incidence of wound infection in cadaveric renal transplant recipients. Urology **31:** 103–106, 1988.
536. KREIS, W., AND SORICELLI, A.: Cyclosporins: immunosuppressive agents with antitumour activity. Experientia (Basel) **35:** 1506–1508, 1979.
537. KROCZEK, R. A., BLACK, C. D. V., BARBET, J., AND SHEVACH, E. M.: Mechanism of action of cyclosporin A in vivo. I. Cyclosporin A fails to inhibit T lymphocyte activation in response to alloantigens. J. Immunol. **139:** 3597–3603, 1987.
538. KROGSTAD, D. J., GLUZMAN, I. Y., KYLE, D. E., ODUOLA, A. M. J., MARTIN, S. K., MILHOUS, W. R., AND SCHLESINGER, P. H.: Efflux of chloroquine from Plasmodium falciparum: mechanism of chloroquine resistance. Science (Wash. DC) **238:** 1283–1285, 1987.
539. KROMBACH, F., HAMMER, C., GEBHARD, F., DANKO, I., SCHOLZ, S., AND GOKEL, M.: The effect of cyclosporine on wolf to dog kidney xenografts. Transplant. Proc. **17:** 1436–1437, 1985.
540. KRÖMER, G., BREZINSCHEK, H. P., FÄSSLER, R., SCHAUENSTEIN, K., AND WICK, G.: Physiology and pathology of an immunoendocrine feedback loop. Immunol. Today **9:** 163–165, 1988.
541. KUNKL, A., AND KLAUS, G. G. B.: Selective effects of cyclosporin A on functional B cell subsets in the mouse. J. Immunol. **125:** 2526–2531, 1980.
542. KUPIEC-WEGLINSKI, J. W., FILHO, M. A., STROM, T. B., AND TILNEY, N. L.: Sparing of suppressor cells: a critical action of cyclosporine. Transplantation **38:** 97–101, 1984.
543. KUPIEC-WEGLINSKI, J. W., HEIDECKE, C. D., ARAUJO, J. L., AMMUD-FILHO, M., TOWPIK, E., ARANEDA, D., STROM, T. B., AND TILNEY, N. L.: Behaviour of helper T lymphocytes in cyclosporine-mediated long-term graft acceptance in the rat. Cell. Immunol. **93:** 168–177, 1985.
544. KUPIEC-WEGLINSKI, J. W., LEAR, P. A., HEIDECKE, C. D., AND TILNEY, N. L.: Modification of function and migration patterns of thymocyte populations by cyclosporine after organ transplantation in rats. Transplantation **37:** 631–633, 1984.
545. KUPIEC-WEGLINSKI, J. W., LEAR, P. A., STROM, T. B., AND TILNEY, N. L.: Population of cyclophosphamide-sensitive T suppressor cells maintain cyclosporine induced allograft survival. Transplant. Proc. **15:** 2357–2363, 1983.
546. KUPIEC-WEGLINSKI, J. W., DE SOUSA, M., AND TILNEY, N. L.: The importance of lymphocyte migration patterns in experimental organ transplantation. Transplantation **40:** 1–6, 1985.
547. KURLANSKY, P. A., SADEGHI, A. M., MICHLER, R. E., COPPEY, L. J., RE, L. P., THOMAS, W. G., SMITH, C. R., REEMTSMA, K., AND ROSE, E. A.: Role of the carrier solution in cyclosporine pharmacokinetics in the baboon. J. Heart Transplant. **5:** 312–316, 1986.
548. KURLANSKY, P. A., SADEGHI, A. M., MICHLER, R. E., SMITH, C. R., MARBOE, C. C., THOMAS, W. A., COPPEY, L., AND ROSE, E. A.: Comparable survival of intra-species and cross-species primate cardiac transplants. Transplant. Proc. **19:** 1067–1071, 1987.
549. KUROMOTO, N., HARDY, M. A., FAWWAZ, R., REEMTSMA, K., AND NOWYGROD, R.: Selective lymphoid irradiation and cyclosporin A in rat allografts. J. Surg. Res. **36:** 428–432, 1984.
550. KURTZ, J. B., AND HOMAN, W. P.: Murine cytomegalovirus infection and cyclosporin A. Transplantation **34:** 215–216, 1982.
551. KUTLU, H. M., SADEGHI, A. M., NORTON, J. E., MARBOE, C. C., SMITH, C. R., REEMTSMA, K., AND ROSE, E. A.: Effect of simultaneous lung transplantation on heart transplant survival in rats. J. Heart Transplant. **6:** 29–33, 1987.
552. KYOGOKU, M., NOSE, M., SAWAI, T., MIYAZAWA, M., TACHIWAKI, O., AND KAWASHIMA, M.: Immunopathology of murine lupus—overview, SL/Ni, and MRL/Mp-1pr/1pr—. In Animal Models: Assessing the Scope of Their Use in Biomedical Research, pp. 95–130, Alan R. Liss, New York, 1987.
553. KYRIAKIDES, G. K., ESQUENAZI, V., OLSON, L., MILGROM, M., AND MILLER, J.: Cyclosporin inhibits nonspecific transfusion-induced immunity in dogs. Transplant. Proc. **19:** 1432–1433, 1987.
554. KYRIAKIDES, G. K., OLSON, L., FLAA, C., AND MILLER, J.: Reversal of kidney and prevention of pancreas transplant rejection with cyclosporine in beagles. Transplant. Proc. **15:** 2950–2952, 1983.
555. LAFFERTY, K. J.: The immunologic network. Transplant. Proc. **20** (suppl. 2): 13–26, 1988.
556. LAFFERTY, K. J., GILL, R., AND BABCOCK, S.: Tolerance induction in adult animals. The ciclosporin anomaly. Prog. Allergy **38:** 247–257, 1986.
557. LAFFERTY, K. J., GILL, R. G., BABCOCK, S. K., AND SIMEONOVIC, C. J.: Activation and expression of allograft immunity. Prog. Transplant. **3:** 55–84, 1986.
558. LAFFERTY, K. J., AND PARIS, L. L.: Cyclosporine and the regulation of autoimmune disease. J. Autoimmun. **1:** 519–532, 1988.
559. LAFFERTY, K. J., PROWSE, S. J., AND SIMEONOVIC, C. J.: Immunobiology of tissue transplantation: a return to the passenger leukocyte concept. Annu. Rev. Immunol. **1:** 143–173, 1983.
560. LAGRANGE, P. H., AND HURTEL, B.: Delayed-type hypersensitivity reactions. Adv. Inflamm. Res. **10:** 275–280, 1986.
561. LAI, C. S., WESSELER, T. A., ALEXANDER, J. W., AND BABCOCK, G. F.: Long-term survival of skin allografts in rats treated with topical cyclosporine. Transplantation **44:** 83–87, 1987.
562. LANCASTER, F., CHUI, Y. L., AND BATCHELOR, J. R.: Anti-idiotypic T cells suppress rejection of renal allografts in rats. Nature (Lond.) **315:** 336–337, 1985.
563. LAND, W., CASTRO, L. A., WHITE, D. J. G., HILLEBRAND, G., HAMMER, C., KLARE, B., AND FORNARA, P.: Ciclosporin in renal transplantation. Prog. Allergy **38:** 293–328, 1986.
564. LANDEGREN, U., RAMSTEDT, U., AXBERG, I., ÖRN, A., AND WIGZELL, H.: Cyclosporin A permits the distinction between specific T and NK activity generated in a human MLC. Int. J. Cancer **28:** 725–730, 1981.
565. LANGER, A., ROSENMANN, E., AND NAOR, D.: The effect of cyclosporin on murine autoreactive delayed-type hypersensitivity induced with syngeneic lymphoblasts. Immunopharmacology **10:** 147–155, 1985.
566. LARSON, D. F.: Mechanism of action: antagonism of the prolactin receptor. Prog. Allergy **38:** 222–238, 1986.
567. LARSON, D. F., COPELAND, J. G., AND RUSSELL, D. H.: Prolactin predicts cardiac allograft rejection in cyclosporin immunosuppressed patients. Lancet **II:** 53 (July 6), 1985.
568. LAU, H., REEMTSMA, K., AND HARDY, M. A.: The use of direct ultraviolet irradiation and cyclosporine in facilitating indefinite pancreatic islet allograft acceptance. Transplantation **38:** 566–569, 1984.
569. LAUPACIS, A., STILLER, C. R., GARDELL, C., KEOWN, P., DUPRÉ, J., WALLACE, A. C., AND THIBERT, P.: Cyclosporin prevents diabetes in BB Wistar rats. Lancet I: 10–12, 1983.
570. LAUTENSCHLAGER, I., HÖCKERSTEDT, K., TASKINEN, E., AHONEN, J., KORSBÄCK, C., SALMELA, K., ORKO, R., SCHEININ, B., SCHEININ, T. M., AND HÄYRY, P.: Fine-needle aspiration cytology of liver allografts in the pig. Transplantation **38:** 330–334, 1984.
571. LAW, P. K., GOODWIN, T. G., AND LI, H. J.: Histoincompatible myoblast injection improves muscle structure and function of dystrophic mice. Transplant. Proc. **20** (suppl. 3): 1114–1119, 1988.
572. LECHLER, R. I., AND BATCHELOR, J. R.: Restoration of immunogenecity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J. Exp. Med. **155:** 31–41, 1982.
573. LEDERMANN, J. A., BEGENT, R. H. J., BAGSHAWE, K. D., RIGGS, S. J., SEARLE, F., GLASER, M. G., GREEN, A. J., AND DALE, R. G.: Repeated antitumour therapy in man with suppression of the host response by cyclosporin A. Br. J. Cancer **58:** 654–657, 1988.
574. LEFKOWITZ, M., KORNBLUTH, J., TOMASZEWSKI, J. E., AND JORKASKY, D. K.: Natural killer-cell activity in cyclosporine-treated renal allograft recipients. J. Clin. Immunol. **8:** 121–127, 1988.
575. LEMAIRE, M., PARDRIDGE, W. M., AND CHANDHURI, G.: Influence of blood components on the tissue uptake indices of cyclosporin in rats. J. Pharmacol. Exp. Ther. **244:** 740–743, 1988.
576. LEMS, S. P. M., CAPEL, P. J. A., AND KOENE, R. A. P.: Rejection of long-surviving mouse skin allografts after withdrawal of cyclosporin A therapy. Transplant. Proc. **12:** 283–286, 1980.
577. LEMS, S. P. M., AND KOENE, R. A. P.: Prolongation of mouse skin allograft survival by cyclosporin A: graft rejection after withdrawal of therapy. IRCS Med. Sci. **7:** 184, 1979.
578. LEONI, P., GARCIA, R. C., AND ALLISON, A. C.: Effects of cyclosporin A on human lymphocytes in culture. J. Clin. Lab. Immunol. **1:** 67–72, 1978.
578a. LESLIE, K., BLAY, R., HAISCH, C., LODGE, A., WELLER, A., AND HUBER, S.: Clinical and experimental aspects of viral myocarditis. Clin. Microbiol. Rev. **2:** 191–203, 1989.
579. LEVINE, S., AND SOWINSKI, R.: Suppression of the hyperacute form of experimental allergic encephalomyelitis by drugs. Arch. Int. Pharmacodyn. Ther. **230:** 309–318, 1977.
580. LÉVY-MARCHAL, C., AND CZERNICHOW, P.: Cyclosporin A in insulin-dependent diabetes mellitus of recent onset: a pilot study in children. Horm. Res. (Basel) **29:** 177–184, 1988.
581. LEWIS, R. M., JOHNSON, P. C., GOLDEN, D., VAN BUREN, C. T., KERMAN, R. M., AND KAHAN, B. D.: The adverse impact of cytomegalovirus infection on clinical outcome in cyclosporine-prednisone treated renal allograft recipients. Transplantation **45:** 353–359, 1988.
582. LEXER, G. H., COOPER, D. K. C., WICOMB, W. N., ROSE, A. G., REES, J.,

KERAAN, M., REICHART, B., AND DUTOIT, E.: Cardiac transplantation using discordant xenografts in a nonhuman primate model. Transplant. Proc. **19:** 1153–1154, 1987.

583. LI, S. Y., AND NELSON, D. S.: Effects of cyclosporin A on the production of experimental anti-erythrocyte autoantibodies in mice. Int. J. Immunopharmacol. **8:** 213–219, 1986.
584. LIKE, A. A., DIRODI, V., THOMAS, S., GUBERSKI, D. L., AND ROSSINI, A. A.: Prevention of diabetes mellitus in the BB/W rat with cyclosporin A. Am. J. Pathol. **117:** 92–97, 1984.
585. LILLEHOJ, H. S.: Effects of immunosuppression on avian coccidiosis: cyclosporine A but not hormonal bursectomy abrogates host protective immunity. Infect. Immun. **55:** 1616–1621, 1987.
586. LIM, S. M. L., WHITE, D. J. G., AND CALNE, R. Y.: Identifying a susceptible period following cyclosporine A-induced tolerance of heart grafts in the rat. Transplant. Proc. **19:** 4218–4220, 1987.
587. LIM, S. M. L., WHITE, D. J. G., AND CALNE, R. Y.: Cyclosporin A-induced acceptance of major histocompatibility complex-incompatible grafts differs immunologically from class I or minor antigen-mismatched grafts accepted in the absence of immunosuppression. Transplant. Proc. **19:** 4252–4253 1987.
588. LIM, S. M. L., WHITE, D. J. G. AND CALNE, R. Y.: Cyclosporin coverage during the risk period leads to 100% long-term graft acceptance in the rat. Transplant. Proc. **20** (suppl. 3): 1013–1015, 1988.
589. LIM, S. M. L., WHITE, D. J. G., AND CALNE, R. Y.: Unresponsiveness to class I antigens is not equal to tolerance to class I antigens induced by cyclosporine. Transplant. Proc. **20** (suppl. 3): 1031–1033, 1988.
590. LINDSEY, N. J., HARRIS, K. R., NORMAN, H. B., SMITH, J. L., LEE, H. A., AND SLAPAK, M.: The effect of cyclosporin A on the primary and secondary immune responses in the rabbit. Transplant. Proc. **12:** 252–255, 1980.
591. LINN, T., VOLKMANN, A., GERMANN, H., WOEHRLE, M., BRETZEL, R. G., BICKER, U., AND FEDERLIN, K.: Ciamexon in the low dose streptozotocin induced diabetes of mice. Diabetes Res. **6:** 113–117, 1987.
592. LIVERSIDGE, J., THOMPSON, A. W., SEWELL, H. F., AND FORRESTER, J. V.: EAU in the guinea pig: inhibition of cell-mediated immunity and Ia antigen expression by cyclosporin A. Clin. Exp. Immunol. **69:** 591–600, 1987.
593. LIVERSIDGE, J., THOMPSON, A. W., SEWELL, H. F., AND FORRESTER, J. V.: Cyclosporine A, experimental autoimmune uveitis, and major histocompatibility class II antigen expression of cultured retinal pigment epithelial cells. Transplant. Proc. **20** (suppl. 4): 163–169, 1988.
594. LÖLIGER, C., AND LEHMANN-GRUBE, F.: Mechanism of recovery from acute virus infection. II. Effect of treatment of mice with cyclosporine A on their ability to eliminate the lymphocytic choriomeningitis virus. Med. Microbiol. Immunol. **174:** 187–196, 1985.
595. LOVETT, J., NYBERG, L. M., BROWN, S., AND MATHUR, S.: Suppression of post-vasectomy cytotoxic sperm antibody formation in rats by a short-term pretreatment with cyclosporine. Am. J. Reprod. Immunol. Microbiol. **11:** 65–68, 1986.
596. LOWDER, J. N., MILLER, R. A., HOPPE, R., AND LEVY, R.: Suppression of anti-mouse immunoglobulin antibodies in subhuman primates receiving murine monoclonal antibodies against T cell antigens. J. Immunol. **138:** 401–406, 1987.
597. MACK, D. G., AND MCLEOD, R.: New micromethod to study the effect of antimicrobial agents on Toxoplasma gondii: comparison of sulfadoxine and sulfadiazine individually and in combination with pyrimethamine and study of clindamycin, metronidazole and cyclosporin A. Antimicrob. Agents Chemother. **26:** 26–30, 1984.
598. MADARA, J. L., AND KIRKMAN, R. L.: Structural and functional evolution of jejunal allograft rejection in rats and the ameliorating effects of cyclosporine therapy. J. Clin. Invest. **75:** 502–512, 1985.
599. MADHOK, R., AND CAPELL, H. A.: Cyclosporine A in rheumatoid arthritis: results at 30 months. Transplant. Proc. **20** (suppl. 4): 248–252, 1988.
600. MAGANTO, P., CIENFUEGOS, J. A., SANTAMARIA, L., CODESAL, J., TENDILLO, F., AND CASTILLO-OLIVARES, J. L.: Effect of ciclosporin on allogeneic hepatocyte transplantation: a morphological study. Eur. Surg. Res. **20:** 248–253, 1988.
600a. MAHLBERG, U., UUSITALO, H., UUSITALO, R., PALKAMA, A., AND TALLBERG, T.: Suppression of experimental autoimmune uveitis in guinea pigs by ethylenediamine tetra-acetic acid, corticosteroids, and cyclosporin. J. Ocul. Pharmacol. **3:** 199–210, 1987.
601. MAHER, E. R., SWENY, P., CHAPPEL, M., VARGHESE, Z., AND MOORHEAD, J. F.: Cyclosporin in the treatment of steroid-responsive and steroid-resistant nephrotic syndrome in adults. Nephrol. Dial. Transplant. **3:** 728–732, 1988.
602. MAHON, J. L., GUNN, H. C., STOBIE, K., GIBSON, C., GARCIA, B., DUPRÉ, J., AND STILLER, C. R.: The effect of bromocriptine and cyclosporine on spontaneous diabetes in BB rats. Transplant. Proc. **20** (suppl. 4): 197–200, 1988.
603. MAIER, T., HOLDA, J. H., AND CLAMAN, H. N.: Natural suppressor (NS) cells. Immunol. Today **7:** 312–315, 1986.
604. MAKOVER, D., FREUNDLICH, B., AND ZURIER, R. B.: Relapse of systemic lupus erythematosus in a patient receiving cyclosporine A. J. Rheumatol. **15:** 117–119, 1988.
605. MARCOS, M. A. R., DE LA HERA, A., GASPAR, M. L., MÁRQUEZ, C., BELLAS, C., MAMPASO, F., TORIBIO, M. L., AND MARTÍNEZ-A, C.: Modification of emerging repertoires by immunosuppression in immunodeficient mice results in autoimmunity. Immunol. Rev. **94:** 51–74, 1986.
606. MARCOS, M. A. R., GASPAR, M. L., TORIBIO, M. L., AND MARTÍNEZ-A., C.: Syngeneic graft-versus-host disease induced by cyclosporine: a reappraisal. Transplantation **47:** 1096, 1989.
607. MARGREITER, R., HUBER, C., SPIELBERGER, M., AND KÖNIG, P.: Cyclosporine in the treatment of acute cadaveric kidney graft rejection refractory to high dose methylprednisolone. Transplantation **36:** 203–204, 1983.
608. MARGREITER, R., KORNBERGER, R., KOLLER, J., STEINER, E., SPIELBERGER, M., AIGNER, F., SCHMID, T., AND VOGEL, W.: Can a liver graft from the same donor protect a kidney from rejection? Transplant. Proc. **20** (suppl. 1): 522–523, 1988.
609. MARKHAM, D. F., GRIFFITH, B. P., LERNER, E., LUCIA, H. L., AND BIA, F. J.: Effects of cyclosporine on chronic cytomegalovirus infection in the guinea pig. Intervirology **28:** 171–180, 1987.
610. MARNI, A., FERRERO, M. E., TIENGO, M., AND GAJA, G.: Graft-versus-host and host-versus-graft reactions after small intestine allografts in hyperimmunized rats: effect of cyclosporine treatment. Transplant. Proc. **19:** 1207–1211, 1987.
611. MARQUET, R. L., WEIMAR, W., HEINEMAN, E., AND JEEKEL, J.: Inhibition of chronic kidney allograft rejection by cyclosporine. Transplant. Proc. **15:** 2953–2955, 1983.
612. MARTIN, D. C., DOWDY, S. F., HEWITT, C. W., BLACK, K. S., DOMINGUEZ, D., AND GONZALEZ, G. A.: Comparison of renal allograft survival in rats pretreated with multiple blood transfusions combined with either cyclosporine or azathioprine. Transplant. Proc. **17:** 1087–1090, 1985.
613. MARTIN, D. C., HEWITT, C. W., BLACK, K. S., DOWDY, S. F., QUINTERO, C. S., AND REYES, M.: Extensive prolongation of rat renal allograft survival following donor or nonspecific transfusions and concomitant immunosuppressant. Transplantation **43:** 790–794, 1987.
614. MARTINELLI, G. P., CHUNG-LOY, R., SHER, L., RACELIS, D., MILLER, C. M., AND SCHANZER, H.: Long-term prolongation of cardiac allografts by subtherapeutic levels of cyclosporine in rats conditioned with pretransplant blood transfusions and cyclosporine. Transplantation **39:** 1–5, 1985.
615. MARTINELLI, G. P., HOROWITZ, C., CHIANG, K., RACELIS, D., AND SCHANZER, H.: Pretransplant conditioning with donor-specific transfusions using heated blood and cyclosporine. Preservation of the transfusion effect in the absence of sensitisation. Transplantation **43:** 140–145, 1987.
616. MARWICK, J. R., CHAMBERS, J. D., HOBBS, J. R., AND PEGRUM, G. D.: Timing of cyclosporin-A therapy for abrogation of HvG and GvH responses in rats. Lancet **II:** 1037–1040, 1979.
617. MASON, D. W., AND MORRIS, P. J.: Inhibition of the accumulation, in rat kidney allografts, of specific—but not nonspecific—cytotoxic cells by cyclosporine. Transplantation **37:** 46–51, 1984.
618. MATAS, A. J., TELLIS, V. A., QUINN, T. A., GLICKLICH, D., SOBERMAN, R., AND VEITH, F. J.: Successful transplantation of highly sensitized patients without regard to HLA matching. Transplantation **45:** 338–342, 1988.
619. MATSUMOTO, Y., PERRY, G., SCHEIBEL, L. W., AND AIKAWA, M.: Role of calmodulin in Plasmodium falciparum: implications for erythrocyte invasion by the merozoite. Eur. J. Cell. Biol. **45:** 36–43, 1987.
620. MATTER, B. E., DONATSCH, P., RACINE, R. R., SCHMID, B., AND SUTER, W.: Genotoxicity evaluation of cyclosporin A, a new immunosuppressive agent. Mutat. Res. **105:** 257–264, 1982.
621. MAYER, G., WATSCHINGER, B., POHANKA, E., GRAF, H., POPOW, T., ULRICH, W., AND KOVARIK, J.: Cytomegalovirus infection after kidney transplantation using cyclosporine A and low-dose prednisolone immunosuppression. Nephrol. Dial. Transplant. **3:** 464–468, 1988.
622. MAYUMI, H., KAYASHIMA, K., SHIN, T., AND NOMOTO, K.: Drug-induced tolerance to allografted mice. V. Prolongation of skin graft survival in tolerant mice with combined immunosuppressive treatments. Transplantation **39:** 335–337, 1985.
623. MCCABE, R. E., LUFT, B. J., AND REMINGTON, J. S.: The effects of cyclosporine on Toxoplasma gondii in vivo and in vitro. Transplantation **41:** 611–615, 1986.
624. MCCABE, R. E., REMINGTON, J. S., AND ARAUJO, F. G.: In vivo and in vitro effects of cyclosporin A on Trypanosoma cruzi. Am. J. Trop. Med. Hyg. **34:** 861–865, 1985.
625. MCGINNES, K., LUCKHURST, E., AND PENNY, R.: Natural killer cell activity in cardiac transplant recipients. Transplantation **44:** 460–463, 1987.
626. MCGONIGLE, R. J. S., BEAMAN, M., STONE, J., YOUNG, J., MICHAEL, J., AND ADU, D.: Does cyclosporin A adversely affect Pneumocystis carinii infection? Postgrad. Med. J. **64:** 659–662, 1988.
627. MCGREGOR, A. M., RENNIE, D. P., WEETMAN, A. P., HASSMAN, R. A., FOORD, S. M., DIEGUEZ, C., AND HALL, R.: The influence of cyclosporine A on experimental autoimmune thyroid disease in the rat. Life Sci. **32:** 97–108, 1983.
628. MCGREGOR, C. G. A., BALDWIN, J. C., JAMIESON, S. W., BILLINGHAM, M. E., YOUSEM, S. A., BURKE, C. M., OYER, P. E., STINSON, E. B., AND SHUMWAY, N. E.: Isolated rejection after combined heart-lung trans-

plantation. J. Thorac. Cardiovasc. Surg. **90:** 623–630, 1985.
629. McGregor, C. G. A., Oyer, P. E., and Shumway, N. E.: Heart and heart-lung transplantation. Prog. Allergy **38:** 346–365, 1986.
630. McIntosh, K. R., and Drachman, D. B.: Induction of suppressor cells specific for AChR in experimental autoimmune myasthenia gravis. Science (Wash. DC) **232:** 401–403, 1986.
631. McIntosh, K. R., and Drachman, D. B.: Properties of suppressor cells induced to acetylcholine receptor using cyclosporine A. Ann. NY Acad. Sci. **505:** 628–638, 1987.
632. McKenzie, F. N., Stiller, C. R., Wall, W. J., Keown, P., Silver, M. D., Painvin, A., and Heimbecker, R. O.: Studies of canine heterotopic heart allografts treated with cyclosporine-A. Transplant. Proc. **15:** 1244–1246, 1983.
633. McKenzie, R. C., Epand, R. M., and Johnson, D. C.: Cyclosporine A inhibits herpes simplex virus-induced cell fusion but not virus penetration into cells. Virology **159:** 1–9, 1987.
634. McWhinnie, D. L., Dallman, M. J., and Morris, P. J.: The influence of cyclosporine on cellular infiltration in rat renal allografts. Transplant. Proc. **19:** 345–347, 1987.
635. Meador, J., Sweet, P., Stupecky, M., Wetzel, M., Murray, S., Gupta, S., and Slater, L.: Enhancement by cyclosporin A of daunorubicin efficacy in Ehrlich ascites carcinoma and murine hepatoma 129. Cancer Res. **47:** 6216–6219, 1987.
636. Metzger, J. M., and Peterson, L. B.: Cyclosporin A enhances the pulmonary granuloma response induced by Schistosoma mansoni eggs. Immunopharmacology **15:** 103–115, 1988.
637. Meyers-Elliott, R. H., Chitjian, P. A., and Billups, C. B.: Effects of cyclosporine A on clinical and immunological parameters in herpes simplex keratitis. Invest. Ophthalmol. & Visual Sci. **28:** 1170–1180, 1987.
638. Meyers-Elliott, R. H., Chitjian, P. A., and Billups, C. L.: Effect of cyclosporine A on the corneal inflammatory response in herpes simplex virus keratitis. Exp. Eye Res. **45:** 281–303, 1987.
639. Michler, R. E., Marboe, C. C., Socha, W. W., Moor-Jankowski, J., Reemtsma, K., and Rose, E. A.: Simian-type blood group antigens in nonhuman primate cardiac xenotransplantation. Transplant. Proc. **19:** 4456–4462, 1987.
640. Michler, R. E., McManus, R. P., Smith, C. R., Sadeghi, A. N., Marboe, C. C., Reemtsma, K., and Rose, E. A.: Prolongation of primate cardiac xenograft survival with cyclosporine. Transplantation **44:** 632–636, 1987.
641. Michler, R. E., Socha, W. W., Marboe, C. C., Smith, C. R., Reemtsma, K., Moor-Jankowski, J., and Rose, E. A.: Macaque-to-baboon cardiac transplantation: model of choice for xenotransplantation in humans. Transplant. Proc. **20:** 327–328, 1988.
642. Miescher, P. A., Favre, H., Mihatsch, M. J., Chatelanat, F., Huang, Y. P., and Zubler, R.: The place of cyclosporin A in the treatment of connective tissue diseases. Transplant. Proc. **20** (suppl. 4): 224–237, 1988.
643. Miescher, P. A., and Miescher, A.: Combined ciclosporin steroid treatment of systemic lupus erythematosus. In Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 337–345, Springer-Verlag, Berlin, 1985.
644. Mihatsch, M. J., Thiel, G., and Ryffel, B.: Hazards of cyclosporine A therapy and recommendations for its use. J. Autoimmun. **1:** 533–543, 1988.
645. Millard, P. R., Garvey, J. F. W., Jefferey, E. L., and Morris, P. J.: The grafted fetal rat pancreas. Features of development and rejection. Am. J. Pathol. **100:** 209–224, 1980.
646. Miller, A. M., Walma, E. P., Klapwijk, W., and van Bekkum, D. W.: The effect of cyclosporine on host-versus-graft disease in canine bone marrow transplantation. Transplant. Proc. **15:** 3046–3049, 1983.
647. Miller, G. P. G., and Lewis, D. E.: In vitro effect of cyclosporine on interleukin-2 receptor expression stimulated by Cryptococcus neoformans. J. Infect. Dis. **155:** 799–802, 1987.
648. Miller, K., Huber, C., Niederwieser, D., and Göttinger, W.: Successful engraftment of high-risk corneal allografts with short-term immunosuppression with cyclosporine. Transplantation **45:** 651–653, 1988.
649. Miller, R. A., Shen, J. Y., Rea, T. H., and Harnisch, J. P.: Treatment of chronic erythema nodosum leprosum with cyclosporine A produces clinical and immunohistologic remission. Int. J. Lepr. **55:** 441–449, 1987.
650. Miller, T. E., and Findon, G.: Modulation of host defenses against pyogenic microorganisms by cyclosporine. Transplantation **42:** 463–466, 1986.
651. Miller, T. E., and Findon, G.: Exacerbation of experimental pyelonephritis by cyclosporin A. J. Med. Microbiol. **26:** 245–250, 1988.
652. Miller, T. E., Findon, G., and Cawley, S.: Cellular basis of host defence in pyelonephritis. III. Deletion of individual components. Br. J. Exp. Pathol. **68:** 377–388, 1987.
653. Milon, G., Truffa-Bachi, P., Shidani, B., and Marchal, G.: Cyclosporin A inhibits the delayed-type hypersensitivity effector function of T lymphocytes without affecting their clonal expansion. Ann. Inst. Pasteur Immunol. **135D:** 237–245, 1984.
654. Milton, A. D., Spencer, S. C., and Fabre, J. W.: The effects of cyclosporine on the induction of donor class I and class II MHC antigens in heart and kidney allografts in the rat. Transplantation **42:** 337–347, 1986.
655. Mirisklavos, A., Mottram, P. L., Dumble, L. J., and Clunie, G. J. A.: Characterisation of cyclosporine-induced suppressor cells in murine delayed-type hypersensitivity responses. Int. Arch. Allergy Appl. Immunol. **79:** 215–219, 1986.
656. Mizoguchi, Y., Kuboi, H., Kodama, C., Sakagami, Y., Seki, S., Kobayashi, K., Yamamoto, S., and Morisawa, S.: The protective effect of cyclosporin A on experimentally-induced acute hepatic injury in mice. Gastroenterol. Jpn. **22:** 743–747, 1987.
657. Mochizuki, M., Fujino, Y., and Okumura, A.: Antigen-specific suppressor cells induced by cyclosporine. Transplant. Proc. **20** (suppl. 4): 158–162, 1988.
658. Mochizuki, M., Nussenblatt, R. B., Kuwabara, T., and Gery, I.: Effects of cyclosporine and other immunosuppressive drugs on experimental autoimmune uveoretinitis in rats. Invest. Ophthalmol. & Visual Sci. **26:** 226–232, 1985.
659. Mody, C. H., Toews, G. B., and Lipscomb, M. F.: Cyclosporin-A inhibits the growth of Cryptococcus neoformans in a murine model. Clin. Res. **35:** 536A (abstract), 1987.
660. Mody, C. H., Toews, G. B., and Lipscomb, M. F.: Treatment of murine cryptococcosis with cyclosporine-A in normal and athymic mice. Am. Rev. Respir. Dis. **139:** 8–13, 1989.
661. Monaco, A. P., Wood, M. L., Maki, T., Hartner, W., De Fazio, S., and Gozzo, J. J.: Cyclosporine and unresponsiveness to allograft induced by polyclonal antilymphocyte serum and donor-specific bone marrow. Transplant. Proc. **20** (suppl. 3): 36–48, 1988.
662. Monden, M., Valdivia, L. A., Gotoh, M., Hasuike, Y., Kubota, N., Kanai, T., Okamura, J., and Mori, T.: Hamster to rat orthotopic liver transplantation. Transplantation **43:** 745–746, 1987.
663. Monnickendam, M. A., Kirton, R. P., and Darougar, S.: The importance of cellular immune responses in an animal model of trachoma. Poster presented at "Cellular Mechanisms in Infection Immunity", Elsinore, June 1988.
664. Monrad, E. S., Matsumori, A., Murphy, J. C., Fox, J. G., Crumpacker, C. S., and Abelmann, W. H.: Therapy with cyclosporine in experimental murine myocarditis with encephalomyocarditis virus. Circulation **73:** 1058–1064, 1986.
665. Mori, Y., Suko, M., Okudaira, H., Matsuba, I., Tsuruoka, A., Sasaki, A., Yokoyama, H., Tanase, T., Shida, T., Nishimura, M., Terada, E., and Ikeda, Y.: Preventive effects of cyclosporin on diabetes in NOD mice. Diabetologia **29:** 244–247, 1986.
666. Morris, P. J.: Cyclosporin A. Overview. Transplantation **32:** 349–354, 1981.
667. Morris, P. J., Mason, D. W., and Hutchinson, I. V.: The effect of cyclosporine A on lymphocytes in animal models of tissue transplantation. Transplant. Proc. **15:** 2287–2292, 1983.
668. Mottram, P. L., Mirisklavos, A., Dumble, L. J., and Clunie, G. J. A.: Effects of cyclosporin A, antilymphocyte serum and donor-specific transfusions on murine delayed-type hypersensitivity and skin graft survival. Int. Arch. Allergy Appl. Immunol. **79:** 296–304, 1986.
669. Mountz, J. D., Smith, H. R., Wilder, R. L., Reeves, J. P., and Steinberg, A. D.: CS-A therapy in MRL-lpr/lpr mice: amelioration of immunopathology despite autoantibody production. J. Immunol. **138:** 157–163, 1987.
670. Moutier, R., Toyama, K., Lamendin, H., and Bach, J. F.: Guérison de l'ostéopétrose par injection de moelle osseuse allogénique chez le rat "op" traité par la cyclosporine A. C. R. Acad. Sci. (Paris) **301:** 483–485, 1985.
671. Movsowitz, C., Epstein, S., Fallon, M., Ismail, F., and Thomas, S.: Cyclosporin-A in vivo produces severe osteopenia in the rat: effect of dose and duration of administration. Endocrinology **123:** 2571–2577, 1988.
672. Mrowietz, U., and Christophers, E.: Effects of cyclosporine A treatment on psoriasis. I. Influence of low-dose cyclosporine on human monocyte function in vitro. Transplant. Proc. **20** (suppl. 4): 53–57, 1988.
673. Mu, L., and Eastman, C. J.: Cyclosporin-induced dysmorphic changes in the BB/W rat. Lancet **II:** 963 (October 24), 1987.
674. Müller, W., and Herrmann, B.: Cyclosporin A for psoriasis. N. Engl. J. Med. **301:** 555, 1979.
675. Müller, C., Zielinski, C., Treiber, G., and Schernthaner, G.: Natural killer cell activity and antibody-dependent cellular cytotoxicity in recent-onset type I diabetes mellitus: a follow-up study under cyclosporin A treatment. Acta Endocrinol. (Kbh.) **111** (suppl. 274): 129 (abstr. 148), 1986.
676. Muller, S., Adorini, L., Appella, E., and Nagy, Z. A.: Lack of influence of cyclosporine on antigen presentation to lysozyme-specific T cell hybridomas. Transplantation **46** (suppl.): 44S–48S, 1988.
677. Müller-Hermelink, H. K., Kaiserling, E., and Sonntag, H. G.: Modulation of epithelioid cell granuloma formation to apathogenic mycobacteria by cyclosporin A. Path. Res. Pract. **175:** 80–96, 1982.
677a. Mukhergee, P., Mastro, A. M., and Hymer, W. C.: Prolastin induction of interleukin-2 receptors on rat splenic lymphocytes. Endocrinology **126:** 88–94, 1990.
678. Murase, N., Todo, S., Lee, P. H., Lai, H. S., Chapman, F., Nalesnik, F. A., Makowka, L., and Starzl, T. E.: Heterotopic heart transplantation in the rat receiving FK 506 alone or with cyclosporine. Transplant. Proc. **19** (suppl. 1): 71–75, 1987.

679. MURPHY, J. R., BAQAR, S., BAKER, R. H., ROBERTS, E., NICKELL, S. P., AND COLE, G. A.: Stage-selective inhibition of rodent malaria by cyclosporine. Antimicrob. Agents Chemother. **32:** 462–466, 1988.
680. MYBURGH, J. A.: Total lymphoid irradiation in transplantation. Transplant. Proc. **20** (suppl. 1)**:** 118–121, 1988.
681. MYBURGH, J. A., MEYERS, A. M., BOTHA, J. R., THOMSON, P. D., SMIT, J. A., AND LAKIER, R.: Total lymphoid irradiation in clinical kidney transplantation. Clin. Transplant. **1:** 65–70, 1987.
682. NAGAO, T., WHITE, D. J. G., AND CALNE, R. Y.: Kinetics of unresponsiveness induced by a short course of cyclosporin A. Transplantation **33:** 31–35, 1982.
683. NAGY-OLTVAI, Z., JENNINGS, T. A., BRADY, T. G., LUCIA, H. L., ARMSTRONG, J. A., AND HSIUNG, G. D.: Effect of cyclosporin A immunosuppression on primary lymphotropic herpes virus infection in the guinea pig. Intervirology **28:** 105–109, 1987.
684. NAKAGAWA, S., OKA, D., JINNO, Y., TAKEI, Y., BANG, D., AND UEKI, H.: Topical application of cyclosporine on guinea pig allergic contact dermatitis. Arch. Dermatol. **124:** 907–910, 1988.
685. NAKAJIMA, Y., KESSLER, M., NAKANO, H., AND LIE, T. S.: Restoration of T cell responsiveness to interleukin-2 in recipients of pancreatic islet xenografts treated with cyclosporine. Transplantation **40:** 73–76, 1985.
686. NAKANE, A., MINAGAWA, T., YASUDA, I., YU, C., AND KATO, K.: Prevention by gamma interferon of fatal infection with Listeria monocytogenes in mice treated with cyclosporin A. Infect. Immun. **56:** 2011–2015, 1988.
687. NAKAYASU, H., OTA, K., TANAKA, H., IRIE, H., AND TAKAHASHI, K.: Suppression of experimental allergic neuritis by cyclosporin A. Ann. NY Acad. Sci. **540:** 546–548, 1988.
688. NAOR, D., AND LANGER, A.: Analysis of the cyclosporine mechanism in an immunological autoreactive model of delayed-type hypersensitivity. Transplant. Proc. **17:** 2706–2708, 1985.
689. NARAYANAN, K. M., SWARTZ, W. M., STARK, B., MOLLER, A., AND MUNGER, B. L.: Preservation of nerve function in an allogenic hand transplant model in primates. Transplant. Proc. **20** (suppl. 1)**:** 335–336, 1988.
690. NASH, J. R., AND BELL, R. P. F.: Islet transplantation synergism between antilymphocyte and antimacrophage agents. J. Surg. Res. **36:** 154–157, 1984.
691. NEILD, G. H., IVORY, K., HIRAMATSU, M., AND WILLIAMS, D. G.: Cyclosporin A inhibits acute serum sickness nephritis in rabbits. Clin. Exp. Immunol. **52:** 586–594, 1983.
692. NEILD, G. H., IVORY, K., AND WILLIAMS, D. G.: Glomerular thrombosis and cortical infarction in cyclosporin-treated rabbits with acute serum sickness. Br. J. Exp. Pathol. **65:** 133–144, 1984.
693. NEILD, G. H., IVORY, K., AND WILLIAMS, D. G.: Severe systemic vascular necrosis in cyclosporin-treated rabbits with acute serum sickness. Br. J. Exp. Pathol. **65:** 731–743, 1984.
694. NEILD, G. H., IVORY, K., AND WILLIAMS, D. G.: Effect of cyclosporine on proteinuria in chronic serum sickness in rats. Clin. Nephrol. **25** (suppl. 1)**:** 186S–188S, 1986.
695. NEUBERGER, J., ALEXANDER, G., AL-AGHBAR, M. N., LUCEY, M. R., AND WILLIAMS, R.: Effect of ciclosporin on suppressor cell function in patients with primary biliary cirrhosis. *In* Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 171–174, Springer-Verlag, Berlin, 1985.
696. NICKELL, S. P., SCHEIBEL, L. W., AND COLE, G. A.: Inhibition by cyclosporin A of rodent malaria in vivo and human malaria in vitro. Infect. Immun. **37:** 1093–1100, 1982.
697. NICKOLOFF, B. J., FISHER, G. J., MITRA, R. S., AND VOORHEES, J. J.: Additive and synergistic antiproliferative effects of cyclosporin A and gamma interferon on cultured human keratinocytes. Am. J. Pathol. **131:** 12–18, 1988.
698. NICOLAS, J. F., COZON, G., AND REVILLARD, J. P.: Some viral infections and related disorders associated with long-term immunosuppressive treatments. J. Autoimmun. **1:** 559–573, 1988.
699. NIESSEN, G. J. C. M.: The effect of blood transfusion and immunosuppression on organ graft survival. A study in dogs and rats. (thesis). Grafische Verzorging Drukkereij taat b.v. Boskoop, Erasmus University, Rotterdam, 1982.
700. NIESSEN, G. J. C. M., MARQUET, R. L., BIJNEN, A. B., OBERTOP, H., AND JEEKEL, J.: The effect of cyclosporin A and blood transfusions on cardiac allograft survival in rats. Surgery **91:** 339–342, 1982.
701. NIESSEN, G. J. C. M., MARQUET, R. L., COBUSSEN-MATHIJSSEN, A., HEIJSLEK, G. A., AND JEEKEL, J.: The blood transfusion effect in rats can be abolished by administration of cyclosporin-A during transfusion. Transplant. Proc. **15:** 1026–1027, 1983.
702. NIESSEN, G. J. C. M., OBERTOP, H., BIJNEN, A. B., AND JOLING, P.: Absence of the beneficial effect of blood transfusion in canine renal allograft recipients treated with low-dose cyclosporin A. Transplantation **31:** 480–481, 1981.
703. NIESSEN, G. J. C. M., OBERTOP, H., BIJNEN, A. B., MARQUET, R. L., AND JEEKEL, J.: Expression of beneficial blood transfusion effect in dogs is dependent upon immunosuppressants used. Transplant. Proc. **14:** 400–402, 1982.
704. NILSSON, L. A., LINDBLAD, R., OLLING, S. AND OUCHTERLONY, O.: The effect of cyclosporin A on the course of murine infection by Schistosoma mansoni. Parasite Immunol. **7:** 19–27, 1985.
705. NILSSON, O., DAHLSTRÖM, A., GRÖNSTAD, K. O., ROSENGREN, L., BRIVING, C., SKOLNIK, G., AND AHLMAN, H.: Successful transplantation of a human midgut carcinoid tumour to the anterior eye chamber of the rat. Acta Physiol. Scand. **120:** 317–319, 1984.
706. NOBLE, R. L., AND STEINMULLER, D.: Blocking of interleukin-2 production, but not the tissue destruction induced by cytotoxic T cells, by cyclosporine. Transplantation **47:** 322–326, 1989.
707. NORDGREN, S., COHEN, Z., MACKENSIE, R., FINKELSTEIN, D., GREENBERG, G. R., AND LANGER, B.: Functional monitors of rejection in small intestinal transplants. Am. J. Surg. **147:** 152–158, 1984.
707a. NORDMANN, J. P., DE KOZAK, Y., LE HOANG, P., AND FAURE, J. P.: Cyclosporine therapy of guinea-pig autoimmune ureoretimitis induced with autologous retiva. J. cul. Pharmacol. **2:** 325–333, 1986.
708. NORIN, A. J.: The immunobiology of experimental lung transplantation with cyclosporine immunosuppression: cytolytic T lymphocytes and delayed type hypersensitivity in rejecting and tolerant recipients. Transplant. Proc. **20** (suppl. 2)**:** 125–130, 1988.
709. NORIN, A. J., EMESON, E. E., PINSKER, K. L., KAMHOLZ, S. L., AND VEITH, F. J.: Studies with T cells from long-term surviving canine lung allograft recipients: reduced lymphocyte-mediated cytotoxicity but not reduced mixed lymphocyte reactivity. Transplant. Proc. **15:** 508–510, 1983.
710. NORIN, A. J., KAMHOLZ, S. L., PINSKER, K. L., EMESON, E. E., AND VEITH, F. J.: Concanavalin A-dependent cell-mediated cytotoxicity in bronchoalveolar lavage fluid. Transplantation **42:** 466–472, 1986.
711. NORIN, A. J., KAMHOLZ, S. L., PINSKER, K. L., EMESON, E. E., AND VEITH, F. J.: Cyclosporin-induced tolerance in experimental organ transplantation. Evidence of diminished donor-specific cytotoxicity relative to donor-specific proliferative response. J. Immunol. **139:** 332–337, 1987.
712. NOVITZKY, D., COOPER, D. K. C., DUTOIT, E., Oudshoorn, M., Langman, E., and Jacobs, P.: Preformed lymphocytotoxic antibodies disappear following cyclosporine therapy. J. Heart Transplant. **4:** 362–363, 1985.
713. NOWAK, J. S., KAI, O., PECK, R., AND FRANKLIN, R. M.: The effects of cyclosporin A on the chicken immune system. Eur. J. Immunol. **12:** 867–876, 1982.
714. NUGENT, K. M., AND KOPP, W. C.: Effects of cyclosporine on pulmonary clearance of Staphylococcus aureus and Pseudomonas aeruginosa. J. Infect. Dis. **154:** 352–355, 1986.
715. NUSSENBLATT, R.: The clinical use of immunosuppressants in the treatment of putative autoimmune intra-ocular diseases. J. Autoimmun. **1:** 615–621, 1988.
716. NUSSENBLATT, R. B., DINNING, W. G., FUJIKAWA, L. S., CHAN, C. C., AND PALESTINE, A. G.: Local cyclosporine therapy for experimental autoimmune uveitis in rats. Arch. Ophthalmol. **103:** 1559–1562, 1985.
717. NUSSENBLATT, R. B., GUNN, H. C., RYFFEL, B., AND BOREL, J. F.: Experimental autoimmunity. Prog. Allergy **38:** 159–180, 1986.
718. NUSSENBLATT, R. B., RODRIGUES, M. M., SALINAS-CARMONA, M. C., GERY, I., CEVARIO, S., AND WACKER, W.: Modulation of experimental autoimmune uveitis with cyclosporin A. Arch. Ophthalmol. **100:** 1146–1149, 1982.
719. NUSSENBLATT, R. B., RODRIGUES, M. M., WACKER, W. B., CEVARIO, S. J., AND SALINAS-CARMONA, M. C.: Cyclosporin A: Inhibition of experimental autoimmune uveitis in Lewis rats. J. Clin. Invest. **67:** 1228–1231, 1981.
720. NUSSENBLATT, R. B., SALINAS-CARMONA, M., WAKSMAN, B. H., AND GERY, I.: Cyclosporin A: alterations of the cellular immune response in S-antigen-induced experimental autoimmune uveitis. Int. Arch. Allergy Appl. Immunol. **70:** 289–294, 1983.
721. NUSSENBLATT, R. B., AND SCHER, I.: Effects of cyclosporine on T-cell subsets in experimental autoimmune uveitis. Invest. Ophthalmol. & Visual Sci. **26:** 10–14, 1985.
722. NYBERG-HANSEN, R., AND GJERSTAD, L.: Immunopharmacological treatment in myasthenia gravis. Transplant. Proc. **20** (suppl. 4)**:** 201–210, 1988.
723. OCHIAI, T., GUNJI, M., NAGATA, M., SUZUKI, T., ENOMOTO, K., ASANO, T., YAMADA, N., SATO, H., AND OHTSUKA, M.: Cyclosporine enhances an effect of donor-specific blood transfusion in canine renal transplantation. Transplant. Proc. **15:** 2939–2941, 1983.
724. O'CONNELL, J. B., REAP, E. A., AND ROBINSON, J. A.: The effects of cyclosporine on acute murine Cocksackie B3 myocarditis. Circulation **73:** 353–359, 1986.
725. O'GRADY, J. G., ALEXANDER, G. J. M., SUTHERLAND, S., DONALDSON, P. T., HARVEY, F., PORTMANN, B., CALNE, R. Y., AND WILLIAMS, R.: Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bileduct syndrome after liver transplantation. Lancet **II:** 302–305 (August 6), 1988.
726. OGUNNAIKE, H. O., STARKEY, T. D., BALDWIN, J. C., PORTER, K. A., BILLINGHAM, M. E., AND JAMIESON, S. W.: An assessment of Nva^2-cyclosporine in primate cardiac transplantation. Transplantation **43:** 13–17, 1987.
727. OH, J. O., MINASI, P., GRABNER, G., AND OHASHI, Y.: Suppression of secondary herpes simplex uveitis by cyclosporine. Invest. Ophthalmol. & Visual Sci. **26:** 494–500, 1985.
728. OKUDAIRA, H., SAKURAI, Y., TERADA, K., TERADA, E., AND OGITA, T.: Cyclosporin A-induced suppression of ongoing IgE antibody formation

in the mouse. Int. Arch. Allergy Appl. Immunol. **79:** 164–168, 1986.
729. Okudaira, H., Terada, E., and Okudaira, K.: Animal models utilised in the research of autoimmune disease control: experimental therapy of glomerulonephritis in NZB/W F_1 mice. Prog. Clin. Biol. Res. **229:** 157–174, 1987.
730. Oluwole, S. F., Chabot, J., Pepino, P., Reemtsma, K., and Hardy, M. A.: Mechanisms of immunologic unresponsiveness induced by ultraviolet-irradiated donor-specific blood transfusions and peritransplant cyclosporine. Transplantation **46:** 352–358, 1988.
731. Oluwole, S. F., Fawwaz, R. A., Reemtsma, K., and Hardy, M. A.: The role of suppressor T lymphocytes in the induction of specific immunologic unresponsiveness to rat cardiac allografts by donor leukocytes and cyclosporine. Transplant. Proc. **20** (suppl. 3): 1038–1044, 1988.
732. Oluwole, S. F., Lau, H. T., Reemtsma, K., and Hardy, M. A.: Effect of ultraviolet-B-irradiated donor-specific blood transfusions and peritransplant immunosuppression with cyclosporine on rat cardiac allograft survival. Transplantation **45:** 293–297, 1988.
733. Opelz, G.: Multicenter impact of ciclosporin on cadaver kidney graft survival. Prog. Allergy **38:** 329–345, 1986.
734. Orosz, C. G., Adams, P. W., and Ferguson, R. M.: Frequency of human alloantigen-reactive T lymphocytes. III. Evidence that cyclosporine has an inhibitory effect on human CTL and CTL precursors, independent of CS-mediated helper T cell dysfunction. Transplantation **46** (suppl.): 73S–79S, 1988.
735. Orosz, C. G., Zinn, N. E., Adams, P. W., Widmer, M. B., and Ferguson, R. M.: Influence of cyclosporine on CTL behavior in vitro and in vivo. Ann. NY Acad. Sci. **532:** 119–127, 1988.
736. Orosz, C. G., Zinn, N. E., Sirinek, L. P., and Ferguson, R. M.: In vivo mechanisms of alloreactivity—IV. Cyclosporine differentially impairs accumulation of donor-reactive CTL but not donor-reactive alloantibody in murine sponge matrix allografts. Int. J. Immunopharmacol. **10:** 305–316, 1988.
737. Osato, M. S., Roussel, T. J., Wilhelmus, K. R., and Jones, D. B.: In vitro and in vivo antifungal activity of cyclosporine. Transplant. Proc. **15** (suppl. 1): 2927–2930, 1983.
738. Osieka, R., Seeber, S., Pannenbäcker, R., Soll, D., Glatte, P., and Schmidt, C. G.: Enhancement of etoposide-induced cytotoxicity by cyclosporin A. Cancer Chemother. Pharmacol. **18:** 198–202, 1986.
739. O'Toole, C. M., Carvalho, G. S., Cranage, M. P., and Large, S.: Immune responses in cardiac transplantation. I. Detection of activated T Ia^+ cells in the blood during herpes virus infections. Clin. Exp. Immunol. **57:** 671–678, 1984.
740. Otto, U., Huland, H., Klöppel, G., and Baisch, H.: Xenogenic transplantation of human bladder- and renal-cell carcinoma into NMRI mice treated with cyclosporin A and into NMRI nu/nu mice. Urol. Int. (Basel) **42:** 1–5, 1987.
741. Padberg, W. M., Kupiec-Weglinski, J. W., Lord, R. H., Towpik, E., and Tilney, N. L.: $W3/25^+$ T cells mediate specific unresponsiveness in enhanced allograft recipients. Transplant. Proc. **19:** 493–494, 1987.
742. Padberg, W. M., Lord, R. H. H., Di Stefano, R., Araneda, D., Tilney, N. L., and Kupiec-Weglinski, J. W.: Synergy between subtherapeutic doses of cyclosporine and immunologic entrancement in rat recipients of cardiac allografts. Transplantation **45:** 162–168, 1988.
743. Paetkau, V., Havele, C., and Shaw, J.: Direct and indirect modes of action of cyclosporine on cytotoxic T lymphocytes. Ann. NY Acad. Sci. **532:** 405–412, 1988.
744. Painvin, G. A., Heimbecker, R. O., Keown, P. A., Stiller, C. R., Silver, M. D., and McKenzie, N.: The side effects of cyclosporin A: clinical and histologic findings following 31 canine heterotopic cardiac allotransplants. Curr. Surg. **40:** 120–122, 1983.
745. Palestine, A. G., Müllenberg-Coulombre, C. G., Kim, M. K., Gelato, M. C., and Nussenblatt, R. B.: Bromocriptine and low-dose cyclosporine in the treatment of experimental autoimmune uveitis in the rat. J. Clin. Invest. **79:** 1078–1081, 1987.
746. Palestine, A. G., Nussenblatt, R. B., and Gelato, M.: Therapy of human autoimmune uveitis with low-dose cyclosporine plus bromocriptine. Transplant. Proc. **20** (suppl. 4): 131–135, 1988.
747. Palestine, A. G., Roberge, F., Charous, B. L., Lane, H. C., Fauci, A. S., and Nussenblatt, R. B.: The effect of cyclosporine on immunization with tetanus and keyhole limpet hemocyanin (KLH) in humans. J. Clin. Immunol. **5:** 115–121, 1985.
748. Pardue, S. L., Fite, K. V., Bengston, L., Lamont, S. J., Boyle, M. L., and Smyth, J. R.: Enhanced integumental and ocular amelanosis following the termination of cyclosporine administration. J. Invest. Dermatol. **88:** 758–761, 1987.
749. Parfrey, N. A., Ste.-Croix, H., and Prud'homme, G. J.: Aberrant major histocompatibility complex (MHC) expression in cyclosporine-induced autoimmunity (CIA). (in submission)
750. Parker, D., Drössler, K., and Turk, J. L.: Kinetics of the effect of a single dose of cyclosporin-A on antibody and cell mediated immune responses in the guinea pig. Int. J. Immunopharmacol. **6:** 67–74, 1984.
751. Patterson, G. A., Cooper, J. D., Dark, J. H., Jones, M. T., and the Toronto Lung Transplant Group: Experimental and clinical double lung transplantation. J. Thorac. Cardiovasc. Surg. **95:** 70–74, 1988.
752. Penn, I., and Brunson, M. E.: Cancers after cyclosporine therapy. Transplant. Proc. **20** (suppl. 3): 885–892, 1988.
753. Pennock, J. L., Reitz, B. A., Bieber, C. P., Aziz, S., Oyer, P. E., Strober, S., Hoppe, R., Kaplan, H. S., Stinson, E. B., and Shumway, N.: Survival of primates following orthotopic cardiac transplantation treated with total lymphoid irradiation and chemical immune suppression. Transplantation **32:** 467–473, 1981.
754. Perfect, J. R., and Durack, D. T.: Effects of cyclosporine in experimental cryptococcal meningitis. Infect. Immun. **50:** 22–26, 1985.
755. Perfect, J. R., Hobbs, M. M., Granger, D. L., and Durack, D. T.: Cerebrospinal fluid macrophage response to experimental cryptococcal meningitis: relationship between in vivo and in vitro measurements of cytotoxicity. Infect. Immun. **56:** 849–854, 1988.
756. Pescovitz, M. D., Sachs, D. H., Lunney, J. K., and Hsu, S. M.: Localization of class II MHC antigens on porcine renal vascular endothelium. Transplantation **37:** 627–630, 1984.
756a. Pierson, R. N., Winn, H. J., Russell, P. S., and Auchincloss, H.: Xenogeneic skin graft rejection is especially dependent on $CD4^+$ T cells. J. Exp. Med. **170:** 991–996, 1989.
757. Pigatto, P. D., Mozzanica, N., Polenghi, M. M., Altomare, G. F., and Finzi, A. F.: Cyclosporine A inhibits polymorphonuclear leukocyte chemotaxis in vivo. Transplant. Proc. **20** (suppl. 4): 91–94, 1988.
758. Pinto, M., Gill, T. J., and Kunz, H. W.: Prolongation of skin graft survival across different genetic barriers in rats with cyclosporine—and its potentiation by Bordetella pertussis vaccine. Transplantation **36:** 171–177, 1983.
759. Pisetsky, D. S.: Inhibition of in vitro NZB antibody responses by cyclosporine. Clin. Exp. Immunol. **71:** 155–158, 1988.
760. Piszczor MacGregor, M., Lucia, H. L., Vine, W., Fitzgerald, P., and Bia, F. J.: Effects of cyclosporine and cortisone on the pathogenesis of primary infection with cytomegalovirus in the guinea pig. J. Infect. Dis. **53:** 503–510, 1986.
761. Pollack, M. S., Short, H. D., Young, J. B., Piwinski, S. E., Callaway, C., and Debakey, M. E.: Graft stability in a heart transplant recipient whose immunosuppressive therapy was discontinued for 8 months. Transplantation **45:** 242–243, 1988.
762. Polman, C. H., Matthaei, I., de Groot, C. J. A., Koetsier, J. C., Sminia, T., and Dijkstra, C. D.: Low-dose cyclosporin A induces relapsing remitting experimental allergic encephalomyelitis in the Lewis rat. J. Neuroimmunol. **17:** 209–216, 1988.
763. Pons, H. A., Adams, S., and Stadecker, M. J.: Schistosoma mansoni: The basis for the antischistosomal effect of cyclosporine A. Exp. Parasitol. **67:** 190–198, 1988.
764. Powles, R. L., Morgenstern, G. R., Kay, H. E. M., McElwain, T. J., Clink, H. M., Dady, P. J., Barrett, A., Jameson, B., Depledge, M. H., Watson, J. G., Sloane, J., Leigh, M., Lumley, H., Hedley, D., Lawler, S. D., Filshie, J., and Robinson, B.: Mismatched family donors for bone marrow transplantation as treatment for acute leukaemia. Lancet **I:** 612–615, 1983.
765. Press, B. H. J., Sibley, R. K., and Shons, A. R.: Limb allotransplantation in the rat: extended survival and return of nerve function with continuous cyclosporine/prednisone immunosuppression. Ann. Plast. Surg. **16:** 313–321, 1986.
766. Prieto, C., Alcazar, J. M., Ruilope, L. M., Bello, I., Andres, A., Morales, J. M., and Rodicio, J. L.: Usefulness of cyclosporine in the treatment of steroid-resistant rejection in renal transplants. Transplant. Proc. **18:** 993–995, 1986.
767. Pritchard, T. J., and Kirkman, R. L.: Small bowel transplantation. World J. Surg. **9:** 860–867, 1985.
768. Pritchard, T. J., Madara, J. L., Tapper, D., Wilmore, D. W., and Kirkman, R. L.: Failure of cyclosporine to prevent small bowel allograft rejection in pigs. J. Surg. Res. **38:** 553–558, 1985.
769. Prop, J., Bartels, H. L., Petersen, A. H., Wildevuur, C. R. H., and Nieuwenhuis, P.: A single injection of cyclosporin A reverses lung allograft rejection in the rat. Transplant. Proc. **15:** 511–513, 1983.
770. Prop, J., Hoyt, E. G., and Jamieson, S. W.: (Nva^2)-cyclosporine—Less potent than cyclosporine A in rats with lung and heart transplants. Transplantation **44:** 5–8, 1987.
771. Prop, J., van den Berg, C., Tazelaar, H. D., DeValeria, P. A., and Billingham, M. E.: Combined heart-lung transplantation in the rat. Transplantation **43:** 614–619, 1987.
772. Raju, S., Didlake, R. H., Cayirli, M., Turner, M. D., Grogan, J. B., and Achord, J.: Experimental small bowel transplantation utilizing cyclosporine. Transplantation **38:** 561–566, 1984.
773. Randolph, M. A., Yaremchuk, M. J., Moore, J. R., Robinson, R. A., and Weiland, A. J.: Experimental vascularized bone alografting. Microsurgery **8:** 210–217, 1987.
774. Rane, L.: *In* Chemotherapy and Drug Resistance in Malaria, ed. by W. Peters, pp. 94–106, Academic Press, New York, 1970.
775. Rapaport, F. T., Meek, A. G., Arnold, A. N., Miura, S., Hayashi, R., and Strober, S.: Preoperative preparation of high-risk, specifically hyperimmunized canine renal allograft recipients with total-lymphoid irradiation and cyclosporine. Transplantation **44:** 185–195, 1987.
776. Reece-Smith, H., Homan, W. P., DuToit, D. F., McShane, P., and Morris, P. J.: A technique for transplanting pancreatic islets as a

vascularized graft and prevention of rejection with cyclosporin A. Transplantation 31: 442–444, 1981.
777. REEMTSMA, K., PIERSON, R. N., MARBOE, C. C., MICHLER, R. E., SMITH, C. R., ROSE, E. A., AND FENOGLIO, J. J.: Will atherosclerosis limit clinical xenografting? Transplant. Proc. 19 (suppl. 5): 108–118, 1987.
778. REIBER, H., KITZE, B., LINK, M., AND WAGNER, R.: Cellular immune reactions and blood cerebrospinal fluid barrier dysfunction in guinea pigs. Neurochem. Res. 13: 463–466, 1988.
779. REIBER, H., AND SUCKLING, A. J.: Cyclosporin-A treatment of experimental allergic encephalomyelitis: changes in immunological regulation and blood-CSF barrier function. J. Neuroimmunol. 12: 121–130, 1986.
780. REICHENSPURNER, H., ERTEL, W., REICHART, B., PETERS, D., WELZ, A., UEBERFUHR, P., KEMKES, B. M., GOKEL, J. M., AND HAMMER, C.: Xenogeneic and allogeneic canine heart transplantation: a model for cytologic and immunologic monitoring of rejection mechanisms. J. Heart Transplant. 5: 471–476, 1986.
781. RICORDI, C., SANTIAGO, J. V., AND LACY, P. E.: Use of culture and temporary immunosuppression to prolong adrenal cortical allograft survival. Endocrinology 121: 745–748, 1987.
782. RICOUR, C., REVILLON, Y., ARNAUD-BATTANDIER, F., GHNASSIA, D., WEYNE, P., LAUFFENBURGER, A., JOS, J., FONTAINE, J. L., GALLIX, P., AND VAIMAN, M.: Successful small bowel allografts in piglets using cyclosporine. Transplant. Proc. 15: 3019–3026, 1983.
783. RINALDO, C. R., DE BIASIO, R. L., HAMOUDI, W. H., RABIN, B., LIEBERT, M., AND HAKALA, T. R.: Effect of herpes virus infections on T-lymphocyte subpopulations and blastogenic responses in renal transplant recipients receiving cyclosporine. Clin. Immunol. Immunopathol. 38: 357–366, 1986.
784. RINGDÉN, O., BÄCKMAN, L., LÖNNQVIST, B., HEIMDAHL, A., LINDHOLM, A., BOLME, P., AND GAHRTON, G.: A randomized trial comparing use of cyclosporin and methotrexate for graft-versus-host disease prophylaxis in bone marrow transplant recipients with haematological malignancies. Bone Marrow Transplant. 1: 41–51, 1986.
785. RINGDÉN, O., GROTH, C. G., ERIKSON, A., BÄCKMAN, L., GRANQVIST, S., MÄNSSON, J. E., AND SVENNERHOLM, L.: Long-term follow-up of the first successful bone marrow transplantation in Gaucher disease. Transplantation 46: 66–70, 1988.
785a. ROBERTSON, R. P., FRANKLYN, G., AND NELSON, L.: Intravenous glucose tolerance and pancreatic islet β-cell function in patients with multiple sclerosis during 2-yr treatment with cyclosporine. Diabetes 38: 58–64, 1989.
786. RODRIGUEZ, M., AND QUDDUS, J.: Effect of cyclosporin A, silica quartz dust, and protease inhibitors on virus-induced demyelination. J. Neuroimmunol. 13: 159–174, 1986.
787. ROGER, M., VIGEANT, P., AND VIENS, P.: The effect of cyclosporin A on Trypanosoma musculi infection in mice. Canad. J. Microbiol. 34: 92–94, 1988.
788. ROITT, I. M.: Prevailing theories in autoimmune disease. In Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 5–15, Springer-Verlag, Berlin, 1985.
789. ROITT, I. M., BROSTOFF, J., AND MALE, D. K.: Immunology, chapters 11, 13, 22 and 24, Gower Med. Publ. Ltd., London, 1985.
790. ROSE, E. A., MICHLER, R. E., SMITH, C. R., MCMANUS, R. P., SADEGHI, A., DRUSIN, R. E., AND REEMTSMA, K.: Present status of human cardiac allografts and prospects for xenografts. ASAIO-Trans. 34: 19–23, 1988.
791. ROSE, N. R.: Current concepts of autoimmune disease. Transplant. Proc. 20 (suppl. 4): 3–10, 1988.
792. ROSE, N. R., HERSKOWITZ, A., NEUMANN, D. A., AND NEU, N.: Autoimmune myocarditis: a paradigm of post-infection autoimmune disease. Immunol. Today 9: 117–120, 1988.
793. ROSENTHAL, J. T., HAKALA, T. R., STARZL, T., IWATSUKI, S., AND SHAW, B. W.: Secondary cadaver kidney transplants: improved graft survival in secondary kidney transplants using cyclosporin A. J. Urol. 131: 17–18, 1984.
794. ROUQUETTE-GALLY, A. M., BOYELDIEU, D., PROST, A. C., AND GLUCKMAN, E.: Autoimmunity after allogeneic bone marrow transplantation. Transplantation 46: 238–240, 1988.
795. ROUSSEL, T. J., OSATO, M. S., AND WILHELMUS, K. R.: Cyclosporine and experimental corneal transplantation. Transplant. Proc. 15: 3081–3083, 1983.
796. ROUTHIER, G., EPSTEIN, O., JANOSSY, G., THOMAS, H. C., SHERLOCK, S., KUNG, P. C., AND GOLDSTEIN, G.: Effects of cyclosporin A on suppressor and inducer lymphocytes in primary biliary cirrhosis. Lancet II: 1223–1226, 1980.
797. RUBEN, L. N.: IgM memory: long lived hapten-specific memory in the newt, *Notophthalmus viridescens*. Immunology 48: 385–392, 1983.
798. RUCKER, J., TOLEDO-PEREYRA, L. H., MACKENZIE, G. H., AND GORDON, D. A.: Improvement of kidney transplant survival after graft pretreatment with cyclosporin A. Transplantation 34: 356–359, 1982.
799. RUDGE, P.: Cyclosporine and multiple sclerosis: the cons. Neurology 38 (suppl. 2): 29–31, 1988.
800. RULLAN, P. P., BARR, R. J., AND COLE, G. W.: Cyclosporine and murine allergic contact dermatitis. Arch. Dermatol. 120: 1179–1183, 1984.
801. RUMJANEK, V. M., SMITH, L. A., AND MORLEY, J.: Modulation by cyclosporin-A of mononuclear cell distribution during experimental allergic encephalomyelitis. Int. J. Immunopharmacol. 6: 99–104, 1984.
802. RUSSELL, D. H.: New aspects of prolactin and immunity: a lymphocyte-derived prolactin-like product and nuclear protein kinase C activation. Trends Pharmacol. Sci. 10: 40–44, 1989.
803. RYFFEL, B. Experimental toxicological studies with cyclosporin A. In Cyclosporin A, ed. by D. J. G. White, pp. 45–75, Elsevier Biomed. Press, Amsterdam, 1982.
804. RYFFEL, B.: Toxicology—experimental status. Prog. Allergy 38: 181–197, 1986.
805. RYFFEL, B., DEYSSENROTH, H., AND BOREL, J. F.: Cyclosporin A: effects on the mouse thymus. Agents Actions 11: 373–379, 1981.
806. RYFFEL, B., FEURER, C., HEUBERGER, B., AND BOREL, J. F.: Immunosuppressive effect of cyclosporin A in two lymphocyte transfer models in rats: comparison of in vivo and in vitro treatment. Immunobiology 163: 470–483, 1982.
807. RYFFEL, B., FOXWELL, B. M., GEE, A., GREINER, B., WOERLY, G., AND MIHATSCH, M. J.: Cyclosporine—relationship of side effects to mode of action. Transplantation 46 (suppl.): 90S–96S, 1988.
808. RYNASIEWICZ, J. J., SUTHERLAND, D. E. R., KAWAHARA, K., AND NAJARIAN, J. S.: Total lymphoid irradiation: critical timing and combination with cyclosporin A for immunosuppression in a rat heart allograft model. J. Surg. Res. 30: 365–371, 1981.
809. RYNASIEWICZ, J. J., SUTHERLAND, D. E. R., AND NAJARIAN, J. S.: Cyclosporin A and prednisolone: augmentation of rat heart allograft survival by combined therapy. Surg. Forum 32: 357–360, 1981.
810. SADEGHI, A. M., ROBBINS, R. C., SMITH, C. R., KURLANSKY, R. A., MICHLER, R. E., REEMTSMA, K., AND ROSE, E. A.: Cardiac xenograft survival in primates. J. Thorac. Cardiovasc. Surgery 93: 809–814, 1987.
811. SAKAGUCHI, S., FUKUMA, K., KURIBAYASHI, K., AND MASUDA, T.: Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J. Exp. Med. 161: 72–87, 1985.
812. SAKAGUCHI, S., AND SAKAGUCHI, N.: Thymus and autoimmunity. Transplantation of the thymus from cyclosporin A-treated mice causes organ-specific autoimmune disease in athymic nude mice. J. Exp. Med. 167: 1479–1485, 1988.
813. SAKAGUCHI, S., AND SAKAGUCHI, N.: Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. J. Immunol. 142: 471–480, 1989.
814. SAKAMOTO, K., OCHIAI, T., SHINOHARA, N., AND SATO, H.: Enforcement of specific unresponsiveness by the long-term presence of allogeneic heart grafts. Transplantation 37: 97–100, 1984.
815. SALAMAN, S.: Cyclosporine mono-drug therapy. Transplant. Proc. 20 (suppl. 3): 117–120, 1988.
816. SALISBURY, J. D., AND GEBHARDT, B. M.: Suppression of corneal allograft rejection by cyclosporin A. Arch. Ophthalmol. 99: 1640–1643, 1981.
817. SAMULACK, D. D., DYKES, R. W., AND MUNGER, B. L.: Neurophysiologic aspects of allogeneic skin and upper extremity composite tissue transplantation in primates. Transplant. Proc. 20 (suppl. 2): 279–290, 1988.
818. SANTOS, G. W., HESS, A. D., AND VOGELSANG, G. B.: Graft-versus-host reactions and disease. Immunol. Rev. 88: 169–192, 1985.
819. SARON, M. F., SHIDANI, B., GUILLON, J. C., AND TRUFFA-BACHI, P.: Beneficial effect of cyclosporin A on the lymphocytic choriomeningitis virus infection in mice. Eur. J. Immunol. 14: 1064–1066, 1984.
820. SARON, M. F., SHIDANI, B., GUILLON, J. C., AND TRUFFA-BACHI, P.: Beneficial use of cyclosporin A in lymphocytic choriomeningitis virus infection in mice: dose and kinetic effects. Ann. Inst. Pasteur Virol. 137E: 243–249, 1986.
821. SARON, M. F., SHIDANI, B., GUILLON, J. C., AND TRUFFA-BACHI, P.: Mechanism of action of cyclosporine A on the lymphocytic choriomeningitis virus infection of mice. Med. Microbiol. Immunol. 175: 125–128, 1986.
822. SAUNDERS, N. R., EGAN, T. M., CHAMBERLAIN, D., AND COOPER, J. D.: Cyclosporin and bronchial healing in canine lung transplantation. J. Thorac. Cardiovasc. Surg. 88: 993–999, 1984.
823. SAYDJARI, R., TOWNSEND, C. M., BARRANCO, S. C., JAMES, E., AND THOMPSON, J. C.: Effects of cyclosporin A and α-difluoromethylornithine on the growth of hamster pancreatic cancer in vitro. J. Natl. Cancer Inst. 77: 1087–1092, 1986.
824. SCHAD, G. A.: Cyclosporine may eliminate the threat of overwhelming strongyloidiasis in immunosuppressed patients. J. Infect. Dis. 153: 178, 1986.
825. SCHAFFNER, A., DOUGLAS, H., AND DAVIS, C. E.: Models of T cell deficiency in listeriosis: the effects of cortisone and cyclosporin A on normal and nude BALB/c mice. J. Immunol. 131: 450–453, 1983.
826. SCHALLER, E., MAILÄNDER, P., BECKER, M., WALTER, G. F., AND BERGER, A.: Nervenregeneration im autologen und allogenen Transplantat des Nervus ischiadicus der Ratte mit und ohne Immunsuppression durch Cyclosporin A. Handchir. Mikrochir. Plast. Chir. 20: 7–10, 1988.
827. SCHEIBEL, L. W., BUEDING, E., FISH, W. R., AND HAWKINS, J. T.: Protease inhibitors and antimalarial effects. In Malaria and The Red Cell, ed. by J. W. Eaton, and G. J. Brewer, pp. 131–142, Lyss, New York, 1984.
828. SCHEIBEL, L. W., COLOMBANI, P. M., HESS, A. D., AIKAWA, M., ATKIN-

son, C. T., and Milhous, W. K.: Calcium and calmodulin antagonists inhibit human malaria parasites (Plasmodium falciparum): implications for drug design. Proc. Natl. Acad. Sci. USA **84:** 7310–7314, 1987.
829. Schellekens, H., Smiers-De Vreede, E., De Reus, A., and Dijkema, R.: Antiviral activity of interferon in rats and the effect of immune suppression. J. Gen. Virol. **65:** 391–396, 1984.
830. Schiltknecht, E., and Ada, G. L.: Influenza virus-specific T cells fail to reduce lung virus titres in cyclosporin-treated, infected mice. Scand. J. Immunol. **22:** 99–103, 1985.
831. Schiltknecht, E., and Ada, G. L.: In vivo effects of cyclosporine on influenza A virus-infected mice. Cell. Immunol. **91:** 227–239, 1985.
832. Schiltknecht, E., and Ada, G. L.: The generation of effector T cells in influenza A-infected, cyclosporine-treated mice. Cell. Immunol. **95:** 340–348, 1985.
833. Schindler, R., ed.: Ciclosporin in Autoimmune Diseases, Springer-Verlag, Berlin, 1985.
834. Schmitz-Rixen, T., Megerman, J., Colvin, R. B., Williams, A. M., and Abbott, W. M.: Immunosuppressive treatment of aortic allografts. J. Vasc. Surg. **7:** 82–92, 1988.
835. Schran, H. F., Hassell, A. E., Baumhefner, R. W., Myers, J. W., Ellison, G. W., Tourtellotte, W. W., and Belendiuk, G. W.: Distribution of cyclosporine in cerebrospinal fluid. Neurology **38** (suppl. 1): 99 (abstract PP8), 1988.
836. Schrijver, G., Wetzels, J. F. M., Robben, J. C. M., Assmann, K. J. M., Koene, R. A. P., and Berden, J. H. M.: Antiproteinuric effect of cyclosporine A in passive antiglomerular basement membrane nephritis in the mouse. Transplant. Proc. **20** (suppl. 4): 304–308, 1988.
837. Schulak, J. A., and Engelstad, K. M.: Humoral presensitization in rat heart allotransplantation. J. Surg. Res. **42:** 454–461, 1987.
838. Schulak, J. A., Monson, D., Shelby, J., and Corry, R. J.: Abrogation of second-set rejection with cyclosporine. Transplantation **36:** 289–293, 1983.
839. Schuller-Levis, G. B., Kozlowski, P. B., and Wisniewski, H. M.: Cyclosporin A treatment of an induced attack in a chronic relapsing model of experimental allergic encephalomyelitis. Clin. Immunol. Immunopathol. **40:** 244–252, 1986.
840. Schwartz, M. Z., Flye, M. W., and Storozuk, R. B.: Growth and function of transplanted fetal rat intestine: effect of cyclosporine. Surgery **97:** 481–486, 1985.
841. Selawry, H., Whittington, K., and Fajaco, R.: Effects of cyclosporine on islet xenograft survival in the BB/W rat. Transplantation **42:** 568–570, 1986.
842. Selgrade, M. K., Daniels, M. J., Burleson, G. R., Lauer, L. D., and Dean, J. H.: Effects of 7,12-dimethyl-benz[a]anthracene, benzo[a]pyrene and cyclosporin A on murine cytomegalovirus infection: studies of resistance mechanisms. Int. J. Immunopharmacol. **10:** 811–818, 1988.
843. Sell, S.: The major histocompatibility complex and the immunoglobulin gene superfamily. *In* Basic Immunology, pp. 169–233, Elsevier, Amsterdam, 1987.
844. Sell, S.: Immune tolerance and autoimmunity. *In* Basic Immunology, pp. 235–259, Elsevier, Amsterdam, 1987.
845. Sender, M. B., Ward, H. J., Glassock, R. J., Rajfer, J., and Koyle, M. A.: Infection patterns in cyclosporine-treated cadaveric renal transplant patients. Transplant. Proc. **20** (suppl. 3): 920–923, 1988.
846. Sestier, C., Odent-Pogu, S., Bonneville, M., Maurel, C., Lang, F., and Sai, P.: Cyclosporin enhances diabetes induced by low-dose streptozotocin treatment in mice. Immunol. Lett. **10:** 57–60, 1985.
847. Settaf, A., Meriggi, F., Van de Stadt, J., Gane, P., Crougneau, S., Reynes, M., Rouger, P., and Houssin, D.: Delayed rejection of liver xenografts compared to heart xenografts in rats. Transplant. Proc. **19:** 1155–1157, 1987.
848. Shaffer, D., Maki, T., DeMichele, S. J., Karlstad, M. D., Bistrian, B. R., Balogh, K., and Monaco, A. P.: Studies in small bowel transplantation. Prevention of graft-versus-host disease with preservation of allograft function by donor pretreatment with antilymphocyte serum. Transplantation **45:** 262–269, 1988.
849. Shaw, J. F. L.: Combined effects of cyclosporin A and sodium salicylate upon survival of rat allografts. IRCS Med. Sci. (Surg. Transplant.) **10:** 827, 1983.
850. Sheil, A. G. R.: The second international congress on cyclosporine. Transplant. Proc. **20** (suppl. 3): 1123–1131, 1988.
851. Shepherd, W. F. I., Coster, D. J., Fook, T. C., Rice, N. S. C., and Jones, B. R.: Effect of cyclosporin A on the survival of corneal grafts in rabbits. Br. J. Ophthalmol. **64:** 148–153, 1980.
852. Sherwood, R. A., Brent, L., and Rayfield, L. S.: Presentation of alloantigens by host cells. Eur. J. Immunol. **16:** 569–574, 1986.
853. Shevach, E. M.: The effects of cyclosporin A on the immune system. Annu. Rev. Immunol. **3:** 397–423, 1985.
854. Shidani, B., Colle, J. H., Motta, I., and Truffa-Bachi, P.: Effect of cyclosporin A on the induction and activation of B memory cells by thymus-independent antigens in mice. Eur. J. Immunol. **13:** 359–363, 1983.
855. Shidani, B., Milon, G., Marchal, G., and Truffa-Bachi, P.: Cyclosporin A inhibits the delayed-type hypersensitivity reaction: impaired production of early pro-inflammatory mediator(s). Eur. J. Immunol. **14:** 314–318, 1984.
856. Shidani, B., Motta, I., and Truffa-Bachi, P.: Mitogenic signals are not required to circumvent the cyclosporin-induced inhibition of TNP-specific B memory cell expression. Ann. Inst. Pasteur Immunol. **136c:** 313–321, 1985.
857. Shidani, B., Motta, I., and Truffa-Bachi, P.: Cyclosporin A does not affect the in vitro induction of antigen-specific delayed-type hypersensitivity-mediating T cells. Eur. J. Immunol. **17:** 291–294, 1987.
858. Shigematsu, H., and Koyama, A.: Suppressive effect of cyclosporin A on the induction of chronic serum sickness nephritis in the rat. Acta Pathol. Jpn. **38:** 11–19, 1988.
859. Shih, W., Hines, W. H., and Neilson, E. G.: Effects of cyclosporin A on the development of immune-mediated interstitial nephritis. Kidney Int. **33:** 1113–1118, 1988.
860. Shimazu, R., Grogan, J. B., and Raju, S.: Long-term survival of orthotopic bowel allografts in the rat treated with short-term low-dose cyclosporine. Transplantation **46:** 673–677, 1988.
861. Shimizu, T., Martin, M. S., Pelletier, H., Lagadec, P., and Martin, F.: Effects of cyclosporin A on progressive and regressive tumors induced by two cancer lines derived from a single colon carcinoma chemically induced in the rat. Immunobiology **178:** 401–415, 1989.
862. Shinozuka, H., Gill, T. J., Kunz, H. W., Witkowski, L. A., Demetris, A. J., and Perrera, M. I. R.: Enhancement of the induction of murine thymic lymphomas by cyclosporine. Transplantation **41:** 377–380, 1986.
863. Shinozuka, H., Hattori, A., Gill, T. J., and Kunz, H. W.: Experimental models of malignancies after cyclosporine therapy. Transplant. Proc. **20** (suppl. 3): 893–899, 1988.
864. Shirakusa, T.: Ultrastructural study on acute rejection of canine allograft using cyclosporin A. J. Thorac. Cardiovasc. Surg. **35:** 361–365, 1987.
865. Sieff, C. A., Chessels, J. M., Levinsky, R. J., Pritchard, J., Rogers, D. W., Casy, A., Muller, K., and Hall, C. M.: Allogeneic bone marrow transplantation in infantile malignant osteopetrosis. Lancet **I:** 437–441, 1983.
866. Siliski, J. M., Simpkin, S., and Green, C. J.: Vascularized whole knee joint allografts in rabbits immunosuppressed with cyclosporin A. Arch. Orthop. Trauma. Surg. **103:** 26–35, 1984.
867. Simeonovic, C. J., Prowse, J., and Lafferty, K.: Reversal of diabetes in outbred mice by islet allotransplantation. Diabetes **35:** 1345–1349, 1986.
868. Slater, L. M., Sweet, P., Stupecky, M., and Gupta, S.: Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J. Clin. Invest. **77:** 1405–1408, 1986.
869. Slater, L. M., Sweet, P., Stupecky, M., Wetzel, M. W., and Gupta, S.: Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. Br. J. Cancer **54:** 235–238, 1986.
870. Smart, L. M., Forrest, E. H., Whiting, P. H., Davidson, R. J. L., and Thompson, A. W.: Cyclosporine A and acute T cell leukaemia in the rat. Transplant. Proc. **20** (suppl. 3): 900–912, 1988.
871. Smit, J. A., Drielsma, R. F., Myburgh, J. A., Laupacis, A., and Stiller, C. R.: Renal allograft survival in the baboon using a pretreatment protocol with cyclosporine. Transplantation **36:** 121–124, 1983.
872. Smit, W. J. F., Dumble, L. J., Francis, D. M. A., and Clunie, G. J. A.: Cyclosporine regulation of blood transfusion sensitizing and enhancing potential of rabbit skin allograft recipients. Transplant. Proc. **20** (suppl. 3): 1105–1109, 1988.
873. Smith, J. A., Mottram, P. L., Mirisklavos, A., Mason, A., Dumble, L. J., and Clunie, G. J. A.: Isograft and allograft control studies for murine cardiac transplantation. Transplant. Proc. **19:** 1039–1042, 1987.
874. Smith, S. W. G., Chappell, L. H., Thomson, A. W., MacGowan, A. G., and Simpson, J. G.: Prophylactic and therapeutic effects of cyclosporin A in murine Schistosomiasis mansoni: studies on bisexual and unisexual infections and the hepatic inflammatory response. Int. Arch. Allergy Appl. Immunol. **85:** 174–179, 1988.
875. Solbach, W., Forberg, K., Kammerer, E., Bogdan, C., and Röllinghoff, M.: Suppressive effect of cyclosporin A on the development of Leishmania tropica-induced lesions in genetically susceptible BALB/c mice. J. Immunol. **137:** 702–707, 1986.
876. Solbach, W., Forberg, K., and Röllinghoff, M.: Effect of T-lymphocyte suppression on the parasite burden in Leishmania major-infected, genetically susceptible BALB/c mice. Infect. Immun. **54:** 909–912, 1986.
877. Solbach, W., Lange, C. E., Röllinghoff, M., and Wagner, H.: Growth, interleukin-2 production, and responsiveness to IL-2 in T_4-positive T lymphocyte populations from malignant cutaneous T cell lymphoma (Sézary's syndrome): the effect of cyclosporine A. Blood **64:** 1022–1027, 1984.
878. Sollinger, H. W., Kamps, D., Cook, K., Warner, T., Glass, N. R., and Belzer, F. O.: Segmental pancreatic allotransplantation with high-dose cyclosporine and low-dose prednisone. Transplant. Proc. **15:** 2997–3000, 1983.
879. Sorokin, R., Kimura, H., Schroder, K., Wilson, D. H., and Wilson, D. B.: Cyclosporine-induced autoimmunity: conditions for expressing disease, requirement for intact thymus, and potency estimates of autoimmune lymphocytes in drug-treated rats. J. Exp. Med. **164:** 1615–1616, 1986.
880. Speck, B., Gratwohl, A., Tichelli, A., and Würsch, A.: Critical

hypotheses on cyclosporine in bone marrow transplantation. Transplant. Proc. **20** (suppl. 3): 505–515, 1988.
881. SQUIFFLET, J. P., SUTHERLAND, D. E. R., RYNASIEWICZ, J. J., FIELD, J., HEIL, J., AND NAJARIAN, J. S.: Combined immunosuppressive therapy with cyclosporin A and azathioprine. Transplantation **34**: 315–318, 1982.
882. STANFOORD, M. R., ATKINSON, E., KASP, E., AND DUMONDE, D. C.: Modulation of experimental retinal vasculitis using dexamethasone, cyclosporin A, and prazosin. Eye **1**: 626–631, 1987.
883. STARK, G. R.: Progress in understanding multidrug resistance. Nature (Lond.) **324**: 407–408, 1986.
884. STARK, G. B., SWARTZ, W. M., NARAYANAN, K., AND MÖLLER, A. R.: Hand transplantation in baboons. Transplant. Proc. **19**: 3968–3971, 1987.
885. STARZL, T. E., HAKALA, T. R., IWATSUKI, S., ROSENTHAL, T. J., SHAW, B. W., KLINTMALM, G. B. G., AND PORTER, K. A.: Cyclosporin A and steroid treatment in 104 cadaveric renal transplantations. In Cyclosporin A, ed. by D. J. G. White, pp. 365–377, Elsevier Biomed. Press, Amsterdam, 1982.
886. STEINBRUCHEL, D., KEMP, E., STARKLINT, H., AND DIEPERINK, H.: Synergistic effect of cyclosporine A, cyclophosphamide, and steorids in rabbit-to-rat skin xenotransplantation. Transplant. Proc. **19**: 1168–1170, 1987.
887. STILLER, C. R.: Ciclosporin and autoimmune disease—an overview. *In* Ciclosporin in Autoimmune Diseases, ed. by R. Schindler, pp. 373–381, Springer-Verlag, Berlin 1985.
888. STILLER, C. R., LAUPACIS, A., KEOWN, P. A., GARDELL, C., DUPRÉ, J., THIBERT, P. AND WALL, W.: Cyclosporine: action, pharmacokinetics, and effects in the BB rat model. Metabolism **32** (suppl. 1): 69–72, 1983.
889. STORB, R., DEEG, H. J., ATKINSON, K., WEIDEN, P. L., SALE, G., COLBY, R., AND THOMAS, E. D.: Cyclosporin A abrogates transfusion-induced sensitization and prevents marrow graft rejection in DLA-identical littermates. Blood **60**: 524–526, 1982.
890. STORB, R., DEEG, H. J., FISHER, L., APPELBAUM, F., BUCKNER, C. D., BENSINGER, W., CLIFT, R., DONEY, K., IRLE, C., MCGUFFIN, R., MARTIN, P., SANDERS, J., SCHOCH, G., SINGER, J., STEWART, P., SULLIVAN, K., WHITERSPOON, R., AND THOMAS, E. D.: Cyclosporine *v* methotrexate for graft-v-host disease prevention in patients given marrow grafts for leukemia: long-term follow-up of three controlled trials. Blood **71**: 293–298, 1988.
891. STORB, R., AND THOMAS, E. D.: Graft-versus-host disease in dog and man: the Seattle experience. Immunol. Rev. **88**: 215–238, 1985.
892. STRAUSS, R., HEYMER, B., AND HOF, H.: Effects of cyclosporin A on experimental infection with Listeria monocytogenes. Clin. Exp. Immunol. **62**: 491–498, 1985.
893. STRIPH, G., DOFT, B., RABIN, B., AND JOHNSON, B.: Retina S antigen-induced uveitis. The efficacy of cyclosporine and corticosteroids treatment. Arch. Ophthalmol. **104**: 114–117, 1986.
894. SUGIMOTO, K., SHELBY, J., AND CORRY, R. J.: The effect of cyclosporine on cardiac xenograft survival. Transplantation **39**: 218–219, 1985.
895. SULLIVAN, K. M., WHITERSPOON, R. P., STORB, R., DEEG, H. J., DAHLBERG, S., SANDERS, J. E., APPELBAUM, F. R., DONEY, K. C., WEIDEN, P., ANASETTI, C., LOUGHRAN, T. P., HILL, R., SHIELDS, A., YEE, G., SHULMAN, H., NIMS, J., STROM, S., AND THOMAS, E. D.: Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-v-host disease. Blood **72**: 555–561, 1988.
896. SUN, D., BEN-NUN, A., AND WEKERLE, H.: Regulatory circuits in autoimmunity: recruitment of counter-regulatory $CD8^+$ T cells by encephalitogenic $CD4^+$ T line cells. Eur. J. Immunol. **18**: 1993–1999, 1988.
897. SUYA, H., FUJIOKA, A., PINCELLI, C., FUKUYAMA, K., AND EPSTEIN, W. L.: Skin granuloma formation in mice immunosuppressed by cyclosporine. J. Invest. Dermatol. **90**: 430–433, 1988.
898. SUZUKI, S., HIJIOKA, T., SAKAKIBARA, I., AND AMEMIYA, H.: The synergistic effect of cyclosporine and mizoribine on heterotopic heart and partial-lung in rats. Transplantation **43**: 743–744, 1987.
899. SUZUKI, S., MIZUOCHI, I., AND AMEMIYA, H.: Immunological study of cyclosporine in heterotopic transplantation of canine hearts. Transplantation **39**: 565–568, 1985.
900. TAKAGISHI, K., KAIBARA, N., HOTOKEBUCHI, T., ARITA, C., MORINAGA, M., AND ARAI, K.: Effects of cyclosporin on collagen induced arthritis in mice. Ann. Rheum. Dis. **45**: 339–344, 1986.
901. TAKASHIMA, T., AND COLLINS, F. M.: Immunosuppressive effect of cyclosporin A on Mycobacterium bovis BCG infections in mice. Infect. Immun. **55**: 1701–1706, 1987.
902. TAKASHIMA, T., AND COLLINS, F. M.: T cell-mediated immunity in persistent Mycobacterium intracellular infections in mice. Infect. Immun. **56**: 2782–2787, 1988.
903. TAKIFF, H., NOVAK, M., IWAKI, Y., AND TERASAKI, P. I.: Low-dose cyclosporine maintenance therapy after immunosuppressive induction in a rat cardiac transplant model. Transplantation **45**: 297–301, 1988.
904. TAKIZAWA, H., SUKO, M., KOBAYASHI, N., SHOJI, S., OHTA, K., NOGAMI, M., OKUDAIRA, H., MIYAMOTO, T., AND SHIGA, J.: Experimental hypersensitivity pneumonitis in the mouse: histologic and immunologic features and their modulation with cyclosporin A. J. Allergy Clin. Immunol. **81**: 391–400, 1988.
905. TAKIZAWA, H., SUKO, M., SHOJI, S., OHTA, K., HORIUCHI, T., OKUDAIRA, H., MIYAMOTO, T., AND SHIGA, J.: Granulomatous pneumonitis induced by Bacille Calmette-Guérin in the mouse and its treatment with cyclosporin A. Am. Rev. Respir. Dis. **134**: 296–299, 1986.
906. TALAL, N.: Cyclosporine as an immunosuppressive agent for autoimmune disease: theoretical concepts and therapeutic strategies. Transplant. Proc. **20** (suppl. 4): 11–15, 1988.
907. TEJANI, A., BUTT, K., TRACHTMAN, H., SUTHANTHIRAN, M., ROSENTHAL, C. J., AND KHAWAR, M. R.: Cyclosporin A induced remission of relapsing nephrotic syndrome in children. Kidney Int. **33**: 729–734, 1988.
908. TELLIDES, G., DALLMAN, M. J., AND MORRIS, P. J.: Synergistic interaction of cyclosporine with interleukin 2 receptor monoclonal antibody therapy. Transplant. Proc. **20** (suppl. 2): 202–206, 1988.
909. TERADA, M., SALZLER, M., LENNARTZ, K., AND MULLEN, Y.: The effect of H-2 compatibility on pancreatic beta cell survival in the nonobese diabetic mouse. Transplantation **45**: 622–627, 1988.
910. TERAOKA, S., MISHIRO, S., EBIHARA, K., SANAKA, T., YAMAGUCHI, Y., NAKAJIMA, I., KAWAI, T., YAGISAWA, T., HONDA, H., FUCHINOUE, S., TAKAHASHI, K., TOMA, H., AGISHI, T., AND OTA, K.: Effect of cyclosporine on proliferation of non-A, non-B hepatitis virus. Transplant. Proc. **20** (suppl. 3): 868–876, 1988.
911. TERASAKA, R., LACY, P. E., BUCY, R. P., AND DAVIE, J. M.: Effect of cyclosporine and low-temperature culture on prevention of rejection of islet xenografts (rat-to-mouse). Transplantation **41**: 661–662, 1986.
912. TERASAKA, R., LACY, P. E., HAUPTFELD, V., BUCY, R. P., AND DAVIE, J. M.: The effect of cyclosporin-A, low-temperature culture, and anti-Ia antibodies on prevention of rejection of rat islet allografts. Diabetes **35**: 83–88, 1986.
913. THE CANADIAN-EUROPEAN RANDOMIZED CONTROL TRIAL GROUP: Cyclosporin-induced remission of IDDM after early intervention. Diabetes **37**: 1574–1582, 1988.
914. THAISS, F., MIHATSCH, M. J., BATSFORD, S., VOGT, A., AND SCHOLLMEYER, P.: Effect of cyclosporine on in situ immune complex glomerulonephritis. Clin. Nephrol. **25** (suppl. 1): 181S–185S, 1986.
915. THEOFILOPOULOS, A. N., AND DIXON, F. J.: Murine models of systemic lupus erythematosus. Adv. Immunol. **37**: 269–390, 1985.
916. THOENES, G. H., UMSCHEID, T., SITTER, T., AND LANGER, K. H.: Cyclosporin A inhibits autoimmune experimental tubulointerstitial nephritis. Immunol. Lett. **15**: 301–306, 1987.
917. THOMAS, J., CARVER, M., SASH, C., CUNNINGHAM, P., AND THOMAS, F.: Induction of allogeneic unresponsiveness to renal transplants in rhesus monkeys. Transplant. Proc. **19**: 4070–4072, 1987.
918. THOMAS, J., CARVER, M., SASH, C., CUNNINGHAM, P., AND THOMAS, F. T.: Beneficial effect of cyclosporine in posttransplantation induction of unresponsiveness of renal allografts in rhesus monkeys. Transplant. Proc. **20** (suppl. 1): 134–136, 1988.
919. THOMAS, J., WHITLEY, T., CARVER, M., AND THOMAS, F.: Sequential use of antithymocyte globulin and cyclosporine augments skin allograft survival. Transplant. Proc. **16**: 1522–1524, 1984.
920. THOMAS, W. G., SADEGHI, A. M., KURLANSKY, P., MICHLER, R. E., SMITH, C. R., REEMTSMA, K., AND ROSE, E. A.: Postoperative bone marrow injections with cyclosporine or antithymocyte globulin in rat cardiac allografts. Transplantation **42**: 441–442, 1986.
921. THOMMEN-SCOTT, K.: Antimalarial activity of cyclosporin A. Agents Actions **11**: 770–773, 1981.
922. THOMPSON, A. W., BROWN, P. A. J., SIMPSON, J. G., WHITING, P. A., AND DAVIDSON, R. J. L.: Inhibition by cyclosporin A of IgE production and cyclophosphamide-induced eosinophilia in rats immunised with non-parasite antigens. Immunol. Lett. **9**: 93–97, 1985.
923. THOMPSON, A. W., AND CHAPPELL, L. H.: Immunophenotypic analysis of blood and spleen lymphocyte subsets in rats protected against schistosomiasis by cyclosporin A. Immunol. Lett. **17**: 169–172, 1988.
924. THOMPSON, A. W., MOON, D. K., GECZY, C. L., AND NELSON, D. S.: Cyclosporin A inhibits lymphokine production but not the responses of macrophages to lymphokines. Immunology **48**: 291–299, 1983.
925. THOMPSON, A. W., MOON, D. K., INOUE, Y., GECZY, C. L., AND NELSON, D. S.: Modification of delayed-type hypersensitivity reactions to ovalbumin in cyclosporin A-treated guinea-pigs. Immunology **48**: 301–308, 1983.
926. THOMPSON, A. W., MOON, D. K., AND NELSON, D. S.: Suppression of delayed-type hypersensitivity reactions and lymphokine production by cyclosporin A in the mouse. Clin. Exp. Immunol. **52**: 599–606, 1983.
927. THOMPSON, A. W., SMITH, S. W. G., AND CHAPPELL, L. H.: Cyclosporin A: immune suppressant and antiparasitic agent. Parasitol. Today **2**: 288–289, 1986.
928. THORNBURG, L. E., PADBERG, W. M., TOWPIK, E., AND TILNEY, N. L.: Suppressor cells influence maintenance and reversal of late rejection of indefinitely surviving skin allografts in cyclosporine-treated rats. Transplant. Proc. **20** (suppl. 3): 1025–1030, 1988.
929. TILNEY, N. L., PADBERG, W. M., LORD, R. H. H., ARENADA, D., STROM, T. B., AND KUPIEC-WEGLINSKI, J. W.: Synergy between subtherapeutic doses of cyclosporine and immunobiological manipulations in rat heart graft recipients. Transplantation **46** (suppl.): 122S–128S, 1988.
930. TILNEY, N. L., STROM, T. B., AND KUPIEC-WEGLINSKI, J. W.: Pharmacologic and immunologic agonists and antagonists of cyclosporine. Transplant. Proc. **20** (suppl. 3): 13–22, 1988.

931. TIMMERMANN, W., SCHANG, T., AND THIEDE, A.: Modelle und Perspektiven der Pankreastransplantation bei der Ratte. II. Der Abstossungsverlauf von pankreaticoduodenalen Transplantaten verschiedener Histoinkompatibilität und unter dem Einfluss temporärer Therapie mit Ciclosporin. Langenbecks Arch. Chir. **363:** 235–243, 1985.
932. TINDALL, R. S. A., ROLLINS, J. A., PHILLIPS, J. T., GREENLEE, R. G., WELLS, L., AND BELENDIUK, G.: Preliminary results of a double-blind, randomised, placebo-controlled trial of cyclosporine in myasthenia gravis. N. Engl. J. Med. **316:** 719–724, 1987.
933. TIPPING, P. G., AND HOLDSWORTH, S. R.: Effect of cyclosporin A on antibody-induced experimental glomerulonephritis. Nephron **40:** 201–205, 1985.
934. TIPPING, P. G., NEALE, T. J., AND HOLDSWORTH, S. R.: T lymphocyte participation in antibody-induced experimental glomerulonephritis. Kidney Int. **27:** 530–537, 1985.
935. TODO, S., UEDA, Y., DEMETRIS, J. A., IMVENTARZA, O., NALESNIK, M., VENKATARAMANAN, R., MAKOWKA, L., AND STARZL, T. E.: Immunosuppression of canine, monkey, and baboon allografts by FK 506: with special reference to synergism with other drugs and to tolerance induction. Surgery **104:** 239–249, 1988.
936. TÖTTERMAN, T. H., DANERSUND, A., NILSSON, K., AND KILLANDER, A.: Cyclosporin A is selectively cytotoxic to human leukemic T cells in vitro. Blood **59:** 1103–1107, 1982.
937. TOWPIK, E., KUPIEC-WEGLINSKI, J. W., SCHNEIDER, T. M., TYLER, D., PADBERG, W., ARANEDA, D., AND TILNEY, N. L.: Cyclosporine and experimental skin allografts. II. Indefinite survival and development of specific immunologic unresponsiveness. Transplantation **40:** 714–718, 1985.
938. TRACHTMAN, H., HAMMERSCHLAG, M. R., TEJANI, A., STEINBERG, S., AND GERSHON, A. A.: A longitudinal study of varicella immunity in pediatric renal transplant recipients. J. Infect. Dis. **154:** 335–337, 1986.
939. TRUFFA-BACHI, P. (ED.): Mode of action of cyclosporin A. 19th forum in immunology. Ann. Inst. Pasteur Immunol. **138:** 605–661, 1987.
940. TRUFFA-BACHI, P.: Cyclosporin A: a tool for dissecting the mechanisms of the immune response. Ann. Inst. Pasteur Immunol. **138:** 644–648, 1987.
941. TUTSCHKA, P. J.: The role of the thymus in regulating tolerance to self and nonself. Transplant. Proc. **19:** 486–489, 1987.
942. TUTSCHKA, P. J., BESCHORNER, W. E., ALLISON, A. C., BURNS, W. H., AND SANTOS, G. W.: Use of cyclosporin A in allogeneic bone marrow transplantation in the rat. Nature (Lond.) **280:** 148–151, 1979.
943. TUTSCHKA, P. J., HESS, A. D., BESCHORNER, W. E., AND SANTOS, G. W.: Cyclosporin A in allogeneic bone marrow transplantation: preclinical and clinical studies. *In* Cyclosporin A, ed. by D. J. G. White, pp. 519–538, Elsevier Biomed., Amsterdam, 1982.
944. TWENTYMAN, P. R.: Modification of cytotoxic drug resistance by non-immunosuppressive cyclosporins. Br. J. Cancer **57:** 254–258, 1988.
945. TWENTYMAN, P. R. A.: A possible role for cyclosporins in cancer chemotherapy. Anticancer Res. **8:** 985–994, 1988.
946. TWENTYMAN, P. R., FOX, N. E., AND WHITE, D. J. G.: Cyclosporin A and its analogues as modifiers of adriamycin and vincristine resistance in a multi-drug resistant human cancer cell line. Br. J. Cancer **56:** 55–57, 1987.
947. UHTEG, L. C., KUPIEC-WEGLINSKI, J. W., ROCHER, L. L., SALOMON, D. R., TILNEY, N. L., AND CARPENTER, C. B.: Systemic natural killer activity following cardiac engraftment in the rat: lack of correlation with graft survival. Cell. Immunol. **100:** 274–279, 1986.
948. UNANUE, E. R., AND ALLEN, P. M.: The basis for the immunoregulatory role of macrophages and other accessory cells. Science (Wash. DC) **236:** 551–557, 1987.
949. UYEMURA, K., DIXON, J. F. P., WONG, L., REA, T. H., AND MODLIN, R. L.: Effect of cyclosporine A in erythema nodosum leprosum. J. Immunol. **137:** 3620–3623, 1986.
950. VAIMAN, M., DABURON, F., AND RÉMY, J.: Prise de la moelle SLA incompatible chez le porc après irradiations fractionnées et administration de cyclosporine A. C. R. Acad. Sci. (Paris) **293** (Série III): 579–581, 1981.
951. VALDERRAMA, R., BANIA, T. C., SCELSA, S. N., MACCABEE, P. J., SELIGER, M. S., AND HANLEY, T. E.: Prevention and treatment of experimental myasthenia gravis (EAMG) with cyclosporin A in rabbits. Neurology **36:** (suppl. 1): 196 (abstract 1), 1986.
952. VALDIVIA, L. A., MONDEN, M., GOTOH, M., HAUIKE, Y., KUBOTA, N., ICHIKAWA, T., OKAMURA, J., AND MORI, T.: Prolonged survival of hamster-to-rat liver xenografts using splenectomy and cyclosporine administration. Transplantation **44:** 759–762, 1987.
953. VAN BEKKUM, D. W.: Conditioning regimens for marrow grafting. Semin. Hematol. **21:** 81–90, 1984.
954. VAN BEKKUM, D. W., KNAAN, S., AND ZURCHER, C.: Effects of cyclosporin A on experimental graft-versus-host disease in rodents. Transplant. Proc. **12:** 278–282, 1980.
955. VAN DEN BOSCH, J. K., KANIS, I. Y. R., ANTONISSEN, A. C. J. M., BUURMAN, W. A., AND VAN BOVEN, C. P. A.: T-cell-independent macrophage activation in mice induced with rRNA from Listeria monocytogenes and dimethyldioctadecylammonium bromide. Infect. Immun. **53:** 611–615, 1986.
956. VAN DER HEYDEN, A. A. P. A. M., VAN OERS, M. H. J., CORNELISSEN, P., YONG, S. L., WILMINK, J. M., AND SCHELLEKENS, P. T. A.: The influence of cyclosporin A treatment on immune responsiveness in vitro and in vivo in kidney transplant recipients. Transplant. Proc. **20** (suppl. 2): 190–195, 1988.
957. VANDERWERF, B. A., AND SEROTA, A. I.: Low-dose cyclosporine for cadaveric renal transplantation. Transplantation **45:** 320–323, 1988.
958. VAN DE STADT, J., VENDEVILLE, B., WEILL, B., CROUGNEAU, S., MICHEL, A., FILIPPONI, F., ICARD, P., RENOUD, M., LOUVEL, A., AND HOUSSIN, D.: Discordant heart xenografts in rats. Additional effect of plasma exchange and cyclosporin, cyclophosphamide, or splenectomy in delaying hyperacute rejection. Transplantation **45:** 514–518, 1988.
959. VAN JOOST, T., BOS, J. D., HEULE, F., AND MEINARDI, M. M. H. M.: Low-dose cyclosporin A in severe psoriasis. A double-blind study. Br. J. Dermatol. **118:** 183–190, 1988.
960. VAYUVEGULA, B., SLATER, L., MEADOR, J., AND GUPTA, S.: Correction of altered membrane potentials. A possible mechanism of cyclosporin A and verapamil reversal of pleiotropic drug resistance in neoplasia. Cancer Chemother. Pharmacol. **22:** 163–168, 1988.
961. VEITH, F. J., NORIN, A. J., EMESON, E., PINSKER, K. L., AND KAMHOLZ, S. L.: Experimental lung transplantation with cyclosporin A. *In* Cyclosporin A, ed. by D. J. G. White, pp. 143–154, Elsevier Biomed., Amsterdam, 1982.
962. VERSLUIS, D. J., BEYER, W. E. P., MASUREL, N., WENTING, G. J., AND WEIMAR, W.: Impairment of the immune response to influenza vaccination in renal transplant recipients by cyclosporine, but not azathioprine. Transplantation **42:** 376–379, 1986.
963. VERSLUIS, D. J., METSELAAR, H. J., BIJMA, A. M., VAESSEN, L. M. B., WENTING, G. J., AND WEIMAR, W.: The effect of long-term cyclosporine therapy on natural killer cell activity. Transplant. Proc. **20** (suppl. 2): 179–185, 1988.
964. VIALETTES, B., BAUME, D., CHARPIN, C., DE MAEYER-GUIGNARD, J., AND VAGUE, P.: Assessment of viral and immune factors in EMC virus-induced diabetes: effects of cyclosporin A and interferon. J. Clin. Lab. Immunol. **10:** 35–40, 1983.
965. VIALETTES, B., SIMON, M. C., LASSMANN, V., AND VAGUE, P.: Prolonged survival of allotransplanted islets of Langerhans after cyclosporin A treatment in rats. Transplantation **28:** 435–436, 1979.
966. VICKERY, A. C., AND NAYAR, J. K.: Brugia pahangi in nude mice: protective immunity to infective larvae is Thy 1.2+ cell dependent and cyclosporin A resistant. J. Helminthol. **61:** 19–27, 1987.
967. VLADUTIU, A. O.: Effect of cyclosporine on experimental autoimmune thyroiditis in mice. Transplantation **35:** 518–520, 1983.
968. VON GRAFFENRIED, B., FRIEND, D., SHAND, N., SCHIESS, W., AND TIMONEN, P.: Cyclosporin A (Sandimmun®) in autoimmune disorders. *In* Cyclosporin: Mode of Action and Clinical Application, ed. by A. W. Thompson, pp. 213–250, Kluwer Acad. Publ., Dordrecht, 1989.
969. VON WARTBURG, A., AND TRABER, R.: Chemistry of the natural cyclosporin metabolites. Prog. Allergy **38:** 28–45, 1986.
970. WANG, B. S., HEACOCK, E. H., CHANG-XUE, Z., TILNEY, N. L., STROM, T. B., AND MANNICK, J. A.: Evidence for the presence of suppressor T lymphocytes in animals treated with cyclosporin A. J. Immunol. **128:** 1382–1385, 1982.
971. WANG, Y., HAO, L., GILL, R. G., AND LAFFERTY, K. J.: Autoimmune diabetes in NOD mouse is L3T4 T-lymphocyte dependent. Diabetes **36:** 535–538, 1987.
972. WANG, Y., MCDUFFIE, M., NOMIKOS, I. N., HAO, L., AND LAFFERTY, K. J.: Effect of cyclosporine on immunologically mediated diabetes in non-obese diabetic mice. Transplantation **46** (suppl.): 101S–106S, 1988.
973. WATSON, J. G., GARDENER, M. D., GOLDFINCH, M. E., AND PEARSON, A. D.: Bone marrow transplantation for glycogen storage disease type II (Pompé's disease). N. Engl. J. Med. **314:** 385, 1986.
974. WATT, D. J., MORGAN, J. E., AND PARTRIDGE, T. A.: Long term survival of allografted muscle precursor cells following a limited period of treatment with cyclosporin A. Clin. Exp. Immunol. **55:** 419–426, 1984.
975. WEBSTER, L. M., AND THOMPSON, A. W.: Cyclosporin A prevents suppression of delayed-type hypersensitivity in mice immunised with high-dose sheep erythrocytes. Immunology **60:** 409–414, 1987.
976. WEBSTER, L. M., AND THOMPSON, A. W.: Augmentation of delayed-type hypersensitivity to high dose sheep erythrocytes by cyclosporin A in the mouse: influence of drug dosage and route of administration and analysis of spleen cell populations. Clin. Exp. Immunol. **71:** 149–154, 1988.
977. WEDDERBURN, N., EDWARDS, J. M. B., DESGRANGES, C., FONTAINE, C., COHEN, B., AND DE THÉ, G.: Infectious mononucleosis-like response in common marmosets infected with Epstein-Barr virus. J. Infect. Dis. **150:** 878–882, 1984.
978. WEINBLATT, M. E., COBLYN, J. S., FRASER, P. A., ANDERSON, R. J., STRAGG, J., TRENTHAM, D. E., AND AUSTEN, K. F.: Cyclosporin A treatment of refractory rheumatoid arthritis. Arthritis Rheum. **30:** 11–17, 1987.
979. WEIR, M. R., IRWIN, B. C., MATERS, A. W., GENEMANS, G., SHEN, S. Y., CHARACHE, P., AND WILLIAMS, G. M.: Incidence of cytomegalovirus disease in cyclosporine-treated renal transplant recipients based on donor/recipient pretransplant immunity. Transplantation **43:** 187–193, 1987.
980. WEISER, J., AND MATHA, V.: The insecticidal activity of cyclosporines on mosquito larvae. J. Invertebr. Pathol. **51:** 92–93, 1988.

981. WELCH, A. M., HOLDA, J. H., AND SWANBORG, R. H.: Regulation of experimental allergic encephalomyelitis. II. Appearance of suppressor cells during the remission phase of the disease. J. Immunol. **125:** 186–189, 1980.
982. WESTRA, A. L., PETERSEN, A. H., PROP., J., NIEUWENHUIS, P., AND WILDEVUUR, C. R. H.: Prolongation of rat heart allograft survival by perioperative injection of donor cells followed by cyclosporine treatment. Heart Transplant. **7:** 18–22, 1988.
983. WHITE, D. J. G., AND CALNE, R. Y.: Cyclosporin A in heart allografting: its immunosuppressive and tolerance-inducing properties. Heart Transplant. **1:** 102–109, 1982.
984. WHITE, D. J. G., CALNE, R. Y., AND PLUMB, A.: Mode of action of cyclosporin A: a new immunosuppressive agent. Transplant. Proc. **11:** 855–859, 1979.
985. WHITE, D. J. G., AND LIM, S. M. L.: The induction of tolerance by cyclosporine. Transplantation **46** (suppl.): 118S–121S, 1988.
986. WICK, G., HÁLA, K., WOLF, H., ZIEMIECKI, A., SUNDICK, R. S., STÖFFLER-MEILICKE, M., AND DEBAETS, M.: The role of genetically-determined primary alterations of the target organ in the development of spontaneous autoimmune thyroiditis in obese strain chickens. Immunol. Rev. **94:** 113–136, 1986.
987. WICK, G., MÜLLER, P. U., AND SCHWARZ, S.: Effect of cyclosporin A on spontaneous autoimmune thyroiditis of obese strain (OS) chickens. Eur. J. Immunol. **12:** 877–881, 1982.
988. WILDER, R. L., ALLEN, J. B., AND HANSEN, C.: Thymus-dependent and -independent regulation of Ia antigen expression in situ by cells in the synovium of rats with streptococcal cell wall-induced arthritis. J. Clin. Invest. **79:** 1160–1171, 1987.
989. WILLIAMS, K. A., ERICKSON, S. A., AND COSTER, D. J.: Topical steroid, cyclosporin A, and the outcome of rat corneal allografts. Br. J. Ophthalmol. **71:** 239–242, 1987.
990. WILLIAMS, K. A., GRUTZMACHER, R. D., ROUSSET, T. J., AND COSTER, D. J.: A comparison of the effects of topical cyclosporine and topical steroid on rabbit corneal allograft rejection. Transplantation **39:** 242–244, 1985.
991. WILLIAMS, J. W., MCCLELLAN, T., PETERS, T. G., NAG, S., DEAN, P., BANNER, B., VERA, S. R., AND STENZ, F.: Effect of pretransplant graft irradiation on canine intestinal transplantation. Surg. Gynecol. Obstet. **167:** 197–204, 1988.
992. WILLIAMS, J. W., PETERS, T. G., HAGGITT, R., AND VAN VOORST, S.: Cyclosporine in transplantation of the liver in the dog. Surg. Gynecol. Obstet. **156:** 767–773, 1983.
993. WILLIAMS, M. D., WALSHAW, R., BULL, R. W., SCHALL, W. D., PADGETT, D. A., GOSSAIN, V. V., AND NACHREINER, R. F.: Effect of cyclosporine on allotransplanted pancreatic islets in DLA-MLC-compatible dogs. Transplant. Proc. **15:** 3004–3010, 1983.
994. WOOD, A., ADU, D., BIRTWISTLE, R. J., BREWER, D. B., AND MICHAEL, J.: Cyclosporin A and anti-glomerular basement membrane antibody glomerulonephritis in rats. Br. J. Exp. Pathol. **69:** 189–195, 1988.
995. WORKING PARTY ON LEUKAEMIA, EUROPEAN GROUP FOR BONE MARROW TRANSPLANTATION: allogeneic bone marrow transplantation for leukaemia in Europe. Lancet **I:** 1379–1382, 1988.
996. WYLER, D. J., BELLER, D. I., AND SYPEK, J. H.: Macrophage activation for antileishmanial defense by an apparently novel mechanism. J. Immunol. **138:** 1246–1249, 1987.
997. XUE, B., DERSARKISSIAN, R. M., BAER, R. L., THORBECKE, G. J., AND BELSITO, D. V.: Reversal by lymphokines of the effect of cyclosporin A on contact sensitivity and antibody production in mice. J. Immunol. **136:** 4128–4133, 1986.
998. YALE, J. F., GROSE, M., ROY, R. D., SEEMAYER, T. A., AND MARLISS, E. B.: Response to cyclosporine administration at onset of diabetes in BB rats. Diabetes Res. **5:** 129–133, 1987.
999. YALE, J. F., GROSE, M., SEEMAYER, T. A., AND MARLISS, E. B.: Immunological and metabolic concomitants of cyclosporin prevention of diabetes in BB rats. Diabetes **36:** 749–757, 1987.
1000. YALE, J. F., ROY, R. D., GROSE, M., SEEMAYER, T. A., MURPHY, G. F., AND MARLISS, E. B.: Effects of cyclosporine on glucose tolerance in the rat. Diabetes **34:** 1309–1313, 1985.
1001. YANAGIHARA, R. H., AND ADLER, W. H.: Inhibition of mouse natural killer activity by cyclosporin A. Immunology **45:** 325–332, 1982.
1002. YASUMURA, T., AND KAHAN, B. D.: Prolongation of allograft survival by repeated cycles of donor antigen and cyclosporine in rat kidney transplantation. Transplantation **38:** 418–423, 1984.
1003. YEE, G. C., SELF, S. G., MCGUIRE, T. R., CARLIN, J., SANDERS, J. E., AND DEEG, H. J.: Serum cyclosporine concentration and risk of acute graft-versus-host disease after allogeneic marrow transplantation. N. Engl. J. Med. **319:** 65–70, 1988.
1004. YEN, C. Y., GREENSTEIN, S. M., LIPKOWITZ, G. S., HONG, J. H., NITTA, K., FRIEDMAN, E. A., AND BUTT, K. M. H.: Daily and alternate-day cyclosporine immunosuppressive regimens and synergism with azathioprine. Transplant. Proc. **19:** 1272–1275, 1987.
1005. YOCUM, D. E., ALLEN, J. B., WAHL, S. M., CALANDRA, G. B., AND WILDER, R. L.: Inhibition by cyclosporin A of streptococcal cell wall-induced arthritis and hepatic granulomas in rats. Arthritis Rheum. **29:** 262–273, 1986.
1006. YOCUM, D. E., KLIPPEL, J. H., WILDER, R. L., GERBER, N. L., AUSTIN, H. A., WAHL, S. M., LESKO, L., MINOR, J. R., PREUSS, H. G., YARBORO, C., BERKEBILE, C., AND DOUGHERTY, S.: Cyclosporin A in severe, treatment-refractory rheumatoid arthritis. A randomized study. Ann. Intern. Med. **109:** 863–869, 1988.
1007. YOSHIMURA, N., AND KAHAN, B. D.: Nature of the suppressor cells mediating prolonged graft survival after administration of extracted histocompatibility antigen and cyclosporine. Transplantation **39:** 162–168, 1985.
1008. YOSHIMURA, N., AND KAHAN, B. D.: Impact of the timing of antigen administration on synergistic immunosuppression with cyclosporine. Transplantation **40:** 108–110, 1985.
1009. YOSHIMURA, N., AND KAHAN, B. D.: The immunosuppressive action of suppressor cells from antigen-cyclosporine-treated hosts on renal allograft survival. Transplantation **40:** 384–389, 1985.
1010. YOSHIMURA, N., AND KAHAN, B. D.: Suppressor cell activity of cells infiltrating rat renal allografts prolonged by perioperative administration of extracted histocompatibility antigen and cyclosporine. Transplantation **40:** 708–713, 1985.
1011. YOSHIMURA, N., AND KAHAN, B. D.: The requirement for the renal transplant to induce allograft unresponsiveness by the combination of extracted histocompatibility antigen and cyclosporine. Transplantation **42:** 642–646, 1986.
1012. YOSHIMURA, N., MATSUI, S., HAMASHIMA, T., KITA, M., AND OKA, T.: The in vivo immunosuppressive action of suppressor cells from alloantigen-cyclosporine-treated mice and the capacity of spleen cells to release interleukins and γ-interferon. Transplantation **45:** 157–162, 1988.
1013. YOSHIMURA, N., OKA, T., AND KAHAN, B. D.: Importance of the presence of allograft in the induction and maintenance of suppressor T cells in antigen and cyclosporine-treated rats. Transplant. Proc. **19:** 3096–3101, 1987.
1014. YOSHINOYA, S., YAMAMOTO, K., MITAMURA, T., AIKAWA, T., TAKEUCHI, A., TAKAHASHI, K., AND MIYAMOTO, T.: Successful treatment of rheumatoid arthritis with low-dose cyclosporine A. Transplant. Proc. **20** (suppl. 4): 243–247, 1988.
1015. YOUNG, E., SCHACHTER, J., PRENDERGAST, R. A., AND TAYLOR, H. R.: The effect of cyclosporine in chlamydial eye infection. Curr. Eye Res. **6:** 683–689, 1987.
1016. ZAHNER, H., AND SCHULTHEISS, K.: Effect of cyclosporin A and some derivatives in Litomosoides carinii-infected Mastomys natalensis. J. Helminthol. **61:** 282–290, 1987.
1017. ZALEWSKI, A. A., AND GULATI, A. K.: Survival of nerve and Schwann cells in allografts after cyclosporin A treatment. Exp. Neurol. **70:** 219–225, 1980.
1018. ZALEWSKI, A. A., AND GULATI, A. K.: Survival of allografts in sensitized rats treated with cyclosporin A. J. Neurosurg. **60:** 828–834, 1984.
1019. ZALEWSKI, A. A., AND GULATI, A. K.: Failure of cyclosporin A to induce immunological unresponsiveness to nerve allografts. Exp. Neurol. **83:** 659–663, 1984.
1020. ZEEVI, A., DUSQUESNOY, R., EIRAS, G., RABINOWICH, H., TODO, S., MAKOWKA, L., AND STARZL, T. E.: In vitro immunosuppressive effects of FK 506 in combination with other drugs. Transplant. Proc. **20** (suppl. 1): 220–222, 1988.
1021. ZHANG, X., ZHANG, J. T., AND HO, M.: Effects of oral ciclosporin on acute and chronic murine cytomegalovirus infection. Intervirology **27:** 130–137, 1987.
1022. ZHAO, X. F., ALEXANDER, J. W., SCHROEDER, T., AND BABCOCK, G. F.: The synergistic effect of low-dose cyclosporine and fluocinolone acetonide on the survival of rat allogeneic skin grafts. Transplantation **46:** 490–492, 1988.
1023. ZIMMERMANN, F. A., WHITE, D. J. G., GOKEL, J. M., AND CALNE, R. Y.: Orthotope Lebertransplantation bei der Ratte. Verlängerung der Ueberlebenszeit von Allotransplantaten durch Cyclosporin A in einem starken Abstossungsmodell. Chir. Forum Exp. Klin. Forsch. 339–344, 1979.
1024. ZWANENBURG, T. S. B., SUTER, W., AND MATTER, B. E.: Absence of genotoxic potential for cyclosporine in experimental systems. Transplant. Proc. **20** (suppl. 3): 931–933, 1988.