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Immunosuppression

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Administration of an antigen initiates a series of events that results in the appearance of serum antibodies and under appropriate conditions the development of cell-mediated immunity. The immune response has evolved apparently to protect the individual from hostile elements from within and without. Humoral antibodies are active against many infectious agents, and cellular immunity of the delayed hypersensitivity type protects against viral and fungal infections as well as against foreign tissue grafts and tumors. However, in a number of situations, the immune reaction can also operate to the detriment of the host. In these situations suppression of the immune response is desirable. A complex series of events evolving over a period of time takes place before an effective reaction is mounted by the host against the immunizing antigen. These events include interaction, differentiation, and multiplication of cells having differing developmental backgrounds. Exposure of the immunized animal to antigen causes production of various mediator molecules from serum components and by immunologically specific and non-specific reactor cells. Suppression of adverse aspects of the immune response can be accomplished at multiple points along this chain of cellular and molecular events. Methods currently in use as well as those being developed often result in relatively non-specific immunological impairment thereby increasing the risk of development of infections and apparently of neoplasms as well. Other approaches involve the use of the reactants in the immune response - antigen and antibody - to achieve a selective inhibition of the response to specific antigens.

In order to present an approach to immunosuppressive therapy that is rational within current immunological paradigms, the cellular events in the immune response will be described briefly. Some of the ways that non-specific therapeutic agents can interrupt these events will then be reviewed. Practical and potential use of antigen and antibody to specifically suppress the immune

response will then be considered. No attempt will be made to be comprehensive since a number of review articles have appeared within the last several years (1-6). Specific reference will be made only to recent publications that usually have not been included in the review papers.

CELLULAR EVENTS OF THE IMMUNE RESPONSE

Events occurring after introduction of an antigen may be conveniently but arbitrarily divided into 4 stages: antigen distribution, antigen reaction with lymphoid cells, proliferation and differentiation of lymphoid cells and generation of effector molecules. These events are influenced by the physical state of the antigen, genetic constitution of the host, prior immunological experience, and by other as yet poorly understood factors that affect the relative preponderance of humoral and cellular immune responses as well as the qualitative nature of the antibody produced.

Most of the cellular events occurring during the development of the immune response take place within the organized lymphoid tissue of the lymph nodes or spleen. Antigen reaching lymphoid organs probably stimulates antibody production although sensitization for cellular immunity appears to occur at least in part outside lymphoid organs. The vast majority of injected antigen appears to be degraded by phagocytic cells of the reticuloendothelial system in ways unrelated to the immune response. It has proven difficult to trace the distribution of the small amount of immunogenic material that initiates the cellular events of antibody formation. Several lines of evidence suggest, however, that it is located extracellularly although uptake of antigen by cells and subsequent "exteriorization" may occur (7).

Receptors for specific antigens have been demonstrated on the surface of lymphoid cells (8). Within lymphoid tissue, there are geographic differences in distribution of lymphocytes having differing developmental backgrounds. Lymphocytes in the paracortical area or deep cortex of the lymph node and in the periarteriolar

lymphoid sheath of the spleen are derived under thymic influence (T cells). Lymphocytes of the germinal centers and lymphoid follicles, however, and plasma cells of the splenic red pulp and medullary cords of lymph nodes are descendants of bone marrow cells (B cells) and do not require thymic influence for development. These B cells of mammals seem to be analogous to cells developing in birds under the influence of the bursa of Fabricius although the mammalian equivalent of the bursa has not been identified.

Most of the information about cellular interactions has been derived from cultures of lymphoid cells. However, a limited range of antigens, usually heterologous erythrocytes have been used because of the ease with which antibody-releasing cells can be detected and enumerated. IgM rather than IgG production has usually been studied. Most of the essential details have been confirmed, however, in intact animals. At least three cell types participate in the development of a "primary" antibody response to heterologous erythrocytes (9); one of these is a T cell. T cells may produce non-specific cell-free factors stimulating an increase in antibody-forming cells (10); other evidence suggests interaction takes place between antigen and T cells having at least a restricted range of reactivity (11). The nature of the antigen receptor on this type of cell is not clear; immunoglobulin light chains but not the usual heavy chains have been detected (12). Increases in number, or at least increase in reactivity, of T cells follows immunization and T cells appear to have reactivity for the carrier rather than the haptenic portion of the antigen (11).

Antibody producing cells are of the B class and have cell surface receptors for antigen that are probably immunoglobulin in nature (8). Immunoglobulin light chains as well as various classes of heavy chains have been detected on surfaces. Cellular division accounts for most of the increase in numbers of

antibody forming cells after immunization (13, 14). At any one point in time, a given cell produces only one kind of immunoglobulin (specificity, light and heavy chain class etc.). However, several kinds of evidence suggest a switch from production of IgM to production of IgG in a cell lineage if not in any one cell (15). There are geographic differences in production of different classes of immunoglobulins: IgA is produced predominantly in the sub-epithelial connective tissue of gastrointestinal and respiratory tracts and other organs; IgG production is associated with germinal centers of lymphoid tissue.

Immunoglobulin classes differ markedly in biological properties. IgM and some IgG subclasses are able to activate complement while other IgG subclasses, IgA and IgE do not. IgE preferentially sensitizes mast cells to cause histamine release upon contact with antigen.

The reactions of cell-mediated immunity involve thymus-derived cells and there is suggestive evidence that there is at least one common cell in the diverging pathways toward antibody formation and cell-mediated immunity (16). Little is known of the cellular interactions involved in development of cell-mediated immunity. It has not been possible to enumerate the cells involved in these reactions and quantification of the response is relatively crude compared with that obtained for the antibody response. The delayed type hypersensitivity reaction remains the classic example of this response which is generally considered to mediate graft rejection. Other reactions thought to be analogous, include the mixed lymphocyte reaction and the graft-versus-host response. In vitro assay systems considered to reflect this type of reactivity include cell-mediated cytotoxicity and production of various lymphokines such as migration inhibitory factor, macrophages aggregating factor, mitogenic factor, cytotoxic factor, and chemotactic factors by sensitized lymphoid cells on contact with specific antigen. With many non-cellular antigens, some type of adjuvant

is required to induce cell-mediated immunity. Other antigens including cell and tissue grafts do not require additional adjuvant. Lymphoid cells from non-sensitized animals respond in some of these reactions, for example in the mixed lymphocyte interaction, when exposed to target cells bearing major allogeneic histocompatibility antigens. However, lymphoid cells from non-sensitized animals do not demonstrate cell-mediated cytotoxicity against target cells bearing these histocompatibility antigens; immunization is necessary for lymphoid cells to acquire this property. Hence, proliferation and the cytotoxicity are manifestations of either different cell populations or different maturation stages of the same cell population. Two kinds of T cells are required for the graft-versus-host reaction (17). It is not known whether there are similar requirements for cell interactions in other cell-mediated immune response. A different sort of interaction is involved in production of many of the manifestations of cell-mediated reactions. Relatively small numbers of specific, sensitized lymphoid cells present in the lesions of delayed-type hypersensitivity, are presumably responsible for the influx of large numbers of normal cells having no recognized immunological specificity (18). Such accumulation and activation of mononuclear cells in vivo have counterparts in some of the in vitro lymphokine-mediated reactions.

In many situations, humoral antibody and cell-mediated immunity develop simultaneously and often both processes are active in immunologically mediated inflammation. Tuberculin hypersensitivity is mediated by lymphoid cells, but antibody contributes to the intensity of the reaction (19). As will be discussed later, there are other situations in which antibody may interfere with development of some manifestations of cell-mediated immunity.

In principle, suppression of development of an immune response may be achieved by any procedure which reduces the number of cells giving rise to an immune response or interferes with the interaction of antigen and the various types of reactive cells. Suppression of developed immune responses may be achieved by procedures which either decrease the number of cells involved in antibody production and cell mediated immunity or interfere with the mediators of the immune response. Ideally, suppression of the immune responses should be specific. Most of the procedures presently used, however, are "non-specific" since they inhibit immune responses regardless of their antigenic specificity.

Unfortunately, side effects including increased susceptibility to infection and neoplasia and interference with the physiologic role of mediators limit the usefulness of nonspecific agents for immune suppression. Nevertheless, it should be clearly acknowledged that many of the non specific agents have been used widely and effectively in clinical situations, and have been useful experimentally in helping to clarify complex immune reactions. They may also be useful in conjunction with other procedures to obtain immunologically specific suppression more readily.

It is of interest that some of the methods used to obtain non specific suppression may, by changing timing or dosage, result in increased immune responses, again non specifically. These effects are interesting and sometimes have provided useful information about the immune response, but this aspect of their actions is beyond the scope of the present discussion.

NON-SPECIFIC IMMUNE SUPPRESSION

Procedures or agents that cause non specific inhibition of the immune response can be categorized according to presumed mechanisms of suppression. Several procedures such as thoracic duct drainage or treatment with anti-lymphocyte serum are directed against the cell types specifically involved in

immune responses. Other procedures or agents are differentially toxic for certain biochemical processes and generally are more injurious to rapidly dividing cells including antigen-stimulated lymphoid cells. Some of the agents have complex effects and mechanisms of action are poorly understood.

Lymphocyte Depletion by Thoracic Duct Drainage or Thymectomy:

Thoracic duct drainage of rats has prolonged skin graft survival and depressed primary but generally not secondary antibody response (20). The results observed in experimental animals will depend upon the relative proportions of T and B cells which differs among animal species. This approach has also been used in human recipients of renal allografts. Although moderately effective, it is a cumbersome procedure. Thymectomy has also been employed in human renal allograft recipients with little evidence of immunosuppressive effect. In rodents, immunosuppressive effects of adult thymectomy are observed only after a period of months after surgery unless drastic measures are taken to deplete thymus-derived cells already present in lymphoid organs.

Anti Lymphocyte Globulin:

Heterologous antisera prepared by immunization with lymphocytes or thymus cells have striking immunosuppressive effects (21). It is not surprising that somewhat varied effects have been observed with different anti lymphocyte sera (ALS) since several sources of antigen and species of animal have been used for immunization. ALS can inhibit primary antibody response but only when given before or at the time of immunization. Although it has little effect on established humoral responses or on secondary antibody formation, ALS suppresses established cell-mediated responses. Its effect on the reaction of the host to tissue grafts is striking; human skin grafts survive on ALS treated mice for over 2 months.

The activity of ALS resides in the IgG fraction although it may be restricted to some subclasses. The mechanism by which it exerts its striking

immunosuppressive effect is not entirely clear. There appears to be selective depletion of thymus-derived lymphocytes; this may be accomplished by complement mediated cytotoxic cell destruction or by phagocytosis of antibody-coated cells. "Sterile activation" of lymphocytes and interference with antigen recognition have also been suggested as possible mechanisms of ALS action.

In any case, treatment with ALS seems to offer a means for achieving relatively selective suppression of cell-mediated responses leaving pre-existing antibody formation generally intact.

Total Body x Irradiation:

Less than lethal doses of total body x irradiation given before immunization profoundly inhibit development of a primary antibody response. As with many other procedures, radiation has less effect on continuing antibody formation and on the secondary response (22). Much larger amounts of irradiation are needed to inhibit some cell-mediated responses and the T-cell helper function is resistant to 1000 rads (23). Total body irradiation is no longer used clinically to achieve immunosuppression, although local irradiation of organ grafts may be helpful in controlling rejection crises.

Corticosteroids:

Adrenal steroids have become accepted therapy for a wide variety of diseases having an inflammatory component. The effects of adrenal steroids are complex and their inhibitory effect on the immune response is probably due to multiple actions (24). Steroids given in large amounts inhibit antibody formation if treatment is begun at the time of initial exposure to antigen. Treatment will also depress ongoing antibody production and suppress the secondary response although the effect is less striking. Steroids may interfere with phagocytosis of particulate materials, although this effect is observed only with very large doses. The rate of degradation of injected material may also be affected by steroids. Corticosteroids may alter the

mechanisms by which antigen stimulates lymphoid cells.

Adrenal steroids have a striking cytolytic effect on some populations of lymphocytes while other lymphocytes are unaffected (25). Lymphocytes mediating the graft-versus-host reaction are resistant to steroids. There are conflicting reports regarding the steroid sensitivity of other T cells and of precursors of antibody forming cells. Antibody forming cells appear to be resistant.

Steroid effects on cell-mediated immune responses may be due mainly to inhibition of effector mechanisms. Steroid treated guinea pigs did not exhibit delayed sensitivity to tuberculin yet peritoneal exudate cells from these animals were able to transfer tuberculin sensitivity to normal recipients (26). Hydrocortisone inhibited in vitro cytotoxic activity of sensitized lymphoid cells (27). Although this effect was not confirmed with another assay system (28). Membrane stabilization by steroids may account for some of these effects (29).

Antimetabolic Drugs:

Alkylating agents apparently interfere with both cell division and differentiation. As with several other immunosuppressive agents, this class of drugs was first used clinically in anti tumor therapy. Various mustards have been shown to interfere with both antibody formation and cell-mediated immunity. Cyclophosphamide, a transport form of nitrogen mustard, has been most widely used recently both clinically and experimentally. Although effective in inhibiting antibody formation when given before antigen, the greatest effect is observed when given 24 to 48 hours after immunization. Cyclophosphamide arrests progression through cell cycle at the synthetic (S) and premitotic resting (G2) phase, but the effect on antibody formation does not seem to be related only to interference with cell division. Since colchicine given at this time has little effect. Cyclophosphamide also has profound effects on development of cell-mediated immunity. Guinea pigs sensitized with

oxazolone two days after beginning treatment with cyclophosphamide did not develop large pyroninophilic cells in draining lymph nodes and hypersensitivity response could not be elicited upon challenge. Although effective in prolonging allograft survival in rabbits and mice, little effect was observed in dogs. It has been used clinically in patients who do not tolerate azathioprine and in recipients of bone marrow grafts.

Folic Acid Antagonists, aminopterin and amethopterin (methotrexate), inhibit dihydrofolate reductase thereby blocking conversion of folic acid to folinic acid, a step essential to many biologic processes including, ultimately, synthesis of nucleic acids and purine-containing coenzymes. In rodents, methotrexate causes maximum suppression of antibody formation when treatment is begun 24 to 48 hours after immunization. Similarly, methotrexate prevents development of delayed type hypersensitivity in guinea pigs when treatment is begun during the first 4 days after sensitization with oxazolone or DNCB. As with other antimetabolites, toxicity is a major problem.

Purine Base Analogs, 6-mercapto-purine and azathioprine (Imuran) are the two most widely used drugs of this class. The major effect of these drugs is to block the synthesis and interconversion of purine nucleotides required for nucleic acid synthesis. However, effects on various enzyme systems and nucleic acid metabolism are numerous and biochemical explanations for the effects on the immune response are incomplete. Treatment with 6-mercaptopurine produces maximal suppression of antibody formation if given from 12 to 72 hours after immunization. IgM production is inhibited and markedly prolonged, and there is little or no progression to IgG formation. This effect may be observed with other immunosuppressive drugs as well. Little effect is produced if drug is given during the height of antibody production and the secondary response is not inhibited. These observations suggest that 6-mercaptopurine may inhibit development of T cell helper function but have little effect once this

has developed.

In rabbits, 6 mercaptopurine has a striking effect in inhibiting delayed type hypersensitivity lesions with treatment shortly after sensitization resulting in complete unresponsiveness. However, some of this effect may be non specific since treatment with 6 mercaptopurine for ^{several} days markedly decreased the inflammatory response induced by a single subcutaneous injection of egg white in the rabbit.

Azathioprine has largely replaced 6 mercaptopurine in clinical use. It has considerably less toxicity for the epithelium of the gastrointestinal tract possibly because it seems to be degraded to the active agent by the liver. There are rather marked species differences in immunosuppressive effects perhaps due to differences in drug metabolism.

Pyrimidine Base Analogs, although useful in cancer chemotherapy, generally have not been effective in suppressing immunity.

SPECIFIC SUPPRESSION OF THE IMMUNE RESPONSE

Current immunological dogma demands that selective interference of the immune response to a given set of antigenic determinants involves the specific reactants in the immune response: antigen or antibody. Both have been used in a wide variety of experimental situations with varied and sometimes conflicting results. One reason for confusion is that attention has often been focused on either antibody formation or cell-mediated immunity, and impairment with one kind of response has been considered, sometimes incorrectly, to represent adequately the immunological status of the animal. In the following discussion, consideration will be given to the effects of passive immunization on antibody formation and on cell-mediated immunity, to the role of active antibody formation and its role in sustaining an unresponsive state and in modulating the usual immune response, and to mechanisms by which antigen can inhibit the immune response.

Antibody-mediated Suppression of Antibody Formation:

Passively administered antibody can profoundly inhibit active antibody formation (1, 3, 5). Clinically, passive immunization has been spectacularly successful in preventing maternal Rh sensitization.

There has been considerable controversy regarding details of the basic mechanisms involved since the specific features of the immunological deficit produced by passive immunization in various experimental systems have been rather widely different. These varying results can be explained in part by the nature of the antigen, the immunoglobulin class administered, the temporal relationship between injection of antigen and antibody, the relative amounts of antigen and antibody, and the sensitivity of the assay methods for various aspects of the immune response. It is certain that antibody can act in several ways that are not mutually exclusive to alter qualitatively and quantitatively the immune response. Several features seem clear. The specificity of immunosuppression produced by passive immunization indicates that combinations of antibody with antigen is an essential part of the inhibitory mechanism. Passive immunization generally suppresses the response involving all immunoglobulin classes. Induction of antibody formation is much more markedly inhibited by passively administered antibody than is an ongoing immune response; the secondary response is usually affected but slightly. Since combination with antigen is involved, it is not surprising that high affinity antibody has a greater inhibitory effect than antibody with lower affinity.

IgG antibodies are generally more suppressive than IgM. This may relate to differences in tissue distribution and biological half-life as well as other properties determined by the Fc portion of the molecule such as cytophilic capabilities. The various IgG subclasses appear to differ in their

effectiveness in suppressing the immune response, but conflicting data have been presented; both IgG2 and IgG1 have been claimed to be more effective. Although $F(ab')_2$ antibody fragments retain the ability to inhibit antibody formation, they have been found to be much less effective than the IgG from which they were prepared (30). This also suggests a major inhibitory role for binding of the Fc portion of antibody molecules to reactive cells. Activation of the complement system by bound antigen-antibody complexes with consequent specific cell injury would be a direct mechanism for rendering a cell population unresponsive; however, this possibility is difficult to assess because of the scarcity of specific reactive cells in lymphoid tissues (about 1:10,000).

In the mouse, IgM antibody may cause augmentation of the antibody response to suboptimal doses of antigen (31) apparently because of greater localization of antigen in antibody-forming tissues (32). This effect does not occur in the rat. However, IgM antibody given after antigen injection or added to cultures of mouse spleen cells inhibits active antibody formation. In these circumstances, antigen distribution is not affected and antibody causes suppression (33).

Passively administered antibody exerts its suppressive effect by preventing the antigen from initiating critical cellular interactions. This can occur as a result of simple "covering" of all antigenic determinants with antibody, but this probably is not a common mechanism. In some situations, passive immunization with antibody against a single antigenic determinant can inhibit the response to all determinants on an antigenic molecule or cell. Usually this effect is observed when passive immunization is carried out before antigen is injected. Diversion of antigen away from antibody-forming sites toward non-specific destruction by phagocytic cells is the usual explanation. This mechanism probably accounts for the inhibition of an anti-carrier antibody response when anti-hapten antibody is administered before

injection of the antigen (34). In other situations, however, passive immunization suppresses the response to a single determinant on an antigenic molecule containing several determinants (35).

Evidence has been presented to indicate that antibody may interact with cells directly to inhibit their responsiveness to subsequent exposure to antigen (36). Antigen-antibody complexes have also been shown to inhibit specifically the reactivity of lymphoid cells in vitro although it is not known why the reactivity is impaired (37). It has been suggested that this phenomenon induced with complexes formed in antigen excess may account for antigen-induced "low zone" tolerance to be discussed below (38).

Although the effect of passive immunization on induction of antibody formation is indeed impressive there are relatively few clinical situations in which antibody-mediated injury is susceptible to this mode of therapy. The effect of passively administered antibody on cell-mediated immunity is of considerably greater practical interest.

Antibody-Mediated Suppression of Cell-Mediated Immunity:

Passive immunization can inhibit development of cell-mediated immunity; in rats, renal allografts survive indefinitely in animals treated with anti-allograft antiserum given only at the time of grafting. However, the extent of suppression of cell-mediated immunity often seems to be less impressive than with humoral immunity. This may reflect a difference in sensitivity of detection methods for the two manifestations of immunity. There are additional factors, however. Adjuvants favor induction of cell-mediated immunity, at least for non-living antigens, and adjuvants exert their augmenting effects in spite of an inhibiting effect of passive immunization (39). The reactions of cellular immunity also apparently involve a larger portion of the antigen molecule than do those of humoral immunity; this rather striking carrier specificity apparently reflects a property of the T cells (11). It seems

possible that antisera may not contain antibodies reactive with appropriate carrier regions of the antigen.

Several of the mechanisms by which passive immunization can inhibit the development of cell mediated immunity are analogous with those operating to inhibit the development of humoral immunity. Generally there is a requirement for antibodies reactive against all determinants on the antigen to which the host can react, since a response to a single "uncovered" determinant can result in lethal injury to allogeneic cells. When passive immunization is exploited to promote allograft survival, there is an additional requirement that the antibody not be harmful for graft cells in the presence of the host complement system. This requirement is easily met in the rat, for most alloantisera seem not to cause graft injury. In other species including dog, rabbit, and unfortunately man, most alloantisera activate the complement sequence and cause hyperacute rejection of vascularized grafts or cytotoxic injury to cells. It may be possible to avoid this problem by using non-complement fixing subclasses of antibody or by preparing $F(ab')_2$ fragments which although less effective seem still able to inhibit the development of cell-mediated immunity.

Passively administered antibody, if not cytotoxic in the presence of host complement, may combine with antigens on grafted cells and prevent injury by immune lymphoid cells. This mechanism has been demonstrated in vitro with several assay systems and may account in part for prolonged survival of organ and tumor allografts.

Sustained Suppression of Antibody Formation and Cell-mediated Immunity:

Suppression of the immune response due directly to passive immunization is of limited duration since passively administered antibody is subject to the usual metabolic decay processes. Sustained suppression of the immune responses may require active formation of antibody although the level of production may be low, by the host. Prolonged suppression of both antibody formation and

cell mediated immunity has been observed following passive immunization in situations in which the presence of antigen is maintained (40, 16). Since small amounts of antibody are actively produced after passive immunization, antigen-antibody complexes formed in antigen excess may be responsible for the sustained impairment of the immune response (37).

However, the indefinite survival of rat renal allografts after passive immunization (41) is associated with production of "serum blocking factors" consisting at least in part of antibodies that are able to inhibit the cell-mediated response which also develops (42). "Blocking" antibody having the appropriate properties has been found in sera from allograft recipients, both human and animal, as well as patients and animals with progressively growing tumors using several assay systems (43). This finding is complicated, however, by data implicating a low molecular antigen fragment as the actual inhibitory factor (44).

Heterologous antiserum prepared by immunizing rabbits with mouse myeloma cells profoundly inhibits both initiation of humoral antibody response and active antibody production but has relatively little effect on cell-mediated immune responses (45). Anti plasma cell serum may have therapeutic usefulness in those situations in which antibody may interfere with a desirable cell-mediated response; progressive growth of tumor in the presence of cellular immunity due to serum "blocking factors" is an obvious example.

It seems likely that actively produced antibody also plays an important role in the regulatory processes controlling the magnitude and character of the usual immune response (46).

Antigen Mediated Immunologic Unresponsiveness:

Antigen has been used in a variety of different experimental situations to inhibit the immune response to a subsequent antigen challenge. The terms used to describe these situations often imply mechanisms thought to be operative. "Immunological tolerance" has often been used to describe acceptance of an

allograft by an adult animal after treatment in the neonatal period with allogeneic cells. Although originally applied to grafts and involving impaired cell-mediated immunity, this designation has also been applied to impairment of antibody formation after injection of non-living antigens. "Immunological Paralysis" describes the failure to detect antibody following immunization of an adult animal with large amounts of antigen; classically, this was observed with pneumococcal polysaccharide. "High and low zone tolerance" describes the specific suppression of antibody formation induced by extremes of antigen dose usually in adult animals. Usually, either antibody formation or cell-mediated immunity have been studied with little regard for other manifestations of the immune response.

The mechanisms by which antigen induces unresponsiveness in these various experimental situations are poorly understood. The reactivity of lymphoid cells from unresponsive animals is often reduced when evaluated in vitro or on transfer to appropriate test animals. T-cells and B-cells seem to differ in their susceptibility to the suppressive effects of antigen treatment with T-cells being inhibited more rapidly and for a longer duration by lower antigen doses than are the B-cells (47). However, the reasons for inhibition of cell reactivity is by no means clear, although several hypotheses suggest a direct antigen effect. In many situations in which antigen treatment results in an impaired immune response, it is difficult to exclude mediation of the observed effect by small amounts of actively produced antibody. Antibody-forming cells have been found when looked for with sensitive methods in experimental models usually thought to be associated with an absence of immune response. Extremely small quantities of antibody effectively inhibit active antibody formation. Recent evidence implicates an essential role for antibody in some instances of "tolerance" (38). Antigen and antibody may have independent but not mutually exclusive roles in inhibition of the immune response.

Although many of these experimental models have been instrumental in the development of immunological concepts, applicability of these approaches to clinical immunosuppression is quite limited. Induction of "neonatal tolerance" is not practical. "Immunological paralysis" and "high and low zone tolerance" are observed only with some antigens. A greater problem, however, is the recent observation that cellular immunity may be heightened at the two extremes of antigen dose which are associated with impaired humoral immunity (48). If true for most antigens, this would seriously restrict the usefulness of this kind of antigen mediated immunosuppression since in many clinical situations, cell-mediated immunity is responsible for the observed adverse effects.

The phenomenon of "immune deviation" involves selective suppression of cell-mediated immunity (49). Intravenous injection of antigen often inhibits the development of the cell-mediated immune response to subsequent sensitization; this has been observed with various kinds of antigens which do not induce a cell-mediated response after intravenous immunization. Although the mechanisms of this phenomenon have not been completely established, sequestration of circulating antigen reactive, probably thymus-derived, cells and involvement of these cells in the interactions of antibody formation seem to play a major role (50). Such a process, however, can operate only during the initial period after antigen injection since newly formed T cells ultimately will be released into the circulating pool to provide "memory cells" which may also be active in cellular immunity. Other mechanisms probably operate to explain the sustained suppression of cell-mediated immunity often observed.

Suppression of Immune Responses with Antibody Against Cell Receptors:

The above discussion has utilized conventional concepts to explain how antigen and antibody may interfere with the immune response. There is, however, an unconventional explanation that should be considered as well. Antibody-

forming B cells secrete immunoglobulins having unique immunological reactivity specified in the particular configuration of amino acids in the molecule. B cells have cell surface receptors for a particular antigen and also have immunoglobulins presented on the surface (8). It is probable that the specific antigen receptors are immunoglobulins of the kind for which the cell has the potential to produce. Antibodies can be produced to the unique "idiotypic" antigenic determinants on particular antibody molecules by immunization with highly homogeneous antibody preparations or with myeloma proteins having antibody activity (15). Although anti-idiotypic antibody usually has been raised in allogeneic animals, such antisera can be produced in syngeneic hosts (51).

Addition of anti-idiotypic antibody reactive against pneumococci to cultures of mouse spleen cells prevents the induction of antibody formation by immunization with pneumococcal vaccine (52). The anti-idiotypic antibody in this case was raised against Balb/c mouse myeloma which has antibody reactivity with phosphoryl choline, the major antigenic determinant of the pneumococcal capsular antigen. Complete inhibition of plaque-formation by spleen cells from mice immunized with pneumococcal polysaccharide and inhibition of antibody lysis of erythrocytes passively sensitized with pneumococcal polysaccharide or phosphoryl choline are indications of the reactivity of the anti-idiotypic antibody with all of the usually produced antibody against the pneumococcus. Inhibition of active antibody formation by passive immunization with the anti-idiotypic antibody apparently occurs because of its reaction with idiotypic determinants on mouse lymphoid cells since these seem to be the only source of idiotypic antigen present in the unimmunized mouse. Ramseier has presented evidence derived from a complicated bioassay system that similar anti-idiotypic antibodies can be produced in an allograft situation (53). Such antibodies are likely candidates for mediation of immunologically specific immunosuppression.

SUMMARY

The growing number of patients surviving because of organ and tissue transplants attests to the relative effectiveness of presently available non-specific immunosuppressive agents. However, the greatest success has been achieved when donor and recipient share major histocompatibility antigens, and this has been possible only with living related donors. The toxic effects of immunosuppressive drugs as well as the risk of infection and neoplasia remain significant hazards. Specific immunosuppressive therapy using antigen and antibody has the potential to inhibit both antibody formation and development of cell-mediated immunity. With present techniques, erythroblastosis fetalis can be effectively prevented by means of passive immunization. As the regulatory roles of antigen and antibody are better characterized, selective manipulation of the immune response already possible in selected experimental models should have clinical application in promoting graft survival on the one hand and in favoring rejection of spontaneous tumors on the other.

References

1. R.S. Schwartz, Prog. Immunol. 1, 1081 (1971).
2. J. Bradley, and Elson, C.J. J. Med. Genetics 8, 321 (1971).
3. J.C. Woodrow, Series Haematologica III #3, 1 (1970).
4. N. Kaliss, Int. Rev. Exp. Path. 8, 241 (1969).
5. J.W. Uhr and G. Möller, Adv. Immunol 8, 81 (1968).
6. A.E. Gabrielsen and R.A. Good, Adv. Immunol. 6, 91 (1967).
7. A. Cruchaud and E.R. Unanue, J. Immunol. 107, 1329 (1971).
8. W.E. Paul and J.M. Davie, Prog. Immunol. 1, 637 (1971).
9. H.N. Claman, and D.E. Mosier, Prog. Allergy 16 (In press) (1972).
10. R.W. Dutton, R. Kolko, J.A. Hirst, M. Hoffman, J.W. Koppler, J.R. Kettman, J.F. Lesley, and D. Vann. Prog. Immunol. 1, 355 (1971).
11. K. Rajewsky, and H. Pohlitz, Prog. Immunol. 1, 337 (1971).
12. J.F. Lesley, J.R. Kettman and R.W. Dutton, J. Exp. Med. 134, 618 (1971).
13. E.H. Perkins, T. Sado, and T. Makinodan, J. Immunol. 103, 668 (1969).
14. D.A. Rowley, F.W. Fitch, D.E. Mosier, S. Solliday, L.W. Coppleston, and B.W. Brown, J. Exp. Med. 127, 983 (1968).
15. J.E. Hopper and A. Nisonoff, Adv. Immunol. 13, 57, (1971).
16. M.A. Axelrad, Immunol 15, 159 (1968).
17. H. Cantor, and R. Asofsky, J. Exp. Med. 135, 764 (1972).
18. R.T. McCluskey, B. Benacerraf, and J. McCluskey. J. Immunol. 90, 466, (1963).
19. G.L. Asherson and G. Loews, Immunol. 11, 277 (1966).
20. J.L. Gowans, Horney Lectures 64, 87 (1970).
21. N.A. Mitchison, Fed. Proc. 29, 222 (1970).
22. W.H. Taliaferro, C.G. Taliaferro, and B.N. Jaroslow, Radiation and Immune Mechanism. Academic Press, N.Y. 1964.
23. J. Kettman and R.W. Dutton, Proc. Nat. Acad. Sci. USA 68, 699 (1971).

24. J.A. Mannick and R.H. Egdahl, Endocrinologic Agents in Human Transplantation
ed. F.T. Rapaport and J. Dansset, p. 472, Grune & Stratton N.Y. 1968.
25. J.J. Cohen, M. Fischbach, and H.N. Claman, J. Immunol. 105, 1146, (1970).
26. M.M. Cummings, and P.C. Hudgins, J. Immunol. 69, 331 (1952).
27. W. Roseman, and H.D. Moon, J. Immunol. 89, 422 (1962).
28. J. Mael, H. Rudolf, B. Chapuis, and K.T. Brunner, Immunol. 18, 517 (1970).
29. G. Weissman, New Eng. J. Med. 273, 1084, (1965).
30. N.R. StC. Sinclair, R.K. Lees, P.L. Chan, and R.H. Khan, Immunol. 19, 105 (1970).
31. C. Henry and N.K. Jerne, J. Exp. Med. 128, 133 (1968).
32. G. Dennert, J. Immunol. 106, 951 (1971).
33. W.M. Wason, Fed. Proc. 31, 769 (1972).
34. J.W. Kappler, M. Hoffman, and R.W. Dutton, J. Exp. Med. 134, 577 (1971).
35. J-C. Cerottini, P.J. McConahey and J.J. Dixon, J. Immunol 103, 268 (1969).
36. D.A. Rowley, and F.W. Fitch, J. Exp. Med. 120, 987 (1964).
37. M. Feldmann and E. Diener, J. Exp. Med. 131, 247 (1970).
38. M. Feldman and E. Diener, Immunol. 21, 387 (1971).
39. D.A. Rowley, F.W. Fitch, M.A. Axelrad and C.W. Pierce, Immunol. 16, 549 (1969).
40. M.E. Neiders, D.A. Rowley and F.W. Fitch, J. Immunol. 88, 718 (1962).
41. F.P. Stuart, T. Saitoh, and F.W. Fitch, Science 160, 1463 (1968).
42. F.P. Stuart, F.W. Fitch, D.A. Rowley, J.L. Biesecker, K.E. Hellstrom, and
I. Hellstrom, Transplantation 12, 331, (1971).
43. K.E. Hellstrom, and I. Hellstrom, Ann. Rev. Microbiol. 24, 373 (1970).
44. H.O. Sjogren, I. Hellstrom, S.C. Bansol, and K.E. Hellstrom, Proc. Nat.
Acad. Sci. USA, 68, 1372 (1971).
45. N.S. Harris, S.M. Jagarlamooty, C.F. McKhann, and J.S. Najarian, J.
Immunol. 108, 958, (1972).
46. J-C. Bystry, I. Schenkein, and J.W. Uhr, Prog. Immunol. 1, 627 (1971).

47. J.M. Chiller, G.S. Habicht, and W.O. Weigle, Science 171, 813 (1971).
48. C.R. Parish and F.Y. Liew, J. Exp. Med. 135, 298 (1972).
49. G.L. Asherson, Immunol, 10, 179 (1966).
50. J. Sprent, J.F.A.P. Miller, and G.F. Mitchell, Cell. Immunol. 2, 171, (1971).
51. B.W. Brient, J. Haimovich, and A. Nisonoff, Proc. Nat. Acad. Sci. USA 68, 3136, 1971.
52. H. Cosenza, and H. Köhler, Science (In press), (1972).
53. A. Ramseier and Lindenmann, J. Exp. Med. 134, 1083 (1971).