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Specific Suppression of Immune Responses

Antibody directed against either antigen or receptors for antigen can suppress immunity specifically.

Donald A. Rowley, Frank W. Fitch, Frank P. Stuart, Heinz Köhler, Humberto Cosenza

A fully responsive immunologic system is essential for survival against encounter with infectious, oncogenic, and toxic agents; however, in particular instances, an immune response may be disadvantageous. Allergy to a common pollen, maternal antibody that causes erythroblastosis in the fetus, and immunologic rejection of a life supporting organ transplant are familiar examples of undesirable responses. Immune reactions, abnormal in magnitude or kind to certain infectious or other agents, apparently are involved in various "autoimmune" diseases affecting diverse tissues or organs (*1*). Antigen-antibody complexes may localize in the kidney causing renal injury (*2*). Furthermore, antibody to tumor antigens may prevent cell mediated immunologic injury to the tumor; thus, suppression of the antibody response to a tumor may be desirable (*3*).

The general approach for preventing or controlling life threatening immune reactions has been to reduce the total number of lymphoid cells, usually by administering cytotoxic drugs or irradiation or, by giving heterologous antibody directed against lymphoid cells (*4*). These procedures, which nonspecifically suppress responses to all antigens, are sometimes remarkably effective in promoting graft survival or controlling autoimmune processes. However, the complications of immunosuppressive therapy are frequent and often severe; they include overwhelming infection, increased risk of malignancy, and injury to nonlymphoid tissue (*5*).

Ideally, immunosuppressive therapy should selectively suppress either the antibody or the cell mediated response to a specific antigen without altering responses to other agents. This ideal has been achieved clinically in one instance. Immunization by fetal Rh antigen is prevented by giving the Rh negative mother antibody against Rh antigen immediately after she has given birth to an Rh positive child (*6*). The mechanism of specific suppression achieved by giving antibody passively has been studied extensively (*7*). Other studies demonstrate that specific regulation can be achieved by

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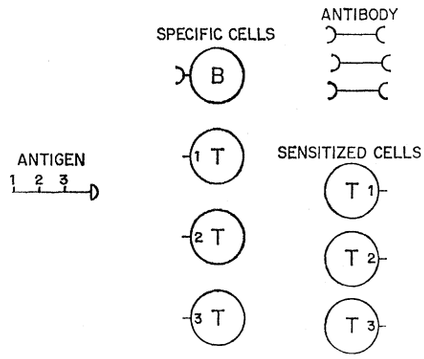

Fig. 1. Antigen includes a carrier having different determinants, indicated here by numbers 1 to 3, and a determinant D. The antigen stimulates B and T lymphocytes having receptors for these determinants. Receptors on T cells are not illustrated because the nature of the T cell receptor is not known; however, it is possible that the general structure of receptors on the two kinds of cells may be similar. Although only one receptor is illustrated on the B cell, such cells are estimated to have on the order of 10^5 receptor molecules per cell. On antigenic stimulation, B lymphocytes proliferate, and synthesize and release antibodies having combining sites for D which are "identical" to the combining sites of the receptors on the B cells specific for D. The T lymphocytes proliferate to give rise to expanded populations of cells having receptors for carrier determinants.

different mechanisms. These findings taken together provide a basis for developing specific immunosuppressive therapy and for understanding the autoregulation of immune reactions occurring in nature.

Reactants and Products

An immune response includes proliferations of lymphoid cells which synthesize and release antibody, or the development of cells responsible for "cell mediated immunity," or both. An antibody response, a cell mediated response, or a combined response may develop depending on the nature of the antigen and the kind of exposure to it. We accept the general assumptions of clonal selection (*8*) which for our purposes can be stated as follows: antigen selects the relatively few lymphoid cells having receptors for it; these few cells proliferate and differentiate giving rise to expanded populations of antigen specific cells.

The term antigen generally refers to diverse and complex agents such as bacteria, viruses, and heterologous cells and proteins, but each such agent contains many different antigenic determinants, that is, the smallest definable units which are recognized, responded to, and with which antibody combines. Those determinants or haptens that have been characterized are relatively simple chemicals such as dinitrophenol, picryl chloride, phosphorylcholine, histamine, or small peptides and saccharides having structures specified by about five or six amino acids or oligosaccharides (*9*). Determinants, though they combine with antibody, do not themselves stimulate an immune response but must be coupled with or be part of a carrier molecule or membrane which has additional different antigenic determinants. If the determinant is coupled with a nonimmunogenic carrier, then to become immunogenic it must be injected with an adjuvant which has multiple antigenic determinants (*10*).

Antigen specific cells are bone marrow–derived lymphocytes (B cells) (*11*) and thymus-derived lymphocytes (T cells). The B cells have immunoglobulins on their cell surface which function as receptors for antigenic determinants (*11*). Some antigens such as pneumococcal polysaccharides, polymerized bacterial flagella, and polyfructase may stimulate B cells directly (*12*). The antibody response to most antigens requires cooperation between B and T cells, but the T cells are "carrier specific" (*13*). Thymus-derived cell receptors have not been characterized, but the density of receptors may be lower on T cells than on B cells or the receptor may be comparable to only a portion of an entire immunoglobulin (*14*). Antibody responses to a determinant may be equally high though a determinant is coupled to unrelated carriers, indicating that T cell interaction with the carrier does not provide a specific stimulus for antibody production by the B cell (*15*).

A third cell type is required for the antibody response to many antigens, at least in vitro. These cells, which tend to adhere to plastic or glass and are usually referred to as A cells, interact with antigen or a product of the interaction of specific T cells and antigen to stimulate B cells (*16*).

Thymus-derived cells are required for the development of cell mediated immunity; B cells are apparently not required, although this possibility is not excluded (*17*). A nonspecific factor which may act to localize or alter the interaction of antigen with T cells is required for development of cell mediated immunity (*18*).

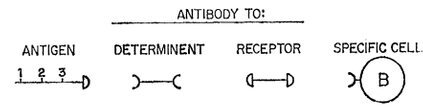

Fig. 2. Interaction of antigen and cells reactive to D can be prevented by antibody directed against either D or the receptor for D.

The specific products of immune responses are antibody, "sensitized" T cells, and cells responsible for "immunologic memory." Antibodies having the same antigenic specificity but differing in other respects are divided into classes and subclasses; IgM, IgG, IgA, and IgE are the classes studied most extensively (*19*).

Sensitized T cells are presumed to have receptors for determinants on the carrier, and interaction of these cells with the antigen causes the release of various mediators which are cytotoxic, inhibit migration of macrophages, or cause increased vascular permeability or other reactions (*20*). It is generally assumed that the mediators are released from sensitized T cells; however, it has been suggested that the interaction of specific T cells and antigen causes release of mediators from another population of T cells which are neither antigen specific nor precursors of such cells (*21*).

Cells that form antibody do not themselves give rise to the population of "memory" cells which are responsible for the continued capability to respond to an antigen; rather, antigen is thought to cause precursor cells to divide and give rise to two populations of B cells; one of antibody forming cells and the other of "memory cells" (*22*). On the other hand, a greater or more rapid antibody response can occur if the number of carrier specific T cells increases independently of any increase in the number of B cells (*23*). Carrier specific T cells recirculate in increased numbers long after immunization with some antigens (*24*), and undoubtedly these cells account in part for the phenomenon of "memory" (*25*).

Conceptual Basis of Specific Suppression

Suppression of the development of immunity could result either from blocking the interaction of antigen with cell receptors or from decreasing the numbers of specific cells that could

interact with antigen. Thus, antibody directed either against the antigen, or against the specific combining site of the cell receptors, might specifically suppress immunity (Figs. 1 and 2). Antibody directed against antigen could suppress immunity either by combining directly with antigen (peripheral block) or by combining with responding cells (central block), in the latter case by combining with antigen-cell complexes to prevent required interaction with other cell types (Fig. 3).

On the other hand, antibody directed against cell receptors would react directly and centrally with responding cells. Similarly, free antigenic determinants or a great excess of antigen might block receptors to prevent necessary cell to cell interactions or act directly with specific cells to cause them to be unresponsive (Fig. 3). These general mechanisms should apply to development of either antibody or cell mediated responses; however, stimulation of either an antibody or cell mediated response to an antigen might suppress development of the other type of response. This could occur because T cells with receptors for an antigen are limited in number and are required for both the antibody and cell mediated responses to some antigens. Diversion of T cells to one type of response should decrease the number available for development of the other type of response. Thus, antigen given alone to stimulate an antibody response might specifically suppress development of cell mediated immunity (Fig. 3).

Other mechanisms of regulation undoubtedly operate. For example, T cells must synthesize receptors for carrier determinants. It is not known whether antigen specific receptors are released or not, but either such agents or T cells themselves should participate in the regulation of immune responses; and indeed, suppression of IgE antibody production by specific T cells has been reported (*26*). It may be worth our noting that a specific response might cause nonspecific suppression to a different antigen. This could occur at any step where an essential element in limited amounts was consumed by the specific response, or alternatively, if products were elaborated which nonspecifically depressed or interfered with B cell function or T cell function, or both. Nonspecific suppression of some immune responses by T cells (*27*) may represent an example of this possibility.

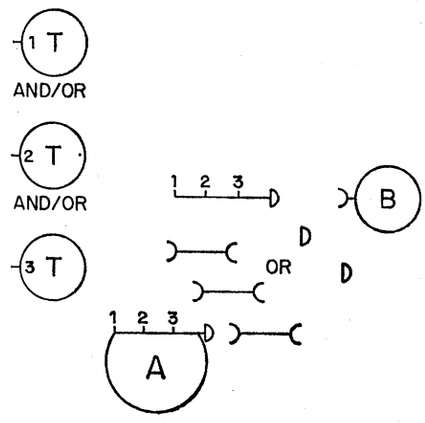

Fig. 3. The interaction of B cells with antigen on A cells could be blocked by antibody to D or by an excess of D. In this way cooperation between B and T cells might be prevented. Some antigens having repeated identical determinants may stimulate B cells directly, or if in excess may cause cross-linkage of receptors to make B cells unresponsive (*44*). The T cell response is the sum of the specific responses to different carrier determinants. If T cells are required for the B cell response, then any procedure which reduces the number of T cells reactive with carrier determinants may reduce the B cell response.

In this article, however, we discuss primarily some models we have worked with which illustrate specific suppression by treatment with antibody to receptors and antigen. We do not attempt to be comprehensive in relating this work to that of others or to discuss in detail alternative interpretations of this work.

Suppression by Antibody

Suppression of the antibody response by passive immunization has been observed for many antigens in many different species, but the nature of the response to sheep erythrocytes (SRBC) and suppression of this response by antibody has probably been studied most extensively. Relatively small amounts of antibody against SRBC given to normal animals specifically suppresses their primary IgM and IgG antibody response. Antibody given up to 24 hours after immunization is almost as effective as when given before or at the time of immunization. When antibody is given more than 24 hours after antigen, it has very little effect on the course of the primary IgM response, though it may alter the development of the IgG response or a secondary response (*28*). The duration of suppression is a function of the biological half-life of the antibody and therefore depends on the amount and class of antibody given. Primary responses can be virtually abolished by small amounts of hyperimmune serum, that is, serum from a donor immunized repeatedly with SRBC. Both IgM and IgG antibodies suppress the antibody response, although IgM antibody can increase responses of mice to suboptimal doses of SRBC under particular circumstances (*29*). The same quantity of the same antibody preparation which so effectively suppresses a first response has little or no effect on previously immunized animals (*30*).

Undoubtedly, antibody suppresses the development of immunity by combining with antigen, but a simple concept of "neutralization" of antigen by antibody cannot account for the phenomenon. For example, if antibody suppressed the immune response by reducing the amount or dose of antigen, then the kinetics of suppressed responses should be equivalent to responses observed with lower antigen doses. This is not the case. A decrease in the dose of antigen decreases the rate of proliferation of antibody producing cells while suppression by antibody decreases the number of cells initially responding without affecting the rate of proliferation of cells that do respond (*31*). This probably occurs because passively given antibody prevents the selection of specific cells in the spleen (*24*), a finding we discuss later.

Admittedly, antibody-SRBC complexes formed in vitro in the presence of excess antibody and washed thoroughly produce very low responses, but such complexes also effectively suppress the response to a large dose of free SRBC given several hours later (*30*). Apparently, a small amount of antibody combined with antigen in critical sites is sufficient to suppress the response. Studies of the response of mouse spleen cells to SRBC in vitro support this contention. The magnitude and kinetics of the plaque-forming cell (PFC) response obtained in vitro are comparable to that obtained in vivo (*32*). The PFC response requires both A cells and a population of nonadherent cells which contains both B and T cells (*33*). The presence of A cells that have reacted with antigen is required for 2 to 3 days of the exponential phase of the response and the addition of antibody to cultures during this period has the same effect as eliminating either A cells or the antigen on A cells

from cultures (*34*). Thus, antibody probably suppresses the PFC response by combining with a small amount of antigen bound to A cells. It is interesting that A cells and antigen are required for shorter periods if cells have been obtained from previously immunized mice (*35*); the relative insensitivity of the secondary response to suppression by antibody may occur because of this decreased requirement (*35*).

Suppression by Antibody against Specific Receptors

We assume that antibody suppresses the development of immunity by combining with antigen and that antigen is exogenous material introduced into the responder. However, recent experiments demonstrate unequivocally that antibody directed against specific receptors causes specific suppression, and in this case the antibody is directed against endogenous antigenic determinants. Whether such antibody to receptors plays a role in the normal regulation of immune responses is not known, but the findings to date are most provocative.

The experiments are based on two assumptions: (i) the specific combining region of a cell receptor for antigen and the antibody produced are very similar or identical, and (ii) the specific combining region is itself potentially antigenic. Thus, an antibody directed against the specific combining region of an antibody would also be directed against the specific receptor, and such antibody could either block the activity of antibody already produced or prevent cells from responding initially. These assumptions have been validated in the following way.

The myeloma protein produced by the TEPC-15 tumor in BALB/c mice combines specifically with the antigenic determinant phosphorylcholine (*36*). The combining region of this myeloma protein is very similar or identical to that of antibody produced by the normal BALB/c mouse to phosphorylcholine (*37, 38*). The normal response is induced by a vaccine of a rough strain of pneumococcus, R36A, having phosphorylcholine as a major determinant or by phosphorylcholine coupled to various carriers. Immunized spleen cells in the presence of complement produce lytic plaques against phosphorylcholine coupled to SRBC (*37*). Mice of the A/He strain that are injected repeatedly with the purified myeloma protein in adjuvant develop precipitating antibody against the myeloma protein which is not removed by absorption with normal BALB/c serum or by a noncross-reacting BALB/c myeloma protein. The antibody has no activity against phosphorylcholine; yet it specifically suppresses plaque formation by cells from BALB/c mice immunized against phosphorylcholine (*37*), and it specifically and completely suppresses the induction of the antibody response to phosphorylcholine in BALB/c mice (*38*). This latter effect is observed if the antibody is given before, but not 1 day after immunization. The response to phosphorylcholine, at least when induced by pneumococcal vaccine, appears to be independent of A or T cells, and therefore the antibody probably suppresses the response by combining with receptors on B cells. Whereas antibody against antigen does not suppress effectively the response of previously immunized animals, the antibody to receptors effectively suppresses the responses of mice immunized 2 weeks previously with phosphorylcholine. Thus, antibody to receptors must suppress "memory" B cells as well as B cells initially responsive to phosphorylcholine.

An individual that produces antibody to receptors must himself have cells with receptors for the combining site of the antibody. Although it might seem unlikely that antibody produced by an individual would be antigenic in the same individual, it is conceivable that antibodies complexed with antigen in the presence of excess antibody might be antigenic with the "complex" serving as carrier and exposed combining sites serving as determinants (Fig. 4). Antibody and anti-antibody would each be the "anti-antibody" to the other, a concept which circumvents the objection that if anti-antibody is possible then anti–anti-antibody, and so on to absurdity must also be possible.

Circumstantial evidence supports the concept that antibody and anti-antibody exist in some hyperimmune serums produced against SRBC and against alloantigens (*39*). The intriguing possibility exists then, that antibody directed to receptors may be synthesized during the course of an immune response, and that such antibody may play an important role in the continuing autoregulation of immune responses to an antigen (*40*).

Suppression by Antigen

Certain antigens such as heterologous serum proteins or bacterial polysaccharides given in large doses cause very low or undetectable serum antibody responses; furthermore, animals may remain specifically unresponsive for prolonged periods of time to doses of the antigen that are normally immunogenic (*41*). Characteristically, such antigens are sequestered or degraded slowly. Unresponsiveness presumably occurs because the antigen blocks receptors and thus interferes with cell-cell interactions or suppresses responding cells directly. Although the evidence that either mechanism operates in vivo is not entirely satisfactory, several investigators have shown both of them to operate in vitro. High doses of free determinant (*42*) or determinant conjugated to syngeneic erythrocytes (*43*) may suppress the immune response. In the latter case, the response is dependent on carrier specific cells, and the determinants coupled to syngeneic cells presumably combine with B cells to interfere with their interaction with T cells. The response to polymerized flagellar antigen does not require T cells, and in this instance a large excess of the antigen presumably suppresses the response by causing cross-linkage of receptor sites on B cells (*44*). These or possibly other mechanisms may account for both the initial and continued suppression of the response observed in vivo after injection of doses of antigen that cause immunologic paralysis of the animal. The other mechanisms may include nonspecific suppression by T cells (*27*) or feedback suppression by small amounts of actively produced antibody. The fact that spleen cells from mice made unresponsive by a large dose of pneumococcal vaccine respond normally in vitro to the antigen indicates that suppression is reversible and that clones of cells have not been eliminated (*45*).

Antigen can also specifically suppress the development of cell mediated immunity, but by a different mechanism that we discuss in the following section.

Suppression of Cell Mediated Immunity by Antibody and Antigen

We have used two models for our studies of suppression of cell mediated immunity: sensitization to SRBC and

renal allograft rejection. Since the models themselves as well as the mechanisms of suppression are complex, brief descriptions of the models may be helpful. Rats injected intradermally with SRBC emulsified in Freund's complete adjuvant (FCA) develop cell mediated immunity to SRBC. Sensitivity, as measured by the local reaction to antigen injected intradermally, is first demonstrable by 5 to 6 days, is severe by 8 to 10 days, and declines to low levels by 3 to 4 weeks after sensitization; both antigen and adjuvant must be reinjected to sustain sensitivity (*46*). Development of sensitivity is partially and specifically suppressed by giving either antibody to SRBC or SRBC alone. Antibody obtained during the early part of the primary response is ineffective whereas hyperimmune serum given 1 day before or at the time of sensitization is maximally suppressive. An immunizing dose of 10^8 SRBC given intravenously 24 hours before sensitization is maximally suppressive. Antigen treatment is ineffective if given 3 to 5 days before or more than 1 day after sensitization. Repeated attempts to obtain complete suppression with either antibody alone or antigen alone have failed, whereas complete suppression is obtained by using the two procedures together (*46, 47*). The success of these procedures suggested that they might be applicable for promoting survival of renal allografts.

Lewis (L) and Brown Norway (BN) are inbred rat strains that have major histocompatibility differences. A kidney from an F_1 LBN donor transplanted into a bilaterally nephrectomized Lewis recipient is invariably rejected, and the recipient dies of uremia, usually within 10 days. Survival is prolonged by either of two procedures: (i) by giving the recipient at the time of surgery antibody directed against graft antigens (that is, antiserum from Lewis rats injected with BN antigen), or (ii) by giving graft antigen intravenously to the recipient 1 day before surgery (that is, BN or LBN lymphoid cells). Each procedure is immunologically specific, and the two used together are more effective than either procedure alone (*48*). Thus, the model is analogous to the model in which SRBC are used as antigen, but it differs in important ways: An adjuvant is required to induce cell mediated immunity to SRBC, whereas both an antibody response and cell mediated immunity develop against graft antigens after kidney grafting. Also, the source and the dose of antigen are not precisely determined since the graft survives for variable lengths of time, and at least some of the grafted cells proliferate.

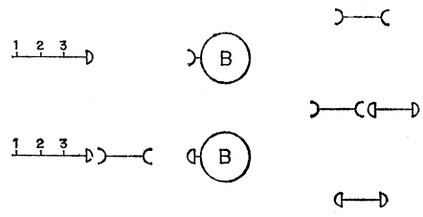

Fig. 4. An individual that produces antibody to receptors (that is, specific anti-antibody) must have cells with receptors that react with the combining site of the antibody. The antibody itself might not stimulate the formation of antibody to receptors because the antibody lacks appropriate carrier determinants; however, the original antigen complexed with the antibody might provide carrier determinants. If both products are produced, antibody and antibody to receptors are probably free and in antibody-antibody complexes.

Recipients treated with antibody alone may survive indefinitely though they often have evidence of impaired renal function. Hyperimmune antiserums effective for prolonging graft survival contain agglutinins for BN erythrocytes and antibody cytoxic for BN lymphoid cells in the presence of complement, but the titers of these antibodies do not necessarily correlate with the effectiveness of antiserums in enhancing graft survival (*49*). Results obtained with small amounts of a very effective antiserum given at the time of surgery are not improved by giving larger amounts or extending the injection schedule, although results with a less effective serum may be improved by giving additional serum for several days after surgery (*49*). However, antiserum treatment does not reverse the rejection process once it has started.

Recipients treated with antigen alone usually do not fare as well as recipients treated with an effective antiserum, but individual rats may survive indefinitely. The timing of antigen injection is important, administration 1 day before surgery being most effective. Recipients given antigen 3 to 5 days before surgery reject kidneys more rapidly than untreated controls; antigen given more than 1 day after surgery has no effect. Doses of antigen in the range of 5×10^7 to 10^8 washed spleen cells, or lymph node cells, or both, are most effective, and these doses of antigen stimulate a moderate circulating antibody response (*50*). The graft itself, particularly the mobile "passenger leukocytes" within the vasculature of the graft, provides a source of antigen which may affect the host's response in the same way as intravenously injected antigen and this may account for the variability of antigen treatment on graft survival (*51*). However, it is important to note that many Lewis recipients treated just once with both an effective antibody preparation and an appropriate dose of allogeneic cells as antigen accept the LBN renal allograft and fare as well as though it were a syngenic graft; they live a full life span without impaired renal function or any evidence of rejection crises.

Antigen given intravenously stimulates an antibody response, and antibody given passively suppresses development of cell mediated immunity. It might be reasoned, therefore, that intravenous antigen suppresses development of cell mediated immunity by stimulating an active antibody response. Several findings do not favor this simple explanation (*52*), however, and as shown in the following section, each procedure has a different effect on recirculating lymphocytes.

Specific Selection of Lymphocytes

Interaction of lymphocytes with antigen at the site of sensitization or in regional nodes is required for the development of cell mediated immunity (*53*). The localization of lymphocytes at these sites by antigen is accomplished by selection of specific cells from the recirculating pool present in lymph and blood. This is deduced from several kinds of observations. For example, thoracic duct lymph collected from rats 24 to 36 hours after they had been sensitized with SRBC in FCA was markedly deficient in cells capable of restoring the antibody response of heavily irradiated rats to SRBC (*24*). Similarly, lymphocytes obtained from blood 24 hours after Lewis rats received an LBN kidney transplant gave greatly reduced mixed lymphocyte interactions against LBN cells (*54*). For both examples, the total number of cells in lymph or blood was not reduced measurably, and the reduced activity was specific for the antigen injected.

Antigens such as SRBC or allogeneic cells injected intravenously also select B or T lymphocytes, or both, from the recirculating pool so that the number of antigen specific cells is reduced in blood and lymph and increased in the spleen (*24, 54, 55*). The reduction in specific cells in blood and lymph is marked from 1 to 3 days but is no longer demonstrable 4 days after antigen injection. Clearly then, antigen injected intravenously 24 hours before sensitization with antigen-adjuvant or by a graft would decrease the number of specific cells that could react with sensitizing antigen and by this mechanism would suppress development of cell mediated immunity. It is worth our emphasizing that antigen injection 24 hours before sensitization neither eliminates all antigen specific cells from the ricirculating pool nor does it abolish development of cell mediated immunity; furthermore, the number of antigen specific cells increases to usual amounts by 3 to 4 days after antigen injection and sensitization at this time is not suppressed.

Antibody given to normal animals prevents selection of recirculating lymphocytes. For example, thoracic duct cells from rats given antibody to SRBC before intravenous injection of SRBC are normally effective in restoring the response of heavily irradiated rats to SRBC (*24*). Passively given antibody also prevents selection of recirculating lymphocytes by alloantigen. Lewis rats were injected intravenously with Lewis anti-BN antibody and several hours later with LBN cells; blood leukocytes that were obtained the following day gave normal mixed leukocyte interaction against LBN lymphoid cells (*54*).

Selective recruitment of recirculating lymphocytes by antigen might be prevented in either of two ways; by antibody directed against antigenic determinants or by antibody directed against specific cell receptors on recirculating lymphocytes. The serums which prevent selection are hyperimmune serums having high titers of antibody directed against the antigen, but, as indicated earlier, such serums may also contain antibodies to receptors. In any event, two other findings are consistent with the general suggestion that antiserums suppress responses of normal animals by preventing selection of recirculating lymphocytes. As noted earlier, the kinetics of responses suppressed by antibody indicate that smaller numbers of cells respond initially. Also, antibody given more than 24 hours after antigen, that is, after selection has occurred, causes much less suppression than when given with the antigen.

All of these findings taken together indicate that intravenous antigen and passive antibody suppress development of cell mediated immunity by different mechanisms. Each procedure has the effect of decreasing the number of specific recirculating lymphocytes which interact with sensitizing antigen: intravenous antigen by reducing the number of such cells in the recirculating pool, and passive antibody by preventing those cells that do circulate from interacting with sensitizing antigen. The fact that the procedures alone do not abolish sensitization is consistent with the observations that intravenous antigen does not eliminate all reactive cells in the recirculating pool, and passive antibody does not totally prevent selection of reactive recirculating cells. It is reasonable to expect that the procedures used together should be more effective than either alone, and such has been observed. It should be emphasized that these mechanisms of suppression pertain only to development of cell mediated immunity, not to sustained suppression which may result without additional antibody being given.

Homeostasis of Suppression

The phenomenon of specific suppression has two phases: one of initial suppression of development, and the other of maintenance of suppression for an extended time. While the first phase is obviously dependent on the particular suppressive treatment used, the second phase is much more complex and is probably dependent on an active response by the host.

Antibody actively produced in small amounts may sustain suppression of the antibody response. For example, rats passively immunized with antibody against SRBC only once and injected with SRBC a day later and once a week thereafter continue to have low antibody responses for as long as injections of SRBC are continued (*56*). Rats given antibody alone 4 weeks previously have no detectable serum antibody, and they respond normally; thus, sustained suppression cannot be attributed to persistence of the antibody given passively. The low titers of serum antibody demonstrable many weeks after passive immunization and weekly antigen injections must therefore be due to active antibody synthesis, and indeed, rats with sustained suppression had significantly more PFC in the spleen than nonimmunized normal animals. If injections of antigen are stopped for a month, the animals are again responsive. It seems reasonable to assume, therefore, that suppression of a large number of potentially responsive cells is sustained by antibody actively produced by the relatively few cells and their progeny which were not suppressed initially (*57*), or by antibody to receptors that were actively produced in response to antigen-antibody complexes.

Such an explanation does not account so readily for continued suppression of cell mediated immunity to SRBC. Severe sensitivity results if injections of both SRBC and adjuvant are made every 8 to 12 days, and such animals have very high titers of antibody to SRBC (*46*). Complete suppression achieved initially with antigen and antibody can be sustained by giving SRBC intravenously 24 hours before each successive intradermal injection of SRBC-FCA. Though such animals develop no detectable cell mediated immunity, they do develop appreciable titers of actively produced antibody. Repeated intravenous injections of antigen cause a sustained reduction in the number of antigen specific cells in the recirculating pool (*24*), and in this way the number of cells which can react with sensitizing antigen is presumably reduced. In any event, it is unlikely that sustained suppression is due to elimination of clones of potentially reactive cells, for if intravenous antigen injections are discontinued prior to injection of antigen and adjuvant, the animals in which the response is suppressed develop cell mediated immunity within 7 to 10 days (*46*).

The mechanisms accounting for continued survival of rat renal allografts are also complex. The evidence is convincing that continuing survival of the kidney is not due to adaptation of the graft to the host (*50*). Continued unresponsiveness of the Lewis recipient is dependent on continued presence of the graft, for if an LBN kidney transplant is replaced by a Lewis kidney, the Lewis recipient 1 month later will cause severe immunologic injury of a second LBN kidney graft (*58*). Presumably, the graft stimulates continued active antibody production, and this antibody prevents rejection of the

graft. Evidence for this, though circumstantial, is reasonably convincing.

1) We are reasonably certain that actively produced antibody can promote graft survival. As pointed out earlier, intravenous antigen given 1 day before grafting promotes survival, but given 3 to 5 days before grafting, causes more rapid rejection of the kidneys. We add here the point that recipients given antigen alone 2 to 4 weeks before grafting have circulating antibody against graft antigen, and they frequently accept a renal allograft for prolonged periods. Apparently then, intravenous allogeneic antigens have three effects: selection of recirculating lymphocytes, stimulation of cell mediated immunity, and stimulation of an active antibody response by the selected lymphocytes. Without a graft or repeated antigen injections, cell mediated immunity wanes and antibody actively produced may partially suppress the reestablishment of cell mediated immunity by the graft (*58*).

2) Rats with suppressed responses do have cell mediated immunity demonstrable in vitro; furthermore, they have serum factors which block cell mediated immunity (*59*). These observations depend on two assays adapted for our rat model; for each assay, lymphoid cells from a Lewis recipient are incubated with appropriate target cells having BN antigens. One test, which depends on the release of chromium-51 from labeled target cells, reveals that antibody given at the time of grafting (which by itself promotes indefinite survival of the graft) delays the onset but not the magnitude of the cell mediated response (*60*). Without passively given antibody, cell mediated immunity reaches a peak by 5 days and kidneys are rejected. With passive antibody, similar levels of immunity develop by 11 days, but the kidney is not rejected. By several weeks cell mediated immunity declines markedly but not completely as long as the allograft remains. The second test, depending on loss of adherence of target cells, demonstrates that both the antiserum used to promote graft survival and serum from rats which have had a renal allograft for many months specifically block cell mediated immunity in vitro (*61*). These findings taken together indicate that those procedures that suppress cause a delay in the onset of development of cell mediated immunity and permit active synthesis of antibody which prevents manifestation of cell mediated injury to the graft. The antibody which is effective may be directed against antigen or specific receptors, or it may include antibody directed against both antigen and receptors.

Tolerance, Paralysis, and Enhancement

Specific suppression can be induced with relative ease in animals that are immunologically unresponsive because of immaturity, or because lymphoid tissue has been partially ablated by x-irradiation, or because they have been injected with antibody to lymphocytes or subjected to other procedures (*62*). With growth or recovery, immunologic responsivity attains normal levels except to those antigens to which exposure occurred during this critical period. Presumably, the cells potentially responsive to these antigens have been eliminated or "turned off," that is, made tolerant, and it is often suggested that tolerance to self-antigens occurs in the same way. Some immunologists might argue that "immunologic paralysis" induced in normal adult animals by large doses of some kinds of antigen such as pneumococcal polysaccharide provides an example of how other antigens may induce tolerance by "turning off" cells. This line of reasoning may lead to the assumption that tolerance and paralysis are fundamentally different from suppression which depends on the products of an immune response for induction and maintenance of the unresponsive state. But the demarcation between these phenomena is often not clear (*41*).

This difficulty is illustrated in the case of "immunologic enhancement" of tumors. It is possible that all tumors, whether induced by chemical, physical, or viral agents, have antigens which are either "new" to the host or are not expressed in the mature individual (*63*). The tumor may grow without apparent immunologic reaction of the host, and the host is apparently "tolerant" to the tumor. Nevertheless, lymphoid cells removed from the host bearing the tumor are specifically cytotoxic to the tumor cells; furthermore, serum factors, which are undoubtedly antibody or antigen-antibody complexes, specifically block this cell mediated injury to the tumor in vitro (*3*). Apparently, a host bearing a growing tumor actively produces antibody in small quantities which "enhances" tumor growth by interfering with cell mediated immunity. The correspondence between the tumor and allograft models is obvious, but the objective of the tumor immunologist is to devise a means for suppressing production of enhancing antibody or to "unblock" enhancing antibody, while the objective of the transplant immunologist is to take advantage of enhancing antibody to promote graft survival.

In many instances it is apparent that tolerance is not due to elimination of specific responding cells. When it can be examined, continued tolerance or paralysis requires persistence of the antigenic stimulus. But even in the presence of the persistent antigen, tolerance may be lost spontaneously or because of treatment with nonspecific agents which cause proliferation of lymphoid cells (*64*). Also, in some models, cells from tolerant or paralyzed animals incubated in vitro or transferred to heavily irradiated recipients may respond as well as normal cells to the antigen (*45*). In other instances antibodies which react with self-antigens are demonstrable in various pathologic states. Thus, in many conditions which are usually considered to be examples of tolerances or paralysis, the capacity to respond must be actively suppressed rather than absent.

A very low, but nevertheless real, active immune response can be demonstrated during the induction and maintenance of tolerance (*57*) or paralysis (*65*) if a sensitive technique such as the PFC response can be used. In some examples of induced tolerance to alloantigens, serum antibody to the alloantigen is present in sufficient quantities to actively suppress responses of normal animals (*66*); in other cases small amounts of antibody in high concentrations at critical sites might suppress, even though no antibody is detectable in the serum. This idea is strengthened by the demonstration that cells from a donor made tolerant to some antigens when transferred to a normal recipient cause the recipient to be specifically unresponsive to the antigen (*67*). In this situation, tolerant cells must elaborate a specific material (antibody to antigen or receptor?) which "turns off" normal cells.

Because of these kinds of findings, we suggest that the many apparent differences between tolerance, paralysis, and enhancement are often only differences of degree. The important underlying problem is to understand how immune responses are regulated at different levels of activity.

Summary

The models we have discussed in detail demonstrate specific suppression of immune reactivity produced in normal adult animals by antibody and antigen. The mechanism of homeostasis of suppression in these models depends on continued exposure to antigen and on an active response by the host. The active response may include production of antibody directed against specific receptors as well as antibody directed against antigen. Thus, specific regulation of both antibody and cell mediated immunity to an antigen might be achieved by the use of only the biological agents of the response: antigen, antibody, and possibly antibody to receptors. The general implication is that these same biological agents are responsible for autoregulation of immune reactions occurring in nature. Presumably, these agents may be used to suppress or reverse immune responses for appropriate clinical objectives.

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39. We have obtained serums which had no detectable antibody against the appropriate antigen but nevertheless specifically suppressed responses to the antigen. For example, hyperimmune serums produced against SRBC or alloantigens were exhaustively absorbed with the appropriate antigen. The serums against SRBC still caused profound suppression of the antibody and delayed hypersensitivity responses of normal rats to SRBC. The serums against BN antigen still enhanced the survival of BN kidney in Lewis recipients. Also, we have obtained serums from rats injected twice weekly for a total of six injections with washed SRBC-antibody complexes produced in the presence of a great excess of antibody. Serums from the recipients of the complexes had no detectable antibody activity against SRBC and when the same rats were injected with free SRBC, their spleen cells showed a very low PFC response to the antigen. Furthermore, serum from the rats injected only with the complexes profoundly suppressed responses of normal rats to SRBC. Finally, T. McKearn,

working in our laboratory, has prepared serum from LBN rats injected with antibody from Lewis rats injected with BN antigen which had been lightly aggregated with gluteraldehyde. This serum specifically blocks a mixed lymphocyte interaction between L and LBN leukocytes, and the blocking activity is specifically reduced by absorption with L spleen cells. This serum also forms precipitin lines in gel with those serums from Lewis rats injected with BN antibody that effectively enhance kidney survival but not with those that are ineffective. Although we cannot confirm that the material causing suppression in any of these models is in fact antibody to receptors, in each case the material was obtained under conditions which we believe might be appropriate for the formation of antibody to receptors.

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Information and the Ecology of Scholars

Thomas R. Blackburn

"What is that very large body with hundreds and hundreds of legs moving across the horizon from left to right in a steady, carefully considered line?"

"That is the tenured faculty crossing to the other shore on the plane of the feasible."

"And this tentacle here of the Underwater Life Sciences Department . . ."

"That is not a tentacle but the department itself."—DONALD BARTHELME, "Brain damage" (*1*).

As the behavior of biological ecosystems becomes increasingly familiar, one is tempted to use ecological phenomena as metaphors for human behavior. The apt and often amusing insights so produced have seemed, however, to offer little basis for the serious consideration of quasi-thermodynamic systems models for society. My object is to suggest a logical basis for the use of ecological concepts in modeling a special subculture: that of scholars (and, in particular, scientists), who produce, barter, and structure information as an ecosystem produces, exchanges, and structures biomass. To go beyond mere analogy, however suggestive, it will be necessary to discuss ecosystems as open, dissipative thermodynamic systems and to point out the relationship between material (thermodynamic) and conceptual (informational) structuring.

I begin with the common observation that the orderly structures found everywhere in nature may be classified into two groups: equilibrium structures, epitomized by crystals, and dissipative structures, epitomized by living cells (*2*). Both kinds of order represent relatively low-entropy states for the matter of which they are composed, although both may persist unchanged for long times. Since only the equilibrium structure represents a local free-energy (or other appropriate potential) minimum state, only such a state may

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