IMMUNOSUPPRESSIVE AGENTS[1]

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INTRODUCTION

By definition, immunosuppressive agents are able to prevent or suppress, to some extent, the immune response. Such agents are of great interest in basic immunological research; some are used in clinical therapy. The development of organ allotransplantation programs in human therapy seems to be largely dependent on the achievement of immunosuppression to prevent the rejection of the transplanted organ. Also, an increasing number of human diseases are now recognized as being due to immunological reactions, involving either heterologous or autologous antigens, thus justifying immunosuppressive treatment. In other diseases, immune mechanisms appear to be involved in the pathogenesis of lesions without objective evidence. A beneficial effect of immunosuppressive drugs in those conditions has been interpreted as indirect evidence of an underlying immunopathogenic mechanism. Moreover, the use of immunosuppressive procedures in experimental research and the understanding of their action at cellular and molecular levels provides a mean for analyzing the different steps involved in the normal immune response (1). Thus, removal of the bursa of Fabricius in birds led to the discovery of a two-cell component immune response (2), and immunosuppression by the use of drugs inhibiting macrophage metabolism stresses their role in antibody formation (3). Furthermore, of great theoretical interest is the enhancing effect of immunosuppression on oncogenesis. This provided a new pathway to study the etiopathogenesis of cancer in general, and its relationship to viral infections in particular.

An immunological response usually begins with the phagocytosis and "processing" of antigen by macrophages. This process leads to coating of the macrophage membrane by antigen molecules or fragments (4). These antigenic determinants would bind to antigenic receptors of corresponding antigen-sensitive small lymphocytes, inducing their differentiation and proliferation. Bone marrow derived small lymphocytes would differentiate into humoral antibody producing cells, while thymus derived lymphocytes would differentiate into cells responsible for cellular immunity. This process

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will also lead to the persistence of "memory" cells responsible for the secondary response to antigenic stimulation.

Immunosuppresive agents must act on one or more steps in the sequence of these events. The processing of antigen by macrophages can be impaired by inhibiting their phagocytizing ability with drugs. Some agents induce profound depletion of the total pool of lymphocytes (X-rays, antilymphocyte serum, corticosteroids). The majority of immunosuppressive agents interfere with cell replication or protein synthesis. They are highly active against primary antibody response and delayed hypersensitivity. Drugs that inhibit synthesis of DNA, DNA-dependent RNA, RNA, or proteins belong to this latter category. In general, immunosuppressive agents are much more active on the primary than on the secondary immune response or on established antibody production.

Immunosuppressive agents can be found among physical agents, simple chemical and biological substances, and living organisms. Furthermore, removal of lymphoid tissues may also suppress the immune responsiveness. Specific immunosuppressive agents inhibit the immune response to a given antigen without impairing the response to other antigens, whereas nonspecific immunosuppressants lead to general immune suppression (Table 1).

This review is focused on present data concerning drugs that inhibit DNA, RNA, and protein synthesis, their effect on the immune response, and their clinical application. The general mechanisms of action of these drugs are summarized in Figure 1.

NONSPECIFIC IMMUNE SUPPRESSION

Alkylating Agents

Alkylating agents interact with nucleophilic centers of molecules, especially with the amino, carboxylic, thio, and phosphate groups, as well as with tertiary nitrogen. Their main site of action is located on the N^7 of guanine residue of DNA (5–10). They alkylate nuclear and cytoplasmic RNA, enzymes, and structural proteins. As they have at least two alkylating groups, active alkylating agents cross-link native DNA together, DNA with proteins, DNA with RNA, RNA with RNA, or RNA with proteins (6, 11, 12). In binding N^7 of guanine in the DNA molecule, alkylating agents labilize the glucosidic bridge between base and deoxyriboside, causing depurination and chain scission. In some instances, the DNA molecule remains intact; during RNA replication it may thus produce an erroneous codon. At the cellular level, alkylating agents therefore interfere with nucleic acid synthesis and cell functions. They block the cell cycle in the G_2 phase (6, 12). Furthermore, they may cause a miscodification of messenger, transfer, and ribosomal RNA, which in turn may lead to impairment of protein synthesis.

As immunosuppressants, alkylating agents are acting on the rapidly dividing cells, i.e., the small sensitized lymphocytes (11); they do not affect long lived lymphocytes (13). The most widely used alkylating agent both in

TABLE 1. IMMUNOSUPPRESSIVE AGENTS

Specific immunosuppression	Immunological unresponsiveness in soluble antigen	low zone paralysis high zone paralysis drug-induced immune tolerance
	Passively administered specific antibody	
Non-specific immunosuppression	Physical agents	Whole or local body irradiation extracorporeal blood irradiation
	Simple chemical agents	alkylating agents purine analogues pyrimidine analogues folic acid antagonists antibiotics plant alkaloids carcinogens
	Biological agents	enzymes hormones normal serum antilymphocyte serum antithymocyte serum antimacrophage serum reticuloendothelial blockade
	Living organisms	viruses microbial endo- exo-toxin parasites
	Removal of lymphoid tissues	thymectomy splenectomy thoracic duct fistula ablation of bursa of Fabricius in birds

experimental and human therapy is cyclophosphamide (Endoxan®, Cytoxan®). Cyclophosphamide is believed to be transformed by tissue phosphoraminidase into the active substance norchloretamine (a nitrogen mustard derivative), but this hypothesis is presently questioned by some authors (*7*, 14–16). The other alkylating agents used in immunosuppression are the nitrogen and uracil mustards (chlorambucil and melphalan, an L-phenylalanine mustard, dimethylsulphonates (busulfan), and polyethyleneimines (thio-TEPA, triethylenemelamine). The action of procarbazine (a methylhydra-

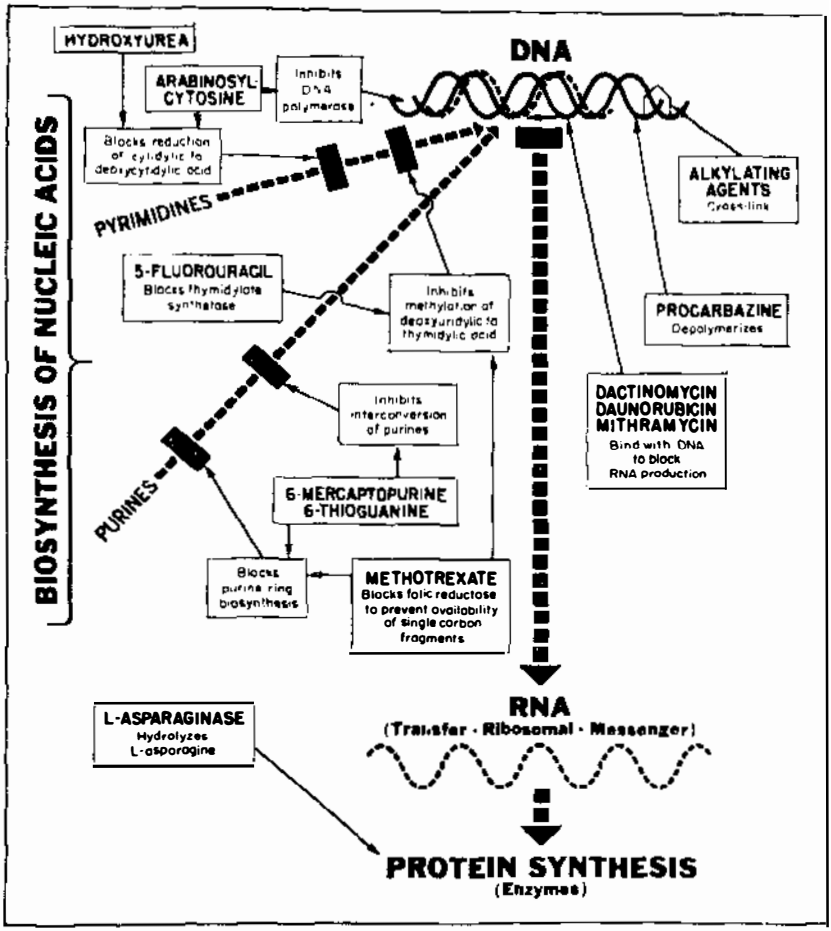

FIG. 1

zine derivative) and mitomycin C on the immune response can also be explained by an alkylating effect (9).

Effect on immunization.—Alkylating agents do not inhibit macrophage phagocytosis but have some effect on the phagocytizing cells themselves, as demonstrated by the slow recovery after a reticuloendothelial system blockade (17). Moreover, they have an effect on the retention of antigen in the spleen of mice (18). Spleen retention is diminished by cyclophosphamide as well as by X-rays, actinomycin D, or cortisone acetate.

Alkylating agents mainly act through their lymphocytolytic effect. *Cyclophosphamide* suppresses the *primary immune response* if administered before, during, or after antigenic stimulation. It affects this primary immune response at different stages (19, 20). Maximal effect is observed in rats when injected before, or 4 days after, antigenic stimulation (12). On the contrary, antibody production can be stimulated when this drug is administered between 11–7 days prior to the antigen (12). In mice stimulated with heterologous red blood cells, the maximum effect is obtained when the immunosuppressive agent is given 1–3 days after the administration of the antigen (21). Cyclophosphamide limits the formation of pyroninophilic blast cells in the primary response (15). In man, cyclophosphamide has also been found to impair immunoglobulin production (22).

Cyclophosphamide may also inhibit the *secondary immune response* and impair on-going antibody synthesis, although to a lower degree than the primary response (19, 23). Cyclophosphamide has been shown to induce immune tolerance if administered simultaneously with high doses of antigen (11, 24, 25). The action of some other alkylating agents on humoral antibody production has also been studied. Berenbaum, Timmis & Braun (26) have analyzed the immunosuppressive potency of sulphonic esters. *Busulfan* has been found to be the best, and a correlation between immunosuppressive activity and lipid-water partition coefficient has been noted. In mice, busulfan displays its maximum suppressive activity when given before antigenic stimulation. In contrast, in the rat antibody formation is enhanced when busulfan is administered before antigenic stimulation. This enhancing effect may be due to the liberation of nucleic acids from damaged cells, which have an adjuvant-like effect (27), or it may be the result of space liberation, permitting cell proliferation (10). *L-phenylalanine mustards* (melphalan), *uracil mustard, nitrogen mustards, chlorambucil,* and *thioTEPA* inhibit antibody production in mice if given before antigenic stimulation, but they have no activity in rats (10). The methylhydrazine *procarbazine* is lymphocytotoxic (28), and has been found to be immunodepressant in mice, rats, and rabbits. In mice, anti-sheep red blood cell antibody formation is suppressed by the administration of procarbazine during a 3-week period prior to antigenic stimulation (29). In rats, the response to sheep red blood cells is not affected but the response to ovalbumin is suppressed (30). When injected before antigen, *methylhydrazine* suppresses the 10S antibody forma-

tion, while when administered after antigenic stimulation, both 1gS and 7S antibody production is inhibited (31). Its effects have been claimed to be greater on the anamnestic response than on the primary response (32).

Delayed hypersensitivity is inhibited by cyclophosphamide when the compound is given during sensitization (8, 14, 33). Similarly, contact sensitivity to inorganic metal compounds is attenuated in guinea pigs (34). Graft survival is prolonged by cyclophosphamide in mice, rabbits, rats, and men, but not in dogs and guinea pigs (5, 8, 14). *Ro-4–6824*, a methylhydrazine derivative, and, to a lesser extent, procarbazine, prolong skin allograft survival in mice (35). A *glycine derivative* alkylating agent seems also to have a beneficial effect on mice skin allograft (36). Finally, it has been found (37) that alkylating agents exert an immunosuppressive activity on the graft, thus attenuating the graft-versus-host disease.

Effect on experimental autoimmune disease.—Studies concerning experimental autoimmune diseases are of great interest since they have definite resemblances to autoimmune diseases in man. Many models are elaborated, such as experimental autoimmune encephalomyelitis and thyroiditis and adjuvant-induced polyarthritis in the rat. The pathogenicity of glomerulonephritis is extensively studied in NZB/W mice, which develop spontaneously antinuclear antibodies and a lupus-like nephritis. Cyclophosphamide readily inhibits the development of experimental autoimmune encephalomyelitis (38, 39), even in the case of an already established disease (40). Salvin & Liauw (41) have observed the same phenomenon when cyclophosphamide is injected simultaneously with allogenic extracts of central nervous system. When the injected antigen is from xenogenic origin, there is little protection. Cyclophosphamide inhibits experimental autoimmune thyroiditis (42). The administration of cyclophosphamide in NZB/W mice suppresses or greatly delays the appearance of antinuclear antibodies, the onset of lupus nephritis, and death rate (44–46), as shown in Table 2. Procarbazine sup-

TABLE 2. CYCLOPHOSPHAMIDE TREATMENT OF FEMALE NZB/W MICE

	Cumulative incidence at 11 months of age		
	Antinuclear antibody[1]	Proteinuria	Death
	%	%	%
Untreated	90	96	95
Treated[2]	0	0	0

[1] Serum diluted 1:10.

[2] Since 4 months of age, 1.8 mg of Cytoxan intraperitoneally weekly.

Taken from *Glomerulonephritis in NZB/W Mice*, P. H. Lambert, Proc. 4th Int. Congr. Nephrol., Stockholm 1969.

presses the adjuvant-induced polyarthritis in rats (47). Its maximum effect appears when it is injected chronically 15 days before to 15 days after antigenic stimulation (30).

ANTIMETABOLITES

Antimetabolites are synthetic substances that have a structural analogy with nucleic acid precursors, and can interfere with the nucleic acid synthesis. Purine and pyrimidine analogs, folic acid antagonists, and analogs of glutamine are the most frequently used antimetabolites.

Purine Analogs

The first synthesized purine analog was 6-mercaptopurine (6-MP), a structural analog of adenine and hypoxanthine, which is a very powerful antimetabolite (48). Its imidazole derivative, azathioprine, is one of the most widely used immune suppressive drugs in basic research and therapy. The sites of action of 6-MP are multiple. In entering a cell, 6-MP is converted by inosinic acid phosphorylase into riboside triphosphate and thioinosinic acid. The latter is transformed into thioxanthylic acid by inosinic acid dehydrogenase, and gives rise to a ribonucleotide (15), as does methylhydrazine (43).

This 6-MP ribonucleotide is converted into 6-methylthiopurine ribonucleotide (49), which can be incorporated into DNA and RNA (50). However, this hypothesis is still controversial (9). The assumed main site of action of 6-MP and its metabolites is at the level of the adenylsuccinate-mediated transformation of inosinic acid into adenylsuccinic acid, the precursor of adenylic acid and xanthylic acid. Thioinosinic acid blocks the action of adenylsuccinate synthetase and competes for inosinic acid dehydrogenase in the conversion into thioxanthylic acid. The 6-MP competes with hypoxanthine for inosinic acid phosphorylase (9). It has a pseudo feed-back effect on purine synthesis by inhibiting incorporation of single or double carbon fragments. The metabolite 6-methylthiopurine ribonucleotide is a strong inhibitor of phosphoribosylpyrophosphate amidotransferase, an enzyme in purine precursors synthesis. The riboside triphosphate, formed along with thioinosinic acid, is an analog of adenosine triphosphate and competes with it (7, 8, 14, 50). Also 6-MP blocks nicotinamide adenine dinucleotide synthesis. In all its effects, 6-MP inhibits primarily *de novo* purine synthesis, thus essentially interfering with DNA synthesis (7). It attacks the cell cycle in S phase.

In vivo, 6-MP is catabolized by xanthine oxidase into thiouric acid. This degradation rapidly diminishes the activity of the cytotoxic drug. It can be blocked by a purine isomer, allopurinol (51). To avoid this reaction, 6-MP derivatives have been synthesized and assayed. A substitution in 9-position, for instance, protects 6-MP against the action of xanthine oxidase. However, it is claimed (7) that this 9-position should be kept free as an essential site in the intracellular conversion of 6-MP into a ribose phosphate. A substitution in position-9 would thus have to be first dealkylated to make the

compound active. Recent findings (52) do not corroborate this conclusion. Although 9-butyl-6MP does not undergo in vivo dealkylation, it is a good immunosuppressant. Moreover, a synergic effect is obtained with 6-MP by competition for xanthine oxidase. The imidazole-substituted 6-MP (azathioprine) which was synthesized under the same premises is split in vivo and liberates 6-MP. This may produce a more delayed action. In some experimental conditions, azathioprine has been found to exert a stronger immune suppressive effect in comparison to an equimolar treatment with 6-MP (53). Conversely (54), 6-MP appears to be more potent than azathioprine when given in high doses.

Effect on immunization.—6-MP diminishes the number of large lymphocytes and macrophages, but seems to have no effect on the phagocytizing ability (14). It strongly inhibits the *primary response* when administered together with or after the antigen. The induced immunosuppression is dependent on the dose of antigen. This profound inhibition of the primary response is found in chicken, mice, rats, rabbits, monkeys, dogs, and men. In guinea pigs, the action is less marked, presumably because of elevated amounts of xanthine oxidase (10). The 7S antibody response is inhibited much more easily than the 19S response. The latter is prolonged when 6-MP is injected before antigenic stimulation (31), and the whole antibody response may be enhanced. The number of plaque-forming cells has been found to be reduced in vitro and invivo (55). In man, *azathioprine* reduces antibody response after a brief enhancing effect (56). 6-MP acts mostly on the inductive phase of the immune response. The *anamnestic response* is depressed only when 6-MP or azathioprine are given in high doses (56). 6-MP has little effect on the on-going antibody synthesis (57).

To a great extent 6-MP also depresses *cellular immune response,* sometimes more than antibody production (15). The antiinflammatory action of 6-MP has been thought to be responsible for this effect (57), however it has not been possible to prevent passive tuberculinic reaction in guinea pigs, even after massive pretreatment with 6-MP (58). The purine analogs inhibit the formation of large pyroninophilic cells and prolong graft survival in rabbit, goldfish, and man. In the mouse and rat, results are less convincing (10). In the dog, pulmonary allograft survival is somewhat prolonged by azathioprine. Better results have been obtained in association with actinomycin C or chloramphenicol (59). It has been shown that 6-MP leads to an increased graft-versus-host reaction, probably by suppressing preferentially the host's immune system (27). Also 6-MP and azathioprine inhibit the onset of experimental autoimmune diseases, such as experimental autoimmune encephalomyelitis (5), and autoimmune thyroiditis. In the latter condition, the experimental disease could not only be prevented, but also, once established, successfully treated by 6-MP (60).

Other Purine Analogs

Many purine analogs have been synthesized and assayed, but few have properties resembling those of 6-MP. Antibody formation can be suppressed by *8-azaguanine,* but it is highly toxic; *6-thioguanine* also inhibits antibody production; *6-bromodeoxyuridine* prolongs graft survival in combination with antilymphocyte serum (61). The purine nucleotide analogs (for instance β-d-ribosyl-6-methylthiopurine) prolong graft survival and reduce the number of plaque forming cells, but appear to have little effect on antibody production (62–66). If injected simultaneously with bovine serum albumin, *6-thioinosine* inhibits antibody formation in rabbits (67). The *9-butyl-6-thiopurines* suppress acute graft rejection in dogs (kidney allograft) and mice (skin allograft). They inhibit hemagglutinin formation in mice (52). To a lesser degree *3-hydroxyxanthine* shares the same effects (68). Some purine analogs with immune suppressive activity have an antipyrimidine effect, as e.g. N^6 ($\triangle^2$-*isopentyl*) *adenosine,* which inhibits the incorporation of uridine into RNA, and thymidine into DNA (69). Other antimetabolites may not be immunosuppressants, but can enhance the action of antipurine. *Duazomycin A,* a glutamine antagonist, increases the effectivity of 6-MP in the suppression of experimental allergic encephalomyelitis (70). *Azaserine,* another glutamine analog, is used in human transplantation (71). Glutamine analogs act at the step where formylglycineamide ribonucleotide is converted into formylglycineamidine ribonucleotide (9).

Pyrimidine Analogs

Pyrimidine analogs can be the structural analogs of uracil and thymidine or of cytosine and cytidine. The former group includes the fluor derivatives of uracil, 5-fluorouracil and its riboside, 5-fluorouracil deoxyriboside. Both inhibit thymidilate synthetase, thus depleting the cell in thymidilic acid, and 5-fluorouracil can be metabolized as uracil but cannot be transformed into the analog of thymidilic acid. It thus acts through thymidine depletion and its effects can be reversed by the administration of thymidine. According to Boesen & Davis (7), it is incorporated into RNA. Other halogenated derivatives, namely bromo-, iodo-, and chloro-deoxyuridine are incorporated into DNA, causing the synthesis of a fraudulent DNA (9). Another analog of uracil, the 2-thiouracil, was found to inhibit DNA synthesis (72). The second group of pyrimidine analogs is represented by arabinosyl-cytosine and 6-azauridine. The inhibiting effect of 6-azauridine on DNA and RNA synthesis is reversible by the administration of cytidine and uridine (5). Cytosine-arabinoside is phosphorylated by deoxycytidine kinase (11, 73). The same enzyme converts adamantoyl-cytarabine (a cytosine-arabinoside derivative) into cytosine-arabinoside (74). Cytosine-arabinoside inhibits the DNA polymerase and the formation of deoxycytidine from cyto-

sine diphosphate. It is converted into a nucleotide (51) and can be incorporated into DNA and RNA. Administration of deoxycytidine inhibits the drug effects.

Effect on immunization.—Both *5-fluorouracil* and *5-fluorouracil deoxyribose* show relatively poor immunosuppressive activity in animals (5), although Merritt & Johnson (75) have been able to suppress antibody formation in mice. In vitro, 5-iodouracil deoxyribose and 5-bromouracil deoxyribose suppress antibody formation but have no effect on the plaque-forming cell number. Perhaps 5-fluorouracile deoxyribose may have an enhancing effect on antibody synthesis (19). Both 5-fluorouracil and 5-fluorouracil deoxyribose depress delayed hypersensitivity. In man, Mitchell & DeConti (76) studied 5-fluorouracil activity in 12 patients treated for cancer and found that 5-fluorouracil inhibited primary response in 8 of 10 patients tested. In the other 2, the produced antibodies were of the 2-mercaptoethanol-sensitive type. In 4 of 9 patients, the secondary response was also suppressed, while in the others it was delayed. The sensitivity to 2,4-dinitrochlorobenzene was abolished and cutaneous reactivity to common bacterial antigens was lowered. It thus appears that 5-fluorouracil exhibits good immunosuppressive activity in man, while being a poor immunosuppressive agent in animals.

Cytosine-arabinoside seems to interfere with the differentiation of macrophages, but does not act on the processing of antigen (73). It suppresses antibody formation in mice, rats, hamsters, and rabbits immunized with heterologous red blood cells (10). The primary response is inhibited if the drug is injected after antigen stimulation. Cytosine-arabinoside inhibits mainly 7S antibody formation. In the rat, 19S antibody production can be enhanced or suppressed, depending on dose of drug and time of administration (77). Cytosine-arabinoside, on the other hand, almost completely suppresses plaque-forming cells in vitro and in vivo, if given 12 hours after the antigen (78), without affecting the in vivo antibody production (55, 79). *Adamantoyl-cytarabine,* a derivative, is a much more powerful immunosuppressant in mice than cytosine-arabinoside. It acts independently of the time of injection and its effects are long-lasting (80). However, in rats challenged with sheep red blood cells or bacterial flagellae, adamantoyl-cytarabine does not affect the primary response. The drug seems to act mostly on thymus-dependent lymphocytes (74). Mitchell et al (81) have tested immune reactivity in 36 patients treated with cytosine-arabinoside: primary and secondary response to E. coli or to tetanus toxoid was inhibited. Two weeks after cessation of therapy, only 14 patients regained a normal reactivity. Cytosine-arabinoside depresses delayed hypersensitivity in rabbits (10). In association with antilymphocyte serum, it can prolong skin allografts for a long time (82). On the other hand cytosine-arabinoside has no effect on canine allograft rejection (83). Adamantoylcytarabine suppresses graft-versus-host disease and prolongs skin allograft survival in mice longer

than cytosine-arabinoside (84). The latter inhibits the onset of experimental encephalomyelitis in rats (10).

Folic Acid Antagonists

Aminopterin and its relative methotrexate (amethopterin) bind firmly dihydrofolate reductase, thus inhibiting the conversion of dihydrofolic acid into tetrahydrofolic acid, i.e. the co-enzyme of the processing of single carbon fragments in the synthesis of purine and thymidilate (7). Methotrexate also blocks the synthesis of thymidilate at the conversion of methanol into methyl. Thus, folic acid antagonists inhibit DNA synthesis and impair cell replication (14). Methotrexate also depresses dehydrogenase activity and blocks transformation of immunoblasts into small lymphocytes without destroying the former (85). An interesting fact is that the effect of folic acid antagonists can be totally reversed by the administration of large amounts of folic or folinic acid. This led to the discovery of the "rescue" technique in therapy, namely the administration in sequence of methotrexate in high doses and folinic acid in an attempt to kill most of the rapidly dividing cells without damaging the others (86). Methotrexate increases the activity of thymidilate synthetase, thus accelerating the recovery of intoxicated cells (87). In rabbits and guinea pigs, methotrexate does not have the same toxicity as in most other species. This is due to elevated amounts of aldehyde oxidase which detoxifies methotrexate (14).

Effect on immunization.—Methotrexate markedly suppresses *primary* and *secondary responses* if injected early after antigenic stimulation (88). This happens in mice and rats immunized with sheep erythrocytes and typhoid vaccine (10); formation of antibodies directed against diphtheria toxoid and ovalbumine is inhibited in guinea-pigs. Antibody suppression occurs also in dogs but not in rabbits (14). Folic acid antagonists show a powerful immunosuppressive effect in man challenged with the V_1 antigen, keyhole limpet hemocyanin or diphtheria toxoid; they inhibit primary and secondary immune responses to E. coli and tetanus toxoid to the same extent as cytosine-arabinoside (81).

Methotrexate can inhibit delayed hypersensitivity in guinea-pigs (34, 89). It can also impair the appearance of tuberculin reaction in an already sensitized animal (5). Folic acid antagonists also depress *delayed hypersensitivity* in man (56). Methotrexate prolongs skin allograft survival in mice, rats, guinea-pigs, dogs, and fish, but not in rabbits (10). It supresses graft-versus-host reaction. Methotrexate inhibits experimental autoimmune encephalomyelitis (90), experimental autoimmune thyroiditis (60), and adjuvant arthritis in rats (91) with equal efficiency as 6-MP.

Hydroxyurea, although not a true antimetabolite, inhibits the enzymatic reduction of purine and pyrimidine nucleotides. It is a specific inhibitor of the DNA synthesis (9). Hydroxyurea slightly affects antibody production

(92), and prolongs graft survival (5). Paget et al (93–95) have studied the immunosuppressive effects of heterocyclic substituted ureas. These substances yield high immunosuppressive activity and one of them seems to inhibit the primary response in mice immunized with heterologous erythrocytes more easily than azathioprine.

ENZYMES

L-Asparaginase.—Little is known about the immunosuppressive effects of L-asparaginase. Usually extracted from E. coli, it hydrolyses L-asparagine. As the amount of asparagine synthetase is low in mammal tissues, cells require exogenous asparagine for their metabolism (96). If the L-asparagine is destroyed, cell metabolism is disturbed, as shown in the experiments of Weksler & Weksler (97) who observed a marked drop in the incorporation of thymidine into DNA.

L-asparaginase is able to suppress primary response in mice and rabbits. This immune suppression occurs regardless of the time of L-asparaginase injection (98–100). The production of 2-mercaptoethanol-sensitive antibodies could be prolonged. Schwartz (101) observed that the drug has immunosuppressive activity if given 24 hours before to 4 hours after antigenic stimulation, while Chakrabarty & Friedman (102) observed no immunosuppressive effect when L-asparaginase is injected before the antigenic stimulation. In rabbits, high doses of L-asparaginase given each day during 21 days after administration of the antigen result in a complete suppression of antibody production. At lower doses, immunosuppression is incomplete and anti-L-asparaginase antibodies begin to appear (103). The best immunosuppressive effects occur when drug and antigen are injected simultaneously. However, if L-asparagine is added, antibody response remains normal. The number of plaque-forming cells is reduced after treatment by L-asparaginase (104), and the phytohemagglutinin-induced blastogenesis of lymphocytes is inhibited (105).

The exact mode of action of the drug is still unknown, but it appears to act on the antigen recognition, before cellular multiplication. L-asparaginase affects the lymphoid tissues directly: it induces lymphopenia, reduces the size of lymph nodes, thymus, and spleen. Lymph node lymphocytes do not migrate normally (97) and delayed hypersensitivity is inhibited (103). The effect of L-asparaginase on the lymphoid tissues may explain the good results obtained in transplantation (106). In mice, skin graft survival is prolonged even across strong histocompatibility barriers, the effect being superior to that of azathioprine (107). These effects on graft survival should depend on a particular mechanism of action of L-asparaginase since similar results are often observed in the presence of L-asparagine. Moreover, graft survival is prolonged when heat-inactivated L-asparaginase is used instead of L-asparaginase with normal enzymatic activity (107). L-asparaginase strongly inhibits the onset of experimental autoimmune encephalomyelitis (108). In man, the enzyme suppresses antibody production, inhibits phyto-

hemagglutinin-induced stimulation of lymphocytes, and delays hypersensitivity (109). The activity of L-asparaginase is in direct relationship with its half-life. It is interesting to note that the immunosuppressive activity of L-asparaginase does not prevent immunization against it.

L-Glutaminase.—It is known that glutamine antagonists have immunosuppressive activity (70). Hersh (110) has shown that L-glutaminase inhibits lymphocyte stimulation induced by phytohemagglutinin (PHA), streptolysin O, and allogeneic antigens. The addition of L-asparagine to the drug does not modify these results.

Papain.—Papain has also immunosuppressive features (111); when injected into rabbits 12 hours before the administration of the antigen, the 3a-1 fraction of papain depresses primary response without impairing the secondary response.

ANTIBIOTICS

Actinomycins.—Actinomycin D, extracted from *streptomyces parvullus,* is one of the most specific cytotoxic agents known (5). At low doses, actinomycin D binds to deoxyguanosine residues of DNA, therefore interfering with RNA polymerase; at higher doses it impairs the transfer of messenger RNA from nucleolus to cytoplasm. Accordingly, actinomycin D suppresses DNA-dependent RNA synthesis, especially that of messenger RNA (9, 112, 113).

Actinomycin D inhibits primary response in rodents and chickens when injected after the antigen (114, 115). In mice, Uteshev et al (116) observed an immunosuppressive effect of actinomycin D whether it is injected 24 hours before or 24 hours after the antigen. The 19 S and 7 S plaque forming cells alone were suppressed. In vitro, actinomycin D impairs also the primary response (117, 118). On the other hand, a dose-dependent enhancement of the primary response following actinomycin D treatment has been observed in mice immunized with sheep red blood cells (119); the 19 S response was increased while 7 S response was lowered. In vitro secondary response can also be inhibited by high doses of actinomycin D, whereas low doses enhance the anamnestic response (79, 120). On-going antibody synthesis is not affected by the antibiotic. Miller & Cole (13) explain this fact by the presence, in plasma cells, of a stable messenger RNA.

In vitro, delayed hypersensitivity is inhibited (3); actinomycin D blocks the lytic action of sensitized lymphocytes (121). On the other hand, actinomycin D has a poor effect in transplantation (122), but it is frequently used as an adjuvant treatment.

Actinomycin C, which contains three antibiotics, is similar in its effects to actinomycin D. It inhibits primary response (123), but as recently shown (124), antibody forming cells may be either enhanced or inhibited depending on the dose of antigen injected simultaneously.

Adriamycin.—Extracted from *streptomyces peucetius,* adriamycin is an antineoplastic agent, which inhibits DNA and RNA synthesis. Adriamycin

inhibits primary response in mice if injected after the antigen; it is lymphocytotoxic and suppresses also the phytohemagglutinin-induced lymphocyte stimulation (125).

Alanosine.—A new antitumoral agent whose mechanism of action still needs to be elucidated. In mice and rabbits it inhibits antibody formation and delayed hypersensitivity when injected simultaneously, or before antigen administration (126-127).

Chloramphenicol.—Chloramphenicol inhibits protein synthesis by binding messenger RNA to ribosomes, or by blocking the availability of template RNA (128). It has the steric configuration of a pyrimidine analog. Chloramphenicol inhibits antibody synthesis in mice and rabbits, particularly the 19 S antibody response (128, 129). It diminishes the number of plaque-forming cells in cell culture (130). In sufficient doses the antibiotic inhibits the anamnestic response in rodents and men (10), does not inhibit delayed hypersensitivity (3), but delays allograft rejection. Some of its analogs seem to improve lupus glomerulonephritis (131). It should be mentioned that antibody depression in patients suffering from typhoid and para-typhoid fever, and treated with chloramphenicol, does not depend on the drug action (132).

Mitomycin C.—Produced by *streptomyces caespitosus,* mitomycin C depolymerizes nucleic acids and inhibits their replication (5). In fact, it has alkylating activity (133). At low doses, it affects DNA formation but not RNA or protein synthesis. At high doses the synthesis of all nucleic acids and proteins is affected (10). Mitomycin C inhibits the primary response in mice in vivo and in isolated lymph nodes (79). In Lewis rats it suppresses the formation of antibodies directed against allogenic tissue antigens (134). Mitomycin C has no action on the on-going antibody synthesis of lymphocytes as assayed in tissue culture (135). Sakauchi & De Witt (134) observed inhibition of DNA synthesis and delayed hypersensitivity as well as lymphopenia by mitomycin C treatment; skin graft survival was prolonged in mice but not in rats.

Ovalicin.—Obtained from *pseudorotum ovalis,* ovalicin reduces the primary immune response and inhibits plaque-forming cells in mice (136). It prevents the onset of experimental autoimmune encephalomyelitis in rats and prolongs skin allograft survival.

Puromycin.—The antibiotic puromycin, extracted from *streptomyes alloniger,* suppresses the aminoacid transfer from soluble RNA to ribosomal protein. Probably it competes with aminoacyl-transfer-RNA, thus eliciting incomplete protein chains (59). Antibody production against polio virus is inhibited by puromycin treatment either before or after antigenic stimulation, while antibody production against human serum albumin is only inhibited when treatment is given after antigen administration. In vitro, it inhibits on-going protein synthesis and suppresses plaque forming cells (55, 112).

Other antibiotics also have immunosuppressive properties. *Rifamycin,* a

well known tuberculostatic, blocks the DNA-dependent RNA polymerase. At high doses, in vivo, it inhibits humoral and cellular immune response (137). *Rubidomycin* inhibits rat adjuvant arthritis (138). *Trimethoprin,* a steric analog of azathioprine has antifolic properties. Its effects on graft survival are comparable to those of azathioprine (139). The immunosuppressive effects of other antibiotics are systematically studied by Russian authors. Thus *Tauromycin* and *Streptothricin* have been found to have an immune suppressive activity (140, 141).

Plant Alkaloids.—Plant alkaloids are substances well known for their antimitotic effect. They are spindle poisons and block cell mitosis in metaphase, probably by interfering with the adenosine triphosphate spindle protein system (7). The mechanism by which these compounds produce immunosuppression is still unclear. It is unlikely that it is due to their antimitotic activity (5). On the other hand, in vitro studies have shown that, at high doses, plant alkaloids inhibit nucleic acid synthesis (7). RNA and protein synthesis appear to be the main target of the action (9). This action could be reversed by the administration of tryptophan and glutamic acid (5).

Colchicine, extracted from the autumn crocus, inhibits phagocytosis and antibody production in mice and rats (10), while in the hamster, it suppresses antibody formation only at high doses. At low doses, antibody production is enhanced. In vitro, colchicine reduces the number of plaque-forming cells in the secondary immune response (142). It has no effect on delayed hypersensitivity. In rabbits, colchicine inhibits the onset of serum sickness if injected before the antigen (143).

The *Vinca alkaloids,* vincristine and vinblastine, seem to act on the circulating precursors of macrophages (144). They suppress antibody production during the time of their administration (5, 12). Vinca alkaloids are effective in mice if administered after the antigen, while they have no activity at all in rabbits; the antibody production can be stimulated if the drugs are given before antigenic stimulation. Vinca alkaloids are furthermore able to depress delayed hypersensitivity and to prolong skin allograft in mice (10).

Little is known about the immunosuppressive activity of *podophyllotoxin.* It has an antimitotic activity comparable to that of colchicine. Its methyl-hydrazine derivative, proserid R, seems to be active in progressive chronic polyarthritis (145).

Recently the mechanism of action and immunosuppressive activity of a new plant alkaloid derived from *Ochronia barbanica:* the *methoxy-9-elliptirine lactate* has been studied (146, 147). This compound inhibits nucleic acid and protein synthesis and reduces the number of plaque-forming cells when injected after antigenic stimulation.

Many other substances that inhibit nucleic acid synthesis are also immunosuppressants, such as imidazole carboximide and triazanoimidazole derivatives (148, 149), β-3-thienylalanine, a phenylalanine analog (150), and acriflavine (5).

HORMONES

Adrenal Steroids

The mechanism of action of corticosteroids is poorly understood. They are known to be lympholytic and it is presumably by this mechanism that antibody suppression occurs. They also have a stabilizing effect on cell and lysosomal membranes.

Effect on antibody production.—Corticosteroids inhibit primary immune response when injected prior to the antigen (151, 152). In mice and rats, the administration can be retarded up to 18 hours after antigenic stimulation without greatly impairing immunosuppressive activity (10). The strongest immunosuppressive effect in mice is obtained (153) when cortisone acetate is given in high doses 3 days before the antigenic challenge. The 7S antibody formation is more affected than 19S antibody formation. When the steroid is injected only after antigenic challenge, the late 7S immune response is reduced. Antibody titers fall progressively, but the height of the immunosuppression is dependent on the doses of antigen (21). Corticosteroids seem to interfere with the "processing" of antigen (18) and reduce the capacity of mouse spleen to retain antigen; they also interfere with antigenic competition by action on short lived lymphocytes (154). Corticosteroids can inhibit the secondary response by depressing the memory cells: if a single large dose of hydrocortisone is given 5 days before antigenic rechallenge, the 7S response is totally abolished, the 19S response enhanced, and the number of plaque-forming cells reduced with an altered morphology (155). Corticosteroids also depress ongoing antibody synthesis, at least temporarily, as shown (156) in mice infected with Trichiuris muris. In this system, a 2-day administration course of cortisone acetate during the primary infection suppresses primary and secondary response.

Corticosteroids do not prevent development of delayed hypersensitivity, but inhibit delayed skin reaction, possibly due to their antiinflammatory effect. However, the effect of steroids on macrophage migration led to the assumption (157) that corticosteroids affect more the afferent limb of delayed hypersensitivity than the efferent one. Corticosteroids moderately prolong skin allograft survival in mice and guinea pigs, but are ineffective in pigs, dogs, and monkeys (14). In rabbits, triamcinolone is superior to other cortisone derivatives for prolongation of skin allograft survival (158).

Adrenal steroids inhibit experimental autoimmune encephalomyelitis, adjuvant-induced arthritis in the rat, and hemolytic anemia in NZB mice but they have no action on autoimmune thyroiditis (60, 159–161). It must be stressed, however, that the guinea pigs' response to corticosteroids in general is very low.

Other Hormones

In the immunological relationship between fetus and mother, some workers have studied the effect of female hormones on immune response and

graft survival. *Medroxyprogesterone* inhibits primary response to bovine gamma globulin in the rabbit (162). A progesteroid derivative, 6-methyl-hydroxy-progesterone, significantly prolongs skin allograft survival in rodents (163). Canine renal allografts as well as skin allografts, are prolonged under medroxyprogesterone treatment (162). Silver et al (164) found an adequate immunosuppression by progesteroids in monkey kidney homografts; they assume that these steroids act through a cortisone-like effect.

It is known that hypopituitary dwarf mice are immunologically deficient. The administration of somatotropic hormone or thyroxine correct the defect. A further treatment of anti-somatotropic hormone or anti-thyroxine suppressed antibody formation (165). On the other hand, pituitary hormones do not seem to be necessary to the integrity of immunological response (166), in hypophysectomised rats. However, the time between hypophysectomy and test challenge may have been too short for an immunological defect to appear. There is a definite relationship (167) between somatotropic hormone and the integrity of the thymus and peripheral lymphatic tissues. Another possible role of somatotropic hormone is in the controlling of maturation and differentiation of the antigen sensitive cells into antibody producing cells (167).

Viral Infections

More than 60 years ago, von Pirquet described the temporary suppression of the tuberculin skin reaction during an intercurrent measles infection (168). Today, we know that many different viruses interfere with immunological reactivity, presumably by infection of lymphoid cells and macrophages (169).

Non-oncogenic viruses.—In animals, Junin virus, murine cytomegalovirus, lymphocytic choriomeningitis virus, and Rinderpest virus (a myxovirus) inhibit the primary and hinder the secondary immune response (170–172). Lymphocytic choriomeningitis virus affects, in mice, both humoral and cellular antibody response, but has no effect on preexisting antibody synthesis (173); the level of immune reactivity in infected animals is closely dependent on the time of infection. Lacticodehydrogenase virus acts as adjuvant if administered prior to antigen, prevents immune tolerance, and prolongs skin graft survival in mice (174). It suppresses graft-vs-host reaction. Some mice infected with lymphocyte choriomeningitis present unimpaired immune reaction toward any antigen but lymphocytic choriomeningitis virus. This fact (175), indicates that a specific depression of immunity might have been achieved.

In mink, chronic infection with Aleutian disease virus reduces circulating antibody response (176), while the secondary response is often enhanced (177). Rubella and Newcastle virus inhibit phytohemagglutinin-induced lymphocytic blastogenesis: measles and influenza depress delayed hypersensitivity (172).

Oncogenic viruses.—The group of Friend and Rausher leukemia viruses inhibit early primary and secondary response, as well as delayed hypersensi-

tivity (172). In mice, cellular immunity is profoundly depressed, while suppression of humoral immunity depends on the time of infection: when injected simultaneously with the antigen, virus causes only a slight decrease of immune response, whereas infection 4 days prior to antigenic challenge may lead to total suppression of antibody production (178). Friend disease virus acts possibly through the infection of the macrophages, where it was shown to impair messenger-RNA synthesis (179). Rauscher leukemia virus probably acts by antigenic competition (180). The activity of Friend and Rauscher leukemia viruses might be explained by an influence on bone marrow precursors of antibody-forming cells (180). Secondary response is inhibited by Friend disease virus administered 15 days before antigenic stimulation, while it is poorly affected by the Rauscher leukemia virus (181).

Many hypotheses have been proposed to explain the viruses' effect on immune response (169). Viruses might act on the "processing" of the antigen or on the lymphoid cell multiplication by infection and damage to the cells involved in these processes. They might also act as competitive antigen, as lymphocytolytic agent through adrenocorticosteroid release, or in the case of leukemia viruses by depleting the pool of lymphocytes available for the immune response.

SPECIFIC IMMUNE SUPPRESSION

Effect of passively administered specific antibody.—It has been known for some time that passive administration of specific antibody either separately or together with the antigen may suppress the primary immune response (182). This type of immunosuppression is highly specific and implies an interaction between the passive antibody and the antigen (182, 183). Indeed, injection of isolated F (ab) 2 or Fab fragments of antibody can inhibit the primary immune response (184–187). Inhibition of antigenic stimulation seems to occur at the level of antigenic determinants and not at the level of the whole molecule (188). In rabbits immunized with human gamma globulin, an injection with anti-human Fab is followed by the production of antibodies reacting only with Fc fragment (189). Immunosuppression can be achieved (190) with sufficient amounts of antibody to cover all the antigenic sites but not to precipitate the molecule as a whole. However, Uhr & Baumann (191) in a system of diphtheria toxoid-antitoxin, find a ratio $AgAb_3$ as effective as $AgAb_5$, thus suggesting that saturation of all antigenic determinants with antibody is not always necessary to suppress the immune response. Interaction between antigen and antibody may also take place after the "processing" of the antigen (192).

Good immunosuppression is obtained when antibody is injected prior to, simultaneously with, or immediately after the antigen (182). Inhibition of the secondary response is much more difficult (185, 191) but could nevertheless be achieved by using sufficient doses of high affinity antibodies (182). Passive antibody administration has no effect on delayed hypersensitivity (191). Conversely, Axelrad & Rowley (193) have been able to suppress

delayed hypersensitivity to sheep red blood cells completely by a simultaneous injection of antigen and antibody. The authors explain this synergic activity of antigen and antibody by stimulation of antigen reactive cells by the antigen, and a presumed action of the antibody on these cells, the result being a limitation of the number of cells available to express the delayed hypersensitivity. This scheme was applied in rats' renal allografting (194). A near indefinite survival of the graft was obtained by injecting intravenously donor spleen cells 1 day before transplantation and by passive immunization with anti-donor spleen and lymph nodes 2 hours before and 1 hour after the transplantation. Prevention of Rh immunization in mother-fetus Rh incompatibility is a direct result of this specific effect of passive antibody administration.

CLINICAL USE OF IMMUNOSUPPRESSANTS

Immunosuppressants are applied in a wide range of clinical states whose pathogenicity is dominated by immunopathologic mechanisms. In general, these drugs are given only in conditions severe enough to justify the use of potentially dangerous agents (see paragraph on side reactions.) Unfortunately, despite many strong recommendations, very few controlled studies have been conducted. Furthermore, immunosuppressants are frequently administered in association with other drugs, which further hampers proper evaluation of results.

Compared with the large number of immunosuppressant agents, the reader will be surprised to discover only a few of them in clinical practice. In view of the above mentioned difficulties one tends to gain experience first with a small number of well defined compounds including 6-MP, azathioprine, cyclophosphamide, chlorambucil, methotrexate, corticosteroids, and actinomycin D.

At the present time most clinicians give preference to corticosteroids. However, since continued therapy with large amounts entails the risk of serious side reactions, more potent immunosuppressants are being increasingly applied, usually in association with corticosteroids, whose antiinflammatory features remain unique. In a recent WHO report an impressive proportion of patients suffering from immunopathological conditions seemed to improve on this regime (Table 3).

In the following paragraphs we summarize experiences with immunosuppressive therapy in the most important clinical conditions.

Autoimmune hemolytic anemia.—Treatment of autoimmune hemolytic anemia differs considerably according to whether the disease is of the warm antibody type or cold antibody type. In the warm antibody type syndromes, the best results are obtained with corticotherapy, eventually associated with splenectomy (195). However, frequent relapses are observed. Antimetabolites are used in patients not responding to the above therapy. Treatment with azathioprine appears moderately encouraging, while alkylating agents give more doubtful results (196). Better results are obtained (197) when

TABLE 3. ANTIMETABOLITE THERAPY IN IMMUNOLOGICAL DISEASES 921 PATIENTS, ALL REFRACTORY TO CORTICOSTEROIDS. WHO REPORT

Disease	Number of cases	Success in %
Ulcerative colitis	62	77.5
Rheumatoid arthritis	128	72.2
Chronic & lupoid hepatitis	93	71
Autoimmune hemolytic anemia	82	62
Idiopathic thrombocytopenic purpura	83	58
Systemic lupus erythematosus	194	56.2
Nephrotic syndrome	232	55.5
Chronic glomerulonephritis	167	47.8
Lupus nephritis	60	55.5

From: *Immunopathology. 5th International Symposium.* Miescher, P. A. & Grabar, P. Schwabe & Co., Basel/Stuttgart 1968.

azathioprine is associated with prednisone. We have obtained a satisfactory result in 3 patients with long standing autoimmune hemolytic anemia who were not responsive to prednisone alone, but responded to combined azathioprine (150 mg per day) and prednisone (10 mg per day) therapy.

The cold antibody type syndromes do not respond well to corticosteroids. Treatment by chlorambucil or cyclophosphamide seems to give best results (195, 198).

Taken as a whole, 62% of autoimmune hemolytic anemias are improved by antimetabolite therapy (Table 3). It should be noted that in autoimmune hemolytic anemias, as in other immunological diseases, there is no direct correlation between clinical improvement and the degree of achieved immunosuppression, an observation which has surprised many workers (56, 199).

Idiopathic thrombocytopenic purpura (ITP).—About 70% of patients with ITP respond favorably to the administration of corticosteroids with or without splenectomy (15, 196). Most clinicians limit use of antimetabolites to cases resistant to this combined approach, or to patients not responding to steroid treatment and exhibiting an increased operation risk. In this selective group of resistant patients, the result of antimetabolite therapy is rather modest. We obtained normalization of the number of platelets in only 2 of 7 patients treated with 6-MP and prednisone. However, the bleeding tendency was improved in 3 additional cases, leaving only 2 complete failures. With the aid of azathioprine and corticosteroids in patients without splenectomy 12 complete remissions were obtained of 17 patients (200).

Rheumatoid arthritis.—Many immunosuppressive agents have been tried in rheumatoid arthritis, mainly corticosteroids, nitrogen mustard, 6-MP, azathioprine, chlorambucil, cyclophosphamide, and methotrexate (196). Improvement has been observed in 75% of cases after a treatment with cyclophosphamide (201). A similar percentage of success is given in the WHO

report (Table 3). Of 60 cases treated by chlorambucil 40% of the patients showing a marked improvement (202). They have found a good correlation between the clinical improvement and the degree of inhibition of the phytohemagglutinin-induced blastogenesis of lymphocytes. Significant improvement is also achieved with methotrexate (203). On the other hand, Mason et al (204) have compared, in a double blind test, azathioprine associated with corticosteroids regimen and administration of corticosteroids alone. In this series, a 36% reduction of steroid doses is observed when given along with azathioprine.

Local treatment with intra-articular injections of thioTEPA seemed, at first, to be promising but was later much disputed (196).

Systemic Lupus Erythematosus (SLE).—With the introduction of steroid treatment, life-threatening episodes of SLE could be controlled for the first time (205–207). Serious side reactions of prolonged steroid administration led clinicians to look for alternative drugs. With the discovery of immune phenomena as the main pathogenic pathway of the disease, immunosuppressive therapy appeared to be the logical new approach (208). A controlled cooperative study comprising various medical centers was planned in 1966, but unfortunately no protocol acceptable by all groups concerned could be worked out. Without the results of a controlled study, it is still impossible to prove the efficacy of immunosuppressive treatment of SLE in statistical terms. However, it became quite obvious to clinicians that patients with kidney involvement did better on combined corticosteroid-immunosuppressive therapy than on steroids alone. Furthermore, the management of severe SLE cases became easier with this new approach. Schubert et al (209) compared a group of patients treated with prednisone alone or with 6-MP. Better results were obtained in the second group. Similar good results were obtained with 6-MP, azathioprine, methotrexate, or cyclophosphamide by others (210–213).

According to a WHO report (Table 3), more than 50% of corticosteroid-resistant cases are improved by antimetabolites. However, doubt is still expressed by some authors (196, 214). According to Gabrielsen & Good (5), 6-MP alone does not give better results than prednisone alone. The association of prednisone with antimetabolites appears to give the best results. Our own approach consists in limiting long term prednisone medication to 10 mg per day in association with either 6-MP (0.8 mg/Kg daily), azathioprine (1.6 mg/Kg daily), cyclophosphamide (2.4 mg/Kg daily) or methotrexate (10–20 mg per week). The relative efficacy of the various antimetabolites is difficult to evaluate. In one severe case, maintenance therapy required either 100 mg of azathioprine or 150 mg of cyclophosphamide in addition to 10 mg of prednisone. Another problem resides in the gradual resistance to 6-MP or azathioprine. Sequential administration of two different immune suppressive agents may prevent this complication (206).

Methotrexate medication is difficult to handle. Individual susceptibility varies greatly from patient to patient. Renal failure precludes administra-

tion of methotrexate, which is eliminated predominantly by the kidneys. Another difficulty with methotrexate is the possibility of a "cumulation toxicity" which may appear after 5–7 weeks with an otherwise well tolerated dose (206).

Most authors agree that lupus nephritis should be treated by a combined prednisone-azathioprine medication, with initially very high prednisone doses (60–100 mg daily) (213, 215, 216). Prior to this approach, prolonged administration of large amounts of corticosteroids has been advocated (217). The longer and more profound the renal lesion is, the more difficult it is to obtain improvement (213).

Chronic glomerulonephritis.—Efficacy of immunosuppressive treatment remains undefined (196) and appears often disappointing. In a study of 62 patients whose renal function had been carefully assessed during treatment with azathioprine and corticosteroids, Herdman, Michael & Good (218) obtained in only 30% a relevant improvement of the renal condition, with 60% complete failures. In a controlled trial of 149 cases, organized by the British Medical Research Council (219) there was no appreciable difference between the control group and the group treated with azathioprine and corticosteroids.

The combined steroid-antimetabolite treatment produces a strong anti-inflammatory activity, which may play a major role in a successful treatment (56). Interesting results have been reported (220) with administration of the anti-inflammatory drug indomethacin alone.

Idiopathic nephrotic syndrome.—This condition responds rather well to corticosteroid therapy, especially in children (196). In steroid resistant patients or in patients requiring high doses for a long period, a 50% remission rate has been reported with 6-MP, azathioprine, and cyclophosphamide (Table 3) (221–223). In a well controlled study, the usefulness of cyclophosphamide was clearly demonstrated (224).

Goodpasture's syndrome.—This rare syndrome appears to respond to the association of corticosteroids with azathioprine (207–226).

Vasculitis.—The vascular purpuras of the Schoenlein-Henoch group as well as the chronic hypergammaglobulinemic variety (two personal cases) appear to respond well to 6-MP, azathioprine, or methotrexate (198, 211).

Periarteritis nodosa.—Some patients respond well, others fail to improve. Three patients out of 5 (personal observations) improved markedly by a combined azathioprine-corticosteroid treatment, including an Australian antigen positive case. The course was unaltered in the 2 other patients with fatal outcome. One female patient discontinued treatment after 18 months; 1 month later she relapsed. Not responding to dipyridamol and acetylsalicylic acid, reinstatement of immunosuppressive therapy led again to a prompt improvement.

Dermatomyositis and systematic scleroderma.—After a surprising success of methotrexate therapy in one 12-year old boy with dermatomyositis

at Bellevue Medical Center (personal observation, 1965), additional cases were treated with methotrexate, with variable results (226).

Our own experience covers 6 patients with primary dermatomyositis. The mean duration of therapy is 2½ years. Four patients improved remarkably while 2 patients resistant to this treatment may be successful (227, 228). In scleroderma, efficacy of immune suppressive therapy is still very controversial (229).

Chronic active hepatitis.—Chronic active hepatitis responds well to corticosteroids, however, the prognosis appears unaffected by this treatment. Immunosuppressive agents have been introduced with promising results (5, 196, 231, 232). More recently a controlled study (233) clearly indicates the efficacy of a combined azathioprine prednisone medication. Out of 46 patients, 40 showed marked improvement with regard to clinical symptoms and signs, liver function as well as needle biopsies. Our personal experience (12 cases) using prednisone (10 mg per day) and 6-MP (50 mg per day) corroborates this result. Azathioprine and 6-MP are not well tolerated if bilirubinemia exceeds 3 mg%. In this case the drop in bilirubin level should be first attempted with steroids alone. The sole administration of prednisone is known to produce a rapid drop in the bilirubin blood level (207). As in lupus nephritis, success of treatment decreases with the length of the preexisting lesion. Once the patient has developed portal hypertension with ascites, the chances of improvement are very slim. Out of 4 patients with such an advanced state of the disease only 1 patient responded favorably to immune suppressive therapy. In the other 3 the outcome was fatal within a 6-month period.

Ulcerative colitis.—Azathioprine and 6-MP have been used in ulcerative colitis with more or less success (196). The effectiveness of the immunosuppressive therapy is difficult to evaluate as, in addition to lack of controlled trials, the disease may resolve spontaneously. Moreover, surgical therapy may be effective. In some instances, careful management of ulcerative colitis by an alternative sequence of azathioprine and corticosteroids may restore the integrity of the terminal intestine (234).

Miscellaneous conditions.—In various forms of uveitis, immunosuppressive therapy appears to be beneficial (235).

Bullous pemphigoid, whose pathogenesis may be mediated by immune phenomena, may well respond to a combined steroid-azathioprine medication (236).

Psoriasis is frequently mentioned as a condition that responds well to immunosuppressive therapy. However, there is no evidence in favor of an underlying immune mechanism. It is generally assumed that the efficacy of treatment is due to the mere antimitotic action of the immune suppressive agents (196, 237, 238).

Myasthenia gravis also appears to respond to immunosuppressive therapy (228, 229). In this condition corticosteroids should be avoided or their

use limited to a minimum since they may produce exacerbation of the disease (239).

Multiple sclerosis is another condition in which immune phenomena are thought to be operative in pathogenesis. Since this condition is characterized by a clinical course in which relapses alternate with remissions, evaluation of immune suppressive therapy is almost impossible without the help of a controlled study (240, also personal communication).

Lastly, an interesting case warrants attention. Green (241) administered VIII globulin simultaneously with Factor VIII and cyclophosphamide to a patient with a circulating anti-factor. Subsequently, the antibody titer to Factor VIII fell to zero and all clinical and biological signs disappeared. The remission was long lasting. Thus, the simultaneous administration of antigen and immunosuppressive agent appears to have induced specific nonresponsiveness.

Transplantation.—One of the major applications of immunosuppressive therapy is human allo-transplantation. No single agent is effective by itself (242). Corticosteroids are currently used as basic regimen, often beginning at the time of transplantation (5, 122). However, steroid trials in human skin allograft have constantly met with failure, a fact that can be related to the resistance of man to the lympholytic action of corticosteroids. Azathioprine is today the main antimetabolite in this field (243, 242). Actinomycin D and azasarine are other immunosuppressive compounds that have been added to azathioprine and prednisone medication. Of the two, azaserine seems to have the more potent effect (244). Cyclophosphamide is difficult to handle, due to its high degree of toxicity. According to a report from the Kidney Transplant Registry, the most successful treatment seems to be association of steroids, azathioprine, actinomycin, or azaserine, and local irradiation (122, 245), all initiated after the operation. Control of threatening rejection is mainly achieved by increase in the doses of corticosteroids (122). Actinomycin D and heparin are recommended for these incidents (243). Concerning antilymphocyte serum, its use in human transplantation is somewhat restricted as the side effects are many (245). Furthermore, the immunosuppressive activity may vary considerably among the various batches.

Rh incompatible pregnancies.—Prevention of the neo-natal isoimmune hemolytic disease associated with Rh-incompatible pregnancies has been the concern of many workers since 1958 (247). Antibody mediated immune suppression has been selected because this method is highly specific and seems to be innocuous. Furthermore, all other means of achieving immunosuppression present undesirable side effects. Specific antibody mediated immunodepression has been extensively analyzed and many controlled trials have been undertaken. Out of 1081 treated women, only 1 developed anti-D IgG antibodies, 6–9 months postpartum, while 51 out of 726 control subjects produced anti-D IgG antibodies. Accordingly, this therapy should reduce the danger of Rh iso-immune hemolytic anemia of the new born "to a vanishing small incidence within a generation" (248), if the antiserum is properly administered with regard to time and quantity.

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