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SELECTIVE SUPPRESSION OF HUMORAL IMMUNITY BY ANTINEOPLASTIC DRUGS

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Within the last few years there has been a tremendous increase in knowledge of clinical and experimental immunology. We are becoming aware of the complexity of immune responses as they really occur, as opposed to the relatively simple responses described in textbooks and studied in model systems. Not only are there humoral (antibody-mediated) and cell-mediated immunities, with their cellular counterparts, the B- and T-cell systems (1), but also there are further subpopulations of lymphoid cells, with differing functions and responses to antigens, and with interaction between them a continuing process. In an immune response to a single antigenic stimulus, there may be, in addition to cells producing antibody, helper T cells that cooperate with B cells in the triggering of antibody production (1), suppressor T cells that somehow limit the extent of the immune response (2), and effector T cells that are involved in the manifestations (such as cytotoxicity, delayed hypersensitivity, contact sensitivity, or skin allograft rejection) of specific cell-mediated immunity (3), presumably through the release of lymphokine(s) (4). It is not known whether all these functions are carried out by the same or different T-cell populations. A similar level of functional complexity has not yet been described for B cells, although the different classes of immunoglobulins and the sensitivity of antibody production to feedback regulation (5) indicate that complexity and interaction are operative here also. Thus, it is apparent that the concept *immunosuppression* is far too naive. However, if ways could be found to affect selectively the different parts of the immune response, the possibility for "fine-tuning" the system would lead to much more subtle immunoregulatory capabilities than now exist.

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The purpose of this paper is to review the relatively limited literature that suggests that selective immunosuppression may be possible. Because the goal of our own work has been to use immunosuppressives in such a way as to maximize host resistance to neoplasia, this review, except for a brief discussion, is limited to observations with antineoplastic agents. Very little work has been published on the suppression of cell-mediated immunity without concomitant antibody suppression (but see 6–8), so we have restricted our review to selective suppression of humoral immunity. As will be seen, selective immunosuppression is barely out of the descriptive stage, and regulation beyond the antibody versus cell-mediated level is just beginning.

SELECTIVE SUPPRESSION OF HUMORAL IMMUNITY: GENERAL DISCUSSION

Before reviewing studies on selective suppression with antineoplastic drugs, we mention a number of other procedures that have been reported to have similar selective effects. Indeed, even the classical methods of immunosuppression may show selectivity. For example, Nossal & Pike (9) have shown that B cells of adult strain CBA mice are more sensitive than T cells to high dose irradiation (800 r). In their study the number of splenic B cells fell by a factor of 200 during the first day after irradiation, whereas the total spleen cell number fell by a factor of 10. Kuening & Bos (10) found that sublethal total body irradiation (450 r) in rabbits selectively destroyed splenic lymphoid follicles and germinal centers (B-dependent areas) without affecting periarteriolar sheath lymphocytes. In our own experience (unpublished observations), 600 r total body irradiation suppressed the ability of strain C_3HeB/FeJ adult male mice to produce 19S antibody to sheep red blood cells (SRBC), as measured by Jerne plaque assay, as long as 30 days after irradiation, whereas the ability to develop contact sensitivity to oxazolone was not significantly affected even when sensitization was assessed 24 hr after irradiation. [Sensitivity to oxazolone was assessed five days after immunization, a time when T cells are principally responsible for the hypersensitivity reaction (11)].

Other immunosuppressive treatments that have been shown to be more effective for both the number and function of B cells than for T cells include prostaglandin E in rats (12, 13) and cytoplasmic components of group A streptococci in mice (14). The immunosuppression induced by graft-vs-host disease has been reported to be longer lasting for humoral immunity than for rejection of skin allografts in mice (15), although cellular immunity seems to be more markedly depressed than humoral immunity in human allogeneic bone marrow chimeras (16). In the latter case, however, the graft recipients were patients with acute lymphocytic leukemia (17).

A most interesting example of selective immunosuppression comes from the work of Jose & Good (18) on the effect of amino acid deficiencies on immunity. Diets moderately deficient in phenylalanine-tyrosine, valine, threonine, methionine-cystine, isoleucine, or tryptophan resulted in depressed production of both hemagglutinating antibodies and serum blocking factors (see below) in strain C_3H mice, although cell-mediated immunity, as measured by an in vitro cytotoxicity test, to allogeneic tumor cells remained intact. Limitation of arginine, histidine, or lysine

had only a slight effect, but limitation of leucine depressed cell-mediated immunity without suppressing antibody production.

Passive administration of specific antibody can be an effective method of inhibiting both antibody production (19) as well as sensitization to allogeneic tissue grafts (19) and development of delayed hypersensitivity to antigens, such as SRBC (20). However, administration of high concentrations of antibody has been found to inhibit antibody production but to enhance delayed hypersensitivity to flagellin, polymerized flagellin, and SRBC in rats, with lower concentrations having the inverse effect (21). This suggests that control of the amount of antibody given could result in regimens selectively suppressive for B- or T-cell immunity. The stage in immunization at which the suppressing serum is taken is also a factor (20).

Another approach to shifting the effective balance between humoral and cell-mediated immunity is preferential stimulation of the T-cell system. This has been accomplished by either acetoacetylation of the antigen (22, 23) or, in the case of red blood cell antigens, by fixation with formaldehyde or glutaraldehyde (24). Thus, acetoacetylation of flagellin turned that antigen from a strong antibody inducer into one that exclusively induced delayed hypersensitivity reactions in rats (22). Likewise, guinea pigs sensitized to acetoacetylated carcinoembryonic antigen (CEA) developed delayed skin reactivity with only low levels of antibody (23). Immunization of mice to fixed chicken red blood cells gave poor primary antibody responses, but subsequent injection of the fixed antigen resulted in normal or better secondary responses, with T-cell specificity, demonstrating that the initial immunization had resulted in effective helper T-cell priming without concomitant B-cell sensitization (24).

This brief survey of selective immunosuppression is not meant to be all-inclusive, but simply to illustrate other approaches to the problem beyond the use of antineoplastic, immunosuppressive drugs. On a practical level, however, it demonstrates that selective effects may be more common than are realized and that analysis of the immune status in experimental animals or patients requires the use of several different types of assays, even in the case of such standard immunosuppressive treatments as X irradiation.

SELECTIVE SUPPRESSION OF HUMORAL IMMUNITY BY ANTINEOPLASTIC DRUGS

6-Mercaptopurine (6-MP)

One of the first reports of selective suppression of humoral immunity was with 6-MP. Kimball, Herriot & Allison (7) found that a dose of 75 mg/kg per day, given on days 0 to 3, only had a minimal effect on retention of strain C_3H skin allografts by AKR mice but markedly suppressed the production of hemagglutinating antibody to SRBC. Stewart (25) likewise found that 6-MP had no significant effect on allograft survival, even when donor and recipient differed only at weak histocompatibility loci and when drug treatment was extended for longer periods of time (15 mg/kg per day; days –1 to 14). On the other hand Schwartz (26) found in guinea pigs that administration of 10 mg/kg per day on days 0 to 4 suppressed delayed

hypersensitivity to a protein antigen (in Freund's adjuvant) without affecting antibody production. Clearly species differences may play a role in these divergent observations.

Cyclophosphamide (CY)

Cyclophosphamide was first used to suppress the B-cell immune system in chickens (27). Birds treated with doses ranging from 4 to 8 mg/day, for the first three days after hatching, showed depressed levels of serum IgM and IgG immunoglobulin levels and also failed to produce normal levels of antibodies to antigens (bovine serum albumin, SRBC, and *S. typhimurium*) at 7 and 11 weeks of age. Their ability to make a cell-mediated immune response (graft vs host reaction) was only slightly affected.

Subsequently, Turk & Poulter (28) showed in adult mice and guinea pigs that either a single i.p. dose of 300 mg/kg, or three such doses given on alternate days, destroyed lymphocytes in B-dependent areas of spleen and lymph nodes, but had much less effect on T-dependent areas. In mice it could also be shown that the proportion of θ antigen (T-cell marker) carrying lymphocytes in peripheral lymphoid organs was over 90% after three injections of CY, as opposed to approximately 24%, 50%, and 57% in control spleens, mesenteric lymph nodes, and peripheral lymph nodes, respectively (29). Dumont (30) essentially confirmed these results, and also identified two T-cell subpopulations, differing in electrophoretic mobility, which also differed in sensitivity to CY. Restoration of T cells began by day 8 after CY (300 mg/kg), but B-cell regeneration was only evident after day 16. Normal levels of both systems were reached by day 30.

In addition to the effect on cell number and lymphoid organ morphology, treatment with 300 mg/kg CY was shown to affect immunological function. Dumont (30) found that responsiveness of spleen cells from treated animals to *E. coli* lipopolysaccharide (LPS), a B-cell mitogen in mice, was initially abolished and then showed the same recovery curve as the B-cell number. Responsiveness to Concanavalin A (Con A), a T-cell mitogen, was also diminished but not to as great an extent as responsiveness to LPS. Recovery of normal Con A responsiveness likewise occurred more quickly than did that of LPS.

Turk and associates (31) showed in guinea pigs that 300 mg/kg of CY greatly enhanced development of contact sensitivity to 2,4-dinitrofluorobenzene or to oxazolone when the antigen was administered three days after the drug. The extent of the reaction following challenge with the antigen seven days after sensitization was greater both in intensity and duration than in control animals. Antibody production to the dinitrophenyl (DNP) hapten, however, was suppressed in the treated animals, compared to antibody production in controls. Lymph node fragments (rich in T cells) from sensitized, nontreated guinea pigs further enhanced the sensitivity of the sensitized, CY-treated animals when placed in their peritoneal cavities; transfer of spleens (rich in B cells) from two such donors, however, partially reversed the effects of CY. One spleen had no effect. Removal of the spleen from guinea pigs four days after sensitization to dinitrofluorobenzene (DNFB) or oxazolone had an effect similar to that of the CY treatment in prolonging the reaction to challenge antigen.

These, and further similar data (32), were interpreted as evidence that B cells normally exercise a suppressor function in the development of T-cell-mediated hypersensitivity. Elimination of the B cells by CY also eliminates this suppression. Efforts to substitute immune serum for spleen cells in the transfer experiment met with no success, although the amount of serum (10 ml from guinea pigs sensitized seven days previously to administration of the antigen) may have been too small. However, it was stated (32) that living B cells are necessary for inhibition of the CY effect.

Further work on selective suppression of antibody formation by CY has been reported by Lagrange, Mackaness & Miller (33). They found that 200 mg/kg CY, given i.v., would permit the use of greater doses of SRBC than normally effective to elicit delayed hypersensitivity to that antigen in strain CD-1 mice. Induction of delayed hypersensitivity to SRBC in mice is strongly dose-dependent, with high doses resulting in failure of sensitization, apparently due to production of an antigen-antibody-complex blocking factor. Mice in whom sensitization has been blocked by high doses of antigen remain refractory to subsequent administration of SRBC. Even in such blocked mice, however, CY treatment can at least partially restore reactivity to further sensitization, as well as inhibit a secondary antibody response to the antigen (34).

It should be emphasized that CY given prior to sensitization does not always enhance the development of delayed hypersensitivity. In studies made along with those using DNFB and oxazolone as antigens (see above), Turk, Parker & Poulter (31) found that 300 mg/kg CY, given three days prior to sensitization with BCG, resulted in depressed reactivity to tuberculin-PPD at challenge seven days later. Jokipii & Jokipii (35) also found suppression of reactivity to azobenzenearsonate-N-acetyl-l-tyrosine (Ars-Tyr) in similarly treated guinea pigs. This latter finding is especially interesting in that the reactivity to Ars-Tyr appears to be a pure T-cell response. Furthermore, Dennert, Hatlen & Tucker (36) have recently examined the time dependence of selective immune suppression by CY. They confirmed the ability of CY to increase selectively the proportion of splenic T cells in unimmunized mice and also showed that pretreatment with the drug before the antigen resulted in spleens with higher T helper cell activity for red cell antigens and greater T cytotoxic cell activity for allogeneic tumor cell antigens. Simultaneous administration of drug and tumor cells also resulted in greater cytotoxicity by spleen cells than in untreated controls. When CY was given after antigen, however, the degree of selectivity was much less. A three-day course (30 mg/kg) resulted in suppression of T helper cell, as well as B cell, response to SRBC, although it only partially inhibited T cytotoxicity to tumor cell antigen. Prolonged drug administration (six days) totally inhibited the cytotoxicity reaction.

Cytosine Arabinoside (ara-C, 1-D-Arabinofuranosylcytosine)

The possibility that ara-C could be used as a selective immunosuppressive was originally seen in studies on development of immune responses to allogeneic tumor cells in mice. Administration of ara-C to strain C_{57}Bl mice early (days 1–5) after injection of strain DBA/2-derived tumor cells resulted in suppression of anti-tumor

cell antibody, but did not alter development of cell-mediated immunity (37). Administration of the drug on days 6 to 10, however, suppressed both responses. Ara-C was also shown to suppress antibody production to SRBC in dogs, without affecting kidney allograft rejection (38).

Extension of these observations resulted in a more complete elucidation of the circumstances under which ara-C can selectively suppress humoral immunity in mice. Similar to the findings in (37), Griswold, Heppner & Calabresi (39) demonstrated that although administration of drug i.p. on days 1–5 or 6–10 after immunization, in doses of 20 or 40 mg/kg per day, suppressed 19S and 7S antibody production in strain C_3H mice, rejection of skin from $C_{57}Bl$ donors was only suppressed with the latter treatment period. Thus, time of administration is an important parameter. Another factor is drug dosage. Induction of contact sensitivity to oxazolone in strain A/J mice was not inhibited by 20 mg/kg per day on days 1 to 5, but was somewhat suppressed by the higher dose of 40 mg/kg per day (40). The third factor contributing to selective suppression by ara-C is the nature of the antigen; the 40 mg/kg per day dose given early suppressed hypersensitivity to oxazolone, but not to methylated bovine serum albumen (MBSA), nor did it affect skin allograft rejection.

As with similar experiments with CY, ara-C, given at times that would selectively suppress antibody production, was found to enhance development of a cell-mediated reaction. For example, mice sensitized to MBSA, and given ara-C early in the sensitization period, showed much greater reactivity than untreated, sensitized mice to antigenic challenge 14 days later (41).

Recently, treatment of unsensitized mice with ara-C has also been found to have a greater effect on levels of B cells in the spleen than on T cells, although the difference is not as great as with CY (see above). Data of A. Anaclerio, G. Heppner, and P. Calabresi (to be published) show that the percentage of splenic B cells in unsensitized C_3H mice treated with the 20 mg/kg per day, days 1 to 5 regimen, has dropped from about 45% to 32% and 35% on days 4 and 5 respectively, with only a slight, insignificant drop in numbers of T cells. No ara-C-induced differences were found in the number of T and B cells in peripheral lymph nodes.

Methotrexate (MTX), Leucovorin (LCV), and 5-Fluorouracil (5-FU)

The discussion above summarizes results from our laboratory on selective humoral suppression by ara-C. It is apparent that there are several difficulties with using ara-C for this purpose. The time, dose, and antigen parameters are quite stringent and make control of the suppression uncertain. In hopes of overcoming these difficulties, we first turned to 5-FU because conflicting reports on its immunosuppressive capacities in the literature suggested that it might be a selective agent. Also, there is a report that 5-FU, and 5-fluoro-2'-deoxyuridine, could potentiate secondary delayed hypersensitivity reactions in cancer patients (42).

At 13 mg/kg per day for the first five days after sensitization, 5-FU suppressed 19S and 7S antibody production to SRBC in C_3H mice, but even higher doses (up to 40 mg/kg per day) did not suppress various cell-mediated immune responses (40, 41). As with ara-C, 5-FU (10 to 30 mg/kg per day, days 1 to 5) enhanced the development of hypersensitivity to MBSA (40), and to oxazolone in some cases (43).

Two problems with 5-FU in mice, however, also interfere with its use as a selective immunosuppressant: (*a*) the degree of suppression of antibody production is variable and oftentimes inadequate, and (*b*) toxicity becomes significant in long-term studies. The combination of MTX, LCV, and 5-FU has been developed to overcome these problems. The combination is administered on day 2 after exposure to antigens [with replicating antigens, such as tumor cells (see below), it is given at weekly intervals]. The doses are 1 mg/kg MTX, 1 mg/kg LCV, and 50 mg/kg 5-FU. MTX and LCV are given simultaneously or separated by a short time interval (15–30 min). 5-FU is given 1 to 6 hr after MTX. The order and timing of the combination are important. If MTX and 5-FU are given simultaneously, only additive suppression of humoral immunity is seen, whereas potentiated inhibition is found when there is an interval between them (43, 44). If 5-FU is given before MTX, the selectivity of the combination becomes equivocal. LCV was added to the combination to counteract potential toxicity problems due to MTX (45). It does not substantially change the immunosuppressive properties (44), although in some cases it seems to enhance the selectivity for humoral immunity (43). This latter is not a consistent finding, however.

In experiments similar to those described above for ara-C, MTX, LCV, and 5-FU have been found to suppress 19S (43) and 7S (44) antibody production to SRBC, but to have no significant effect on skin allograft rejection or contact sensitivity to oxazolone (43, 44). Hypersensitivity to MBSA is markedly stimulated. For example, in an experiment in which sensitized, saline-treated C_3HeB/FeJ mice developed 19 ± 6 μliters of edema upon footpad challenge with MBSA, mice given MTX, LCV, and 5-FU developed 90 ± 8 μliters. Studies on the effect of the combination on lymphoid organ morphology or on numbers of T and B cells have not yet been made.

Bisdioxopiperazines

Two other agents with demonstrated selective immunosuppressive activity are 1,2-bis(3,5-dioxopiperazin-1-yl) ethane (ICRF 154) and (±)-1,2-bis(3,5-dioxopiperazin-1-yl) propane (ICRF 159) (36, 42, 43). These agents are comparable to CY in increasing the proportion of splenic T cells in unsensitized mice, and also, if given prior to immunization with red blood cells, in increasing T helper cell activity (36). Dennert, Hatlen & Tucker (36) found increased T cytotoxic cell activity to allogeneic tumor cell antigen in mice pretreated with ICRF 154, but not with ICRF 159. Turk & Parker (47), however, found that ICRF 159 enhanced development of contact sensitivity in guinea pigs. As with CY, enhanced T-cell function is only clearly demonstrated when these drugs are given prior to, or simultaneously with, antigen, although administration after antigen seems to have a somewhat greater suppressive effect on B than on T cells (36, 46).

Other Drugs

The above drugs are those that, to our knowledge, have been investigated for selective immune effects in some detail. It may be that many agents will be shown to have similar activity, especially when advantage is taken of dose and timing

parameters. For example, Turk & Parker (47) have reported that melphalan and azathioprine can enhance induction of contact sensitivity in guinea pigs.

Mechanisms of Selective Immunosuppression of Humoral Immunity by Antineoplastic Drugs

A full understanding of the mechanisms involved in selective immunosuppression will not be possible until more complete information is available as to which drugs are, or are not able, to produce the effect, and under what circumstances (timing, dose, antigen, etc). It is already apparent, however, that no single class of anti-tumor drugs is involved (48); some selective activity has been found in all groups, including alkylating agents and antimetabolites; folic acid antagonists, antipurines, and antipyrimidines; phase-specific and nonspecific agents. All the agents so-far identified, however, have greater activity on cells undergoing proliferation than on resting cells. The influence of cell proliferation on sensitivity to immunosuppressive agents has long been appreciated (49). Indeed, the rationale cited by Turk & Poulter (28) for looking for selective effects by CY was that it was more likely to be active on short-lived than on long-lived lymphocytes. Since B cells, and some T cells, are shorter lived than the majority of T cells, agents that discriminate on the basis of frequency of division should be selective. In this regard, the fact that CY and ICRF 154, when given at appropriate times, eliminate B cells and an electrophoretically (30) and functionally (36) distinct class of T cells, seems to confirm this hypothesis. Likewise, the sensitivity of selective suppression to the time of drug administration in relation to antigen exposure also suggests that differential proliferation is involved in the effect. However, different drugs are selectively active at different times in the immune response: CY and the bisdioxopiperazines when given before antigen; ara-C, 5-FU, and MTX, LCV, and 5-FU when given after. It may also be that different subpopulations of lymphocytes differ in certain metabolic pathways that render them more or less sensitive to different drugs.

From the immunological point of view one of the most interesting features of selective suppression of humoral immunity is that it is frequently accompanied by an actual increase in cell-mediated immunity. This has been noted in studies with antibody-induced suppression of humoral immunity and acetoacetylated antigen induction of cell-mediated immunity (22) as well as with selectively suppressive drug treatments (31, 36, 41). The opposite effect, depressed cell-mediated immunity accompanied by enhanced antibody synthesis, has also been seen (50). This seesaw relationship, which has been discussed by Bretscher (50), could have quite complicated mechanisms, including feedback interrelationships, perhaps involving antigen-antibody complexes, and the sharing of common nutrients, informational molecules, or cells that can be diverted from one immune system to another. The preferential stimulation of suppressor cells could also be involved (32). These mechanisms would work at the levels of induction or production of immune factors. Heightened cell-mediated reactivity, under conditions of antibody suppression, could also be due to removal of an efferent block, in particular antigen-antibody complexes (serum blocking factors) which in many in vitro circumstances have been shown to be capable of inhibiting expression of cell-mediated immune reactions (41).

USES OF SELECTIVE IMMUNOSUPPRESSION OF HUMORAL IMMUNITY BY ANTINEOPLASTIC DRUGS

As stated above, the ability to suppress selectively one or another type of immune reactivity would allow "fine-tuning" of the immune response. So far our abilities in this regard are quite primitive. Nevertheless selective suppression is becoming increasingly used in three types of experimental work: (*a*) analysis of the nature of complex immune reactions, (*b*) investigation of the site of action of other immunological modifiers, and (*c*) development of ways to strengthen cell-mediated immune reactivity to solid tumors.

The Nature of Complex Immune Reactions

Although delayed hypersensitivity and contact sensitivity are classified as cell-mediated immune responses, many such reactions are in actuality mixed, with B-cell, T-cell, and also macrophage participation. In mixed reactions the B-cell response may act to augment the T-cell immunity, as in the case of macrophage-cytophilic antibodies (51), or counteract it, as in the case of serum blocking factors (antigen-antibody complexes). Clearly, selective suppression of humoral immunity could be used, in the analysis of mixed reactions, first, for identifying them, and then for assessing which type of humoral response is involved. This approach has been reported in at least two studies. Turk & Parker (47), using CY, melphalan, and ICRF 159, demonstrated that the "Jones-Mote" type of hypersensitivity in guinea pigs was in all likelihood a mixed reaction in which B-cell reactivity acted to suppress the T-cell component. Guinea pigs were treated with either drug, and then three days later immunized with 1 μg ovalbumin (OA) in Freund's incomplete adjuvant. Seven days after sensitization they were challenged by intradermal injection of 100 μg OA. The degree of skin reactivity in the drug-treated animals was greater than that in controls at 24, 48, 72, and 96 hr after challenge, although the extent and time course of the difference varied among the three drugs.

Griswold, DiLorenzo & Calabresi (11) used selective suppression with ara-C and 5-FU to show that oxazolone-induced contact sensitivity in mice does not involve a humoral blocking reaction, in addition to the already described cytophilic antibodies and T lymphocytes (51, 52). Early (days 1–5) administration of low doses of ara-C or 5-FU had no effect on the development of sensitivity to oxazolone, in contrast to a great stimulation of reactivity to MBSA.

The Site of Action of Immunological Modifiers

So far the major use of selective humoral suppression to aid in investigation of the site of action of other immunological modifiers has been the work of Mackaness, Lagrange and co-workers. They showed that the effects of infection with BCG (the relatively nonvirulent bacillus Calmette-Guérin strain of tubercle bacilli) and administration of CY were synergistic in augmenting the induction of delayed hypersensitivity to SRBC in mice (53). Previously (see above), they had shown that CY alone could enhance hypersensitivity to that antigen, apparently by inhibiting the development of anti-SRBC antibodies. BCG infection was likewise shown to en-

hance hypersensitivity, but also to enhance antibody production. However, the delayed hypersensitivity was less subject to suppression by humoral blocking factors (antigen-antibody complexes) in BCG-infected animals, than in uninfected controls, possibly as a result of enhanced clearing of these factors by a stimulated reticuloendothelial system. Thus, the synergistic effects of the combination BCG and CY in producing heightened hypersensitivity were explained by an absolute increase in cell-mediated immunity (BCG), a reduction in susceptibility of the cell-mediated immunity to antibody modulation (BCG), and an inhibition of antibody production (CY).

More recently this same group has used selective suppression by CY in their studies on the ability of LPS to either stimulate or depress immunity in mice. LPS, given three days prior to antigen, diminished the development of delayed hypersensitivity to SRBC (54). LPS is a B-cell mitogen in mice, and indeed it could be shown that antibody production was increased in the treated animals. To show that the increase in antibody was causally related to the depressed cell-mediated reaction, CY was administered simultaneously with LPS. Under these circumstances the delayed hypersensitivity reaction was enhanced.

Strengthening of Cell-Mediated Immunity to Solid Tumors

Our major interest in selective inhibition of humoral immune responses relates to work on the nature of immunity to solid cancers. It has been shown that factors, probably antigen-antibody complexes (55), exist in sera of tumor-bearing animals and cancer patients that can block in vitro the expression of cell-mediated cytotoxic or cytostatic immunity (56, 57). Since these serum blocking factors seem to be associated with progressing disease (58, 59), prevention of their development might strengthen host defense reactions against cancer. We have tested this hypothesis by selective immunosuppression with ara-C (60), 5-FU (40), and MTX, LCV, and 5-FU (to be published).

Strain C_3H mice were implanted with syngeneic, early transplants of spontaneously arising mammary tumors and treated for the first five days with low (10 or 20 mg/kg per day) or high (40 mg/kg per day) doses of ara-C. In a typical experiment the tumors grew to palpable size after about four weeks in control mice. In mice given high doses of drug, the tumors appeared after about two weeks. In mice treated with low doses tumor appearance took about six weeks. The exact times for tumor outgrowths to appear differed from experiment to experiment. Also, with some tumors a dose of 20 mg/kg per day was a high dose, resulting in early tumor appearance. In these cases, however, 10 mg/kg per day delayed tumor growth.

Ara-C only influenced tumor growth when tumors capable of inducing cell-mediated immunity were used. With a tumor that was shown by in vitro techniques to be incapable of inducing cell-mediated cytotoxicity (although serum blocking factors were produced) neither 20 or 40 mg/kg per day of ara-C had any effect.

Using in vitro assays we were able to demonstrate directly the selective inhibition of serum blocking factors. Sera from mice given low doses of ara-C, which demonstrated retardation of tumor growth, failed to block cell-mediated immunity until

about two weeks prior to tumor appearance. Sera from mice given high doses of drug also failed to block cell-mediated immunity; however, lymph node cells from these mice failed to kill tumor cells in vitro, whereas lymph node cells from mice given low doses of ara-C not only killed tumor cells but also continued to do so when reactivity of lymph node cells from untreated mice could no longer be demonstrated.

Selectively suppressive treatments with 5-FU and MTX, LCV, and 5-FU have shown effects similar to those of ara-C on the outgrowth of syngeneic mammary tumor cells. In the latter case, treatment, given at weekly intervals, has been effective in slowing, and even preventing, tumor growth, not only when begun soon after tumor implantation, but also after tumors have been allowed to grow unchecked for several weeks before treatment. In fact, MTX, LCV, and 5-FU is moderately effective in delaying reappearance of the tumor following incomplete surgical removal of large tumors. Earlier, low doses of ara-C were also found to be effective in preventing regrowth after surgery (60).

In experiments analogous to these, CY has been found, under appropriate circumstances, to interfere with growth of established polyoma virus–induced sarcomas in rats (61). Sequential in vitro assays have demonstrated serum blocking factors prior to treatment, and in untreated rats with progressively growing tumors, but not after treatment, when the tumors have regressed to about 50% of their pretreatment size (62).

That ara-C, MTX, 5-FU, or CY should interfere with tumor growth is not surprising, for they have all been originally developed as antineoplastic agents. What is surprising is that they inhibit tumor growth under circumstances in which they selectively inhibit antibody production, but may actually increase it if both humoral and cell-mediated immunity are suppressed. Clinically these drugs are often used under conditions thought to be advantageous for killing of tumor cells. These conditions, however, may not be optimal for the selectivity of the drugs for humoral immunity, and thus the host's ability to withstand tumor growth may be weakened. Perhaps in the future, chemotherapeutic regimens will be designed to maximize host reactivity to cancer, as well as to kill tumor cells directly (63).

CONCLUSION

Immune responses are complex, consisting of humoral and cell-mediated immunity, with both augmenting and suppressing interactions between them. The ability to suppress selectively one type of immune response, without directly affecting others, would allow more sensitive and precise immunoregulation than is now possible. Selective suppression of humoral immunity has been reported under a number of circumstances, but, in particular, with several antineoplastic drugs, including 6-mercaptopurine, cyclophosphamide, cytosine arabinoside, 5-fluorouracil, a combination of methotrexate, leucovorin, and 5-fluorouracil, and the bisdioxopiperazines, ICRF 154 and 159. Exploitation of the selective effects of these drugs is leading to new ways of analyzing complex immune responses, investigating the action of other immunological modifiers, and using antineoplastic drugs to strengthen host defense reactions against cancer.

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