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Autoregulation of Immune Responses via Idiotype Network Interactions†

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INTRODUCTION

Classically, the immune system has been perceived as a system that evolved to ward off infectious diseases. It is clear that the immune system functions quite well in this regard. The recent world-wide eradication of smallpox and the decline in the incidence of polio and numerous other diseases attest to the potential efficiency of the immune system.

Data collected during the past 5 to 6 years suggest not only that the immune system operates by recognizing epitopes on cells or molecules that are foreign to an immunocompetent host but also that cells or molecules native to the host (self-epitopes) can and do elicit immune responses in the host. (In this article, I use the word epitope, suggested by Jerne, in place of the terms hapten, haptenic group, and antigenic determinant site.) Evidence suggests that all normal immunocompetent individuals can mount autoimmune responses, but an active regulator of such responses normally is present (109).

Classically, the production of any autoantibody and the related disease states that accompany this production have been regarded as detrimental to the individual. However, recent data suggest that, along with a response to foreign epitopes, there are autoimmune responses which are specific for areas of antibody molecules or T-cell receptors that interact specifically with the foreign epitopes. Furthermore, these autoimmune responses, which limit or regulate the quantitative expression of the response to the original epitope, appear to be a necessary and beneficial part of the total immune response.

The basis for this mechanism of regulation was established by Oudin and Michel (119) and Kunkel et al. (104), who described epitopes on antibody molecules that are located in, or very close to, the antigen-binding site of the antibody molecule. These epitopes now are referred to as idiotypic markers or idiotypes.

The idiotypic markers or idiotypes constitute the general class of immunoglobulin epitopes that are the basis of the regulation mechanism. Since the original description of idiotypes, more than 200 papers have been published on idiotypic markers or on using them as a tool to study diverse molecular and cellular processes. Idiotypes have been used to study the genetics of antibody V-regions, to estimate the total repertoire of antibody specificities, to confirm formally the two genes-one polypeptide chain concept, to show the discontinuous arrangement of V and C genes in the germ line, and, recently, to study the relationship of the T-cell antigen-receptor to the antibody molecule.

The concept that idiotypes may play a major role in regulating immune responses suggests

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that the significance of idiotypes in immunobiology may exceed their use as convenient tools to study molecular and cellular processes. If immunoregulation within an individual can function via idiotype–anti-idiotype interactions, this process could have an impact equal to that of clonal selection on our understanding of the immune system. The role of this regulatory system in formulating strategies for treating numerous immunological disorders could be tremendously important.

This paper focuses on recent data which argue strongly that idiotype regulation may be a real component of most immune responses, although some data presented can be interpreted as arguing against this idea. A major goal is to convince the reader that there is sufficient evidence supporting the concept of idiotype regulation that this regulatory system should be considered in evaluating data. Moreover, experiments should be designed such that participation of idiotype regulation can be evaluated. The data summarized in this paper are based on my limited knowledge and on my biases. Many excellent data were not included, and numerous references were omitted as repetitious and to save space.

IMMUNOGLOBULIN EPITOPES

Antibodies or immunoglobulin molecules have epitopes on their surfaces or buried within them that can elicit the production of antibodies in another species (xenogeneic antisera), in the same species (alloantisera), in another member of the same inbred strain (isoantisera), or in the same individual that synthesized the antibodies (autoantisera). These epitopes are usually classified into the following three families: isotypes, allotypes, and idiotypes. Below, I briefly summarize the properties and characteristics of isotypes and allotypes and review idiotypes in somewhat greater detail.

Isotypes

Isotypes or isotypic markers are epitopes that define the different immunoglobulin heavy-chain classes and subclasses (γ, α, μ, ϵ, and δ chains) and the two light-chain types (κ and λ). These markers are normally invariant in their expression; for instance, the markers are the same on γ chains from all rabbits and the same on the γ chains of antibodies of different specificities within the same individual or between different individuals. Isotypic markers for the different immunoglobulin heavy-chain classes do not cross-react within a species (i.e., antiserum specific for rabbit γ chains does not react with rabbit α, μ, ϵ, or δ chains), but cross-reactions between related species are not uncommon (i.e., antiserum specific for sheep γ chains reacts with goat γ chains but not with goat μ or α chains). These markers are normally detected with immunological assays, such as immunodiffusion. The anti-isotypic antiserum is normally made by injecting another species with a purified preparation of antibodies of one isotype or with a purified myeloma protein (e.g., immunoglobulin M [IgM]) and then absorbing the antiserum with an immunoglobulin of another isotype or a myeloma protein (e.g., IgG) to remove anti-light-chain antibodies or any antibodies that cross-react with heavy chains, thus making the antiserum heavy chain (μ in this example) specific.

Allotypes

Allotypic markers or allotypes are epitopes that are genetic variants of molecules (immunoglobulin molecules here) found on the immunoglobulins of some but not all members of a species. The genetic transmission of immunoglobulin allotypes is Mendelian, with codominant expression in heterozygotes. Immunoglobulin allotypic variants have been found in all species in which they have been sought. All allotype epitopes, except rabbit *a*, *x*, and *y* markers, are located in constant regions of the heavy chain or the light chain. These markers are the same on antibodies from two individuals of a species that have the same allotype, but the markers differ from the allotype epitopes of antibodies or immunoglobulins from a different individual of the species which has an allelic variant at that locus. The allotype epitopes are not related to antibody specificity. Antibodies of different specificities in the same homozygous individual have identical allotype epitopes. Also, antibodies of the same or different specificities from different individuals with the same allotype do not vary in their expression of allotypic epitopes.

Common assays for allotypic markers include immunodiffusion assays, radioimmunoassays, and passive hemagglutination assays. The antiallotype antisera normally are made by cross-immunization within the same species. For example, antibody or total IgG from one individual is injected into another individual, which is matched with the donor for all alleles at all loci except the one for which antiserum is desired. Such sera need not be absorbed because the same isotypic markers are present in the donor molecules and in the recipient. Moreover, since the two are matched for all markers except one, the recipient recognizes all epitopes as self except the one that is different. Specific antiallotype antisera can be made in a xenogeneic host.

The antiallotype titers are usually very low, however, because the isotypic epitopes seem to be more immunogenic than allotypic epitopes in xenogeneic hosts. These antisera can be made by injecting the immunoglobulin of a rabbit into goats, for instance, followed by exhaustive absorption with rabbit immunoglobulin that does not contain the allotype for which antiserum is needed. These absorptions must be done very carefully, and all assays with these antisera must be accompanied by numerous specificity controls.

Idiotypes

Idiotypic markers or epitopes differ significantly in several ways from both isotypes and allotypes. Many of their individual characteristics have been described in the original papers on this subject.

Oudin and Michel (119) elicited antibodies in rabbits by injecting *Salmonella typhi*. They mixed the resulting antiserum with *S. typhi* to bind the antibodies to the intact bacteria and then injected the washed agglutinate into a recipient rabbit. Serum from the recipient reacted with donor serum containing anti-*S. typhi* antibodies but not with pre-inoculation serum from the same rabbit or with serum taken after anti-*S. typhi* antibodies had disappeared. These authors also demonstrated that the antiserum specific for the anti-*S. typhi* antibodies from one rabbit did not react with the anti-*S. typhi* antiserum taken from any of 17 other rabbits. After the decline of antibodies specific for *S. typhi*, the original rabbits were immunized with type II pneumococci. The antiserum containing the antipneumococcal antibodies was not reactive with the antiserum from recipients immunized with anti-*S. typhi* antibodies from the same rabbit.

Kunkel et al. (104) studied human anti-A antibodies from seven individuals who developed high titers after immunization with hog gastric substance A. Purified anti-A antibodies were injected into rabbits, and antisera were absorbed with normal human serum or normal gamma globulin. Antiserum specific for anti-A antibodies from donor Th reacted with the antibodies after absorption. This antiserum did not react with either pooled gamma globulin or with any of seven other purified anti-A antibodies from different individuals. The antiserum did react with whole Th serum containing anti-A antibodies, but absorption of Th serum with substance A removed the activity. Two antisera specific for antilevan antibodies were each idiotype specific, as were one antidextran, one cold agglutinin, and two anti-gamma globulin antibodies.

Kelus and Gell (90) studied the idiotypic specificities of rabbit anti-*Proteus* antibodies. They immunized rabbits with *Proteus*, and the antiserum was absorbed with whole *Proteus* cells for injection into recipient rabbits. Each donor of the anti-*Proteus* antibodies and each recipient rabbit were carefully matched for known allotypes. A total of 10 different anti-*Proteus* antisera were used to elicit anti-idiotypic antisera, and a panel of 60 anti-*Proteus* antisera were studied for idiotypic reactions. Each anti-idiotypic antiserum reacted only with the homologous anti-*Proteus* antiserum and not with preimmune serum from the antibody donor. The idiotypic determinants were localized on the Fab fragments of the anti-*Proteus* antibodies.

These studies showed that, in contrast to isotypic and allotypic epitopes, identical idiotypes appeared to be present only on antibodies of one specificity. Antibodies with different specificities either in the same individual or in different individuals did not bear the same idiotypes. Antibodies from different individuals with the same specificity bore different idiotypes. The epitope was found on antibodies in serum only after immunization. It was absent from preimmune sera and after antibodies had declined in titer.

The idiotypic epitope is located in or very near the antigen-binding site (75, 119, 155). This immunoglobulin fragment, termed Fv, is made up of a heavy-chain V-region and a light-chain V-region. Several studies (47, 62, 74, 100, 134, 153) have shown that the idiotype epitope can be located on heavy-chain V-regions or light-chain V-regions or can depend on a combination of heavy and light chains for its expression. Thus, this property is not consistent, but must be determined for each idiotypic antibody preparation. Most idiotypes require the correct combination of heavy and light chains for their expression.

It has been found that some, but not all, idiotypes are in or very close to the antigen-binding site of the molecule. Brient and Nisonoff (25) elicited anti-*p*-azobenzoate antibodies in rabbits, purified them, and injected them into allotype-matched recipients to make anti-idiotypic antiserum. These authors assayed the effects of adding increasing molar concentrations of free haptens to a reaction between labeled anti-azobenzoate antibodies and anti-idiotypic antiserum. Benzoate derivatives strongly inhibited the reaction. The combining affinities of the benzoate derivatives used correlated closely with their effectiveness as inhibitors. Brient and Nisonoff interpreted their results to indicate that the antigen-binding site of the antibody is part

of an important idiotypic determinant, which is sterically blocked by the free hapten, or that the hapten induced a conformational change that altered idiotypic determinants that were not located in the antigen-binding site. They pointed out that if such conformational changes do occur, they are quite restricted since such binding had no effect on the structure or expression of the isotypic or allotypic epitopes on the antibody molecule.

Other studies (24, 26, 28, 32–34, 114, 136, 137) have shown that some, but not all, idiotypic epitopes are intimately associated with the antigen-binding sites on the antibody molecules. The most extreme examples, where idiotypes are not associated with the antigen-binding sites of the antibody molecules, also suggest that in some cases the idiotype may even be associated with an antibody of different specificity (14, 30, 49, 53, 87, 118). The extreme examples are exceptions to the rule that idiotypic epitopes are associated only with antibody molecules of a given specificity. Oudin and Cazenave (118) and Cazenave et al. (30) immunized rabbits with hen ovalbumin. The specifically purified antibodies were then used to make anti-idiotypic antisera in other rabbits. Antiovalbumin antibodies from other immunized rabbits did not cross-react idiotypically, and anti-idiotypic antiserum did not react with preimmune serum from the rabbit immunized with ovalbumin. These authors found that absorption of antiovalbumin antibodies from the immune serum did not completely remove the capacity of that serum to react with anti-idiotypic antiserum. Four other reports have confirmed this observation. Karol et al. (87) showed cross-idiotypic specificity between goat antibody populations with different specificities for human sickle cell hemoglobin. Eichmann et al. (49) showed that one-half of the A5A idiotype-producing clones in A/J mice which responded to streptococcal carbohydrate lacked specificity for the carbohydrate. Enghofer et al. (53) showed that one-half of a group of levan-immunized CWB mice produced twice as much antibody of the U10-173 idiotype as antibody of antilevan specificity. Bona et al. (14) showed that some idiotype-positive antibodies synthesized in response to inulin could not be absorbed from serum with inulin immunoabsorbents. Taken together, these reports strongly suggest that there is no obligatory relationship between antibody specificity and idiotype. Although these two properties have been found associated in many systems, such an association cannot always be assumed. This is particularly true for induced antibodies because recent data (54, 105) have shown that idiotypic antibodies in induced responses can be extremely heterogeneous when hybridoma proteins are compared. In these studies, serological analyses with an anti-idiotypic antiserum prepared against an anti-*p*-azophenylarsonate cross-reactive-idiotype (CRI)-positive hybridoma suggested that each monoclonal antibody may possess individual antigenic specificities which are different from the determinants detected with conventional anti-idiotype antiserum.

The concept that idiotypes are epitopes on antibodies of a single specificity from a single individual was advanced before extensive studies were carried out which showed that anti-idiotypic antisera can detect similar or identical idiotypes in all individuals of an inbred strain and, more rarely, in some members of outbred animals. Consenza and Köhler (37, 38) showed that anti-idiotypic antibodies specific for the myeloma protein TEPC-15, which has antipneumococcal (anti-phosphorylcholine [anti-PC]) specificity, inhibit plaque formation by spleen cells from BALB/c mice in the primary in vitro response to the pneumococcal polysaccharide; in this system, the myeloma protein is an IgA molecule and the antibody induced by immunization is an IgM molecule, yet the two molecules bear similar or identical idiotypes. Kuettner et al. (102) studied an idiotype associated with anti-*p*-azophenylarsonate antibodies found in A/J mice. All A/J mice immunized with this epitope coupled to keyhole limpet hemocyanin respond with anti-*p*-azophenylarsonate antibodies that bear a cross-reactive idiotype, which can be detected with rabbit antiserum prepared against specifically purified antibodies isolated from individual mice. Eichmann (43) has shown strong intrastrain idiotypic cross-reactions among antibodies to group A streptococcal carbohydrate from individual A/J mice. Such examples of idiotypic cross-reactions indicate that they are valuable tools for studying the genetics of immunoglobulin molecules. These and other cross-reactive systems in inbred mice have been used extensively for such studies.

Examples of idiotypic cross-reactions in outbred animals are less numerous than those in inbred animals, but they are no less significant. Oudin and Bordenave (117) and Bordenave (17, 18) studied cross-reactions of anti-idiotypic antisera prepared by injection of rabbit antibodies specific for *Salmonella abortus-equi*. Bordenave (18) showed that the frequency of these cross-reactions was about 3% of the 5,036 reactions studied. Braun and Krause (22) made an anti-idiotypic antiserum in a goat, which was rendered specific for purified anti-group C streptococcal antibodies from one rabbit. The goat antiserum reacted best with antistreptococcal antibodies from the homologous donor and gave a

line of partial coalescence by agar gel immunodiffusion with antistreptococcal antibodies from one other rabbit. Eichmann and Kindt (51) showed strong idiotypic cross-reactions in a partially inbred family of rabbits. Brandt and Jaton (20, 21) detected identical idiotypes in antipneumococcal antisera from two rabbits and showed that the V_L sequences were identical in these two antisera. Recently, Roland and Cazenave (130) studied cross-reactions of anti-idiotypic antisera specific for purified rabbit antiallotype antibodies. Cross-reactions here were extensive and frequent, even in this outbred system.

There is a potential pitfall that cannot be avoided easily, particularly in outbred animal studies when an anti-idiotype reagent antiserum is made. It concerns allotype matching of donor and recipient. The basic problem is that a donor and a recipient are matched only for known allotypes. However, an antibody with an unrecognized allotype marker could be elicited in very large quantities. If the donor and recipient were inadvertently mismatched at that locus, the antiserum would react with the antibodies from the donor but would not recognize that marker on antibodies of the same or different specificity from other animals because the few clones representing that allotype might not be stimulated in other animals with that allotype. The amount of immunoglobulin in normal or even immune serum bearing the allotype in other animals or in pre-inoculation serum from the same animal might be so small that inhibition assays could not detect the molecules. This is not a problem if the system being studied is inhibitable with free epitope. However, it seems to be a potential problem in idiotype systems where epitope inhibition cannot be done or in systems that are not epitope inhibitable. This problem can be neatly eliminated by making the anti-idiotype antiserum in the same individual that synthesized the idiotypic antibody.

For further reading on idiotypes and the utilization of idiotypic markers, I highly recommend the book by Nisonoff et al. (115) and the reviews by Eichmann (48), Consenza et al. (36), Rajewsky and Eichmann (124), Binz and Wigzell (7), Ramseier et al. (126), Nisonoff et al. (116), and Mäkelä and Karjalainen (108).

NETWORK OF IDIOTYPES

Network Models

The most widely known form of the idiotype (or lymphocyte) network concept is that conceived by Niels Jerne. He formulated the idea (80–85) after considering three outstanding features of the immune system. The first feature was that the total repertoire of available antigen-binding sites in a normal animal is extremely large. (Kunkel [103] coated erythrocytes with a myeloma protein and attempted to inhibit the agglutination of such cells by anti-idiotype, using normal human sera or pooled preparations of human gamma globulins. His data suggested that the total number of antigen-binding sites exceeds 1×10^7 to 3×10^7.) The second point was the property of dualism displayed by the immune system. A dual set of effector lymphocytes (T- and B-lymphocytes) make up the system, and under appropriate circumstances these cells can function either synergistically or antagonistically. Antibody molecules can recognize foreign epitopes and be recognized by other lymphocytes because of the idiotypic epitopes on those antibodies. The third point which Jerne emphasized is the crucial role of suppression in the immune system. Numerous studies have shown that either antiallotypic or anti-idiotypic antibodies can suppress the synthesis of antibodies by the appropriate clones of B-cells that bear the allotype or idiotype. Jacobson et al. (77) have shown that T-cells mediate allotype suppression by recognizing the allotype of the B-cell receptor. The suppressive effect is exerted on the helper T-cell (69). Jerne suggested the terms epitope (to denote antigenic determinants or haptenic sites on antigen molecules), paratope (for the contact area of the antigen-binding site of the antibody molecule), and idiotope (for the antigenic determinant on an antibody molecule that bears the determinant recognized by anti-idiotypic antibodies or anti-idiotypic T-cells).

In a quantitative sense, Jerne argues that antibody molecules normally are in the blood at a concentration of about 5×10^{16} molecules per ml, meaning that the concentration of idiotopes (and paratopes) is 10^{17} sites per ml (two idiotopes per molecule of IgG). Assuming that each animal expresses 10^7 idiotopes (103), then each idiotope would be in serum at a concentration of 10^{10} sites per ml. Jerne points out that studies of low-zone tolerance (1, 112, 135) have demonstrated that epitope concentrations of only 10^6 to 10^{12} sites per ml suppress or paralyze lymphocytes that have specific receptors for that epitope. He also suggests that lymphocytes are subject to continual suppressive effects by other lymphocytes or antibodies that have idiotopes or paratopes that fit onto either the paratope or idiotope on the cell to be suppressed. Foreign epitopes on environmental or mutant self-antigens are also recognized with different degrees of precision by paratopes in the immune system, and their concentrations increase. These molecules can also recognize and react with a set of idiotypes that constitute the "internal image" of the foreign epitope within the immune system. Including

this internal image element in the network suggests that no epitope is really foreign but that all epitopes that induce immune responsiveness may be represented in the system before antigen stimulation. The lymphocytes that bear the internal image idiotopes would then be affected secondarily, and they could affect other lymphocytes in successive recognition waves throughout the network, as illustrated in Fig. 1.

The net effect of such idiotype modulation would be to suppress the initial antibody response. Once stimulated by epitope, cells carrying immunoglobulin receptors bearing paratope 1 and idiotype 1 (the P_1 - i_1 set) are stimulated to divide and become mature antibody-secreting plasma cells. The resulting increase in antibody titer or concentration is defined classically as a primary antibody response. The increase in the concentration of the P_1-i_1 molecule then stimulates cells with receptors bearing paratope 3 and idiotype 3 to divide and become mature antibody-secreting plasma cells. These antibodies can then react with cells bearing P_1-i_1 receptors to suppress synthesis of the antibody. Each cell receptor is presumed to be capable of recognizing an internal image idiotype, as well as a foreign epitope, and bears an idiotype that is recognized by an anti-idiotypic paratope.

The model of Jerne relies on the assumption that the idiotope and the paratope constitute two separate entities, with the idiotope of one molecule fitting the paratope of another molecule and the idiotope of the second molecule fitting the paratope of a third molecule, etc. Thus, a functional, self-regulating network of idiotopes and paratopes was conceived.

The network model of Hoffmann (71) makes no distinction between idiotypic and paratypic patterns of immunoglobulin V-domains. This author assumes that an epitope on a foreign antigen is recognized by a heterogeneous population of receptor V-domains and that if the three-dimensional shapes of all of the V-domains could somehow be averaged, the resulting shape would be complementary to the shape of the original epitope. He refers to the set of lymphocytes representing such a population of antigen-binding V-domains as the positive set. He postulates that there is a set of cells in the immune system that represents an equally heterogeneous

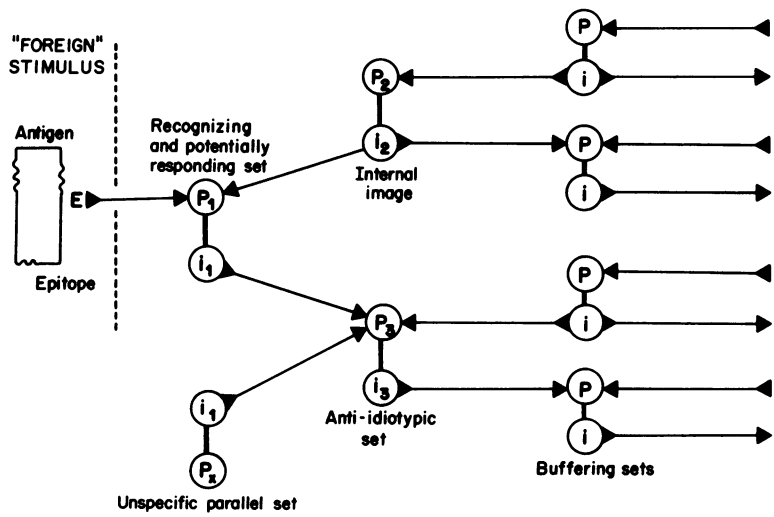

FIG. 1. *Eigen behavior of the immune system. The immune system contains a set (P_1) of combining sites (paratopes) on immunoglobulin molecules and on cell receptors, which recognize a given epitope (E) of an antigen. This recognizing set includes the potential responding lymphocytes. The molecules of set P_1 also display a set (i_1) of idiotopes. Apart from recognizing the foreign epitope, the set P_1 likewise recognizes set i_2 of idiotopes, which thus constitutes within the immune system a kind of internal image of the foreign epitope. This set i_2 occurs in molecular association with set P_2 of paratopes. Likewise, set i_1 is recognized within the immune system by set P_3 of paratopes, which represent anti-idiotypic antibodies. Beside the recognizing set P_1i_1, there is a parallel set P_xi_1 of immunoglobulins and cell receptors, which display idiotopes of set i_1 in molecular association with combining sites that do not fit the foreign epitope. As a first approximation, the arrows indicate a stimulatory effect when idiotopes are recognized by paratopes on cell receptors and a suppressive effect when paratopes recognize idiotopes on cell receptors. Successive groups of ever-larger sets encompass the entire network of the immune system. (From reference 83 and used with the permission of N. K. Jerne and Masson Publishing, Paris.)*

population of V-domains that are anti-idiotypic with respect to the V-domains of the positive set. These he refers to as the negative set. If the shapes of the negative set V-domains were averaged, a shape resembling the original epitope should be obtained. T-cells are assumed to proliferate more rapidly and to be triggered more readily than B-cells. Both T- and B-cells are assumed to be effective immunogens because of the large number of identical V-domains on each cell. T-cells are assumed to produce and secrete monovalent V-domains. These molecules, which are produced by a positive set of T-cells, can block the receptors of the corresponding negative set of both T- and B-cells and inhibit the mutual stimulation of positive and negative sets of cells. Because of the monovalency of the T-cell product, it cannot cross-link with either the monovalent T-cell cell-bound receptors or the di- or multivalent receptors of B-cells to cause patching, capping, and internalization of the complexes. B-cells produce and secrete di- or multivalent antibody molecules (immunoglobulin class differences), which can suppress or kill both T- and B-cells of the complementary set due to effective patching (with a loss of receptors) or to complement-mediated killing.

The model of Hoffmann suggests different types of stable states that can be reached in immune processes. In the virgin (unstimulated) state, the concentrations of both positive and negative sets are so low that mutual stimulation of the complementary sets of cells is not significant. In the immune (immunized) state, the positive B-cell population dominates, thereby suppressing the negative sets of both B- and T-cells. A second dose of antigen would transiently trigger the unimpeded positive sets, but the epitope on the antigen would soon be masked, preventing unlimited stimulation. In the tolerant state, enough positive and negative T-cells are present to stimulate one another to produce their blocking factors continuously and thereby effectively prevent stimulation of the corresponding B-cell populations. Hoffmann suggests that tolerance to self-epitopes is established when there is a balance between the concentration of the positive set of T-cells and the sum of the concentration of the complementary negative set plus the self-epitope concentration. Hoffmann (72) recently incorporated the nonspecific T-cell-dependent factor into his general model.

Richter suggested a network model (127) that regards the immune system as a functional network of V-domains, coupling various sets of lymphocytes and antibody molecules by stimulatory, suppressive, and inhibitory interactions. His basic premise is that an antigen elicits the production of a set of antibody molecules (Ab-1), which, because of the idiotopes exhibited, can act as immunogens to elicit the production of anti-idiotypic antibody (Ab-2). Ab-2 molecules are immunogenic to a third set of lymphocytes, which are stimulated to secrete Ab-3. This cascade is open ended. Negative feedback is assumed because the lymphocytes that are stimulated are aggressive toward the lymphocytes that produce the stimulating antibodies. Therefore, Ab-2 can suppress the lymphocytes producing Ab-1, and Ab-3 can suppress the lymphocytes producing Ab-2, etc. Network interactions here are assumed to be inactive, as the amounts of the different components remain below threshold until exogenous antigen appears and upsets the balance. Three types of dynamic immune responses (low-zone tolerance, normal immune responsiveness, and high-zone tolerance) and immunological memory are predicted by this model. Low-zone tolerance can result if antigen stimulates the production of Ab-1, but Ab-2 does not reach a concentration sufficient to trigger the synthesis of Ab-3. A normal immune response would occur if the Ab-2 concentration exceeded the Ab-3 threshold because Ab-3 would then suppress the Ab-2-producing cells, permitting Ab-1 cells to continue unimpeded proliferation. High-zone tolerance may be a form of low-zone tolerance at the level of Ab-3 or a normal response at the level at which Ab-2 suppresses Ab-1.

Adam and Weiler (2) have suggested a network model that uses interactions among V-domains of antibodies to explain the generation of antibody diversity and the existence of a large set of different lymphocyte sets early in ontogeny. These authors assume that one consequence of a high rate of lymphocyte proliferation is the emergence of mutant lymphocytes that synthesize altered immunoglobulin V-domains. These mutated sets of lymphocytes can inhibit or eliminate previously established (nonmutant) sets of cells and can themselves be inhibited or eliminated by the nonmutant sets by suppressive effects of paratope-idiotope interactions. These interactions lead to a steady-state equilibrium, where the rate of appearance of mutant sets is equal to the rate of appearance or depletion of sets of lymphocytes. This model does not deal with the effects of adding exogenous epitopes to the system.

Herzenberg et al. (68) have suggested a model system in which the cells and cell products are organized into several integrated circuits, which ultimately function to regulate all aspects of antibody production. This system utilizes the idiotype–anti-idiotype recognition system and

goes a step further by providing for the operation of other systems which allow specific interactions between individual circuit elements, such as B-cells, antibodies, suppressor and helper T-cells, macrophages, and soluble regulatory products.

Idiotype-Anti-Idiotype Complementarity

A crucial aspect of the network concept where Jerne and Hoffmann disagree regards the separation of paratope from idiotope. It is critical in the network concept of Jerne that the two sites are separate and distinguishable, as the paratope of one molecule interacts with the idiotope of a second molecule, whose paratope reacts with the idiotope of a third molecule, etc. Therefore, a network is established that theoretically links molecules by the interaction of one area of a molecule with a separate area on another molecule.

Hoffmann uses only the designations epitope and paratope (omitting idiotope), and he considers the paratope of the molecule to be the most important epitope. In its simplest form, the model of Hoffmann suggests that one paratope elicits a group of anti-idiotypic antibodies, whose paratopes each are to some degree complementary to the paratope of the first antibody. A network would exist if one or more of the anti-idiotypic antibodies could also react with other similar epitopes on another set of antibodies.

A general theoretical problem that occurs when it is proposed that the paratope is also the epitope (or part of it) is that the paratope has been viewed for so long as being a cavity in the end of the Fab arms of antibodies. Recent X-ray crystallographs have verified this concept for five or six molecules, so it is difficult to accept the idea of complementarity of two objects, each of which forms a cavity.

The idea of complementarity of the idiotypic and anti-idiotypic molecules now is more palatable, as paratopes are known to have shapes other than cavities. Cisar et al. (31) described two binding sites specific for dextran; one of these is specific for the terminal end of the dextran molecule and has a cavity structure, and the other is specific for the extended internal sugars in the dextran molecule and is in the shape of a groove instead of a cavity. Schalch et al. (131) described the same two kinds of molecules in induced antibodies specific for group A variant streptococcal carbohydrate. Thus, a paratope or a part of a paratope could exist as a slight protrusion and thus may be complementary to a cavity (or a part of a cavity) on another molecule.

The assumption that the paratope of an antibody is a negative image of the epitope on the antigen and that some part of the anti-idiotype antibody paratope is complementary makes it reasonable to argue that the paratope or some part of the paratope on the anti-idiotypic antibody should be a good image of the original epitope. The internal image idiotope (Fig. 1) in the model of Jerne also fulfills this criterion. The internal image or the paratope of the anti-idiotype should elicit antibodies that react with the original epitope. Using the terminology of Richter, Ab-3 should be reactive with the original epitope that elicited Ab-1.

The problem of complementarity in idiotype-anti-idiotype structures has been studied in several laboratories. Cazenave (29) immunized a rabbit with ribonuclease (RNase). The anti-RNase antibodies (Ab-1) were purified and used to prepare anti-idiotypic antibodies (Ab-2) in two recipient rabbits matched for allotypes of groups a and b. The Ab-2 of one recipient was used to prepare an anti-(anti-idiotype) in two other recipients (Ab-3). After a 4-month rest period, the rabbits that had made Ab-3 were immunized with RNase. The resulting anti-RNase antibodies (Ab-1′) were cross-reactive with both Ab-2 sera that had been elicited with Ab-1. This showed a similarity of idiotypes on Ab-1 and Ab-1′. Control experiments showed no such cross-reaction between Ab-2 and any of 12 unrelated anti-RNase sera. The data showed that immunizing with Ab-2 idiotypes before RNase immunization primed these rabbits to favor the expression of Ab-1 idiotypes on the anti-RNase (Ab-1′) antibodies.

Urbain et al. (152) immunized a rabbit with *Micrococcus lysodeikticus* and injected the purified anticarbohydrate antibodies (Ab-1) into recipients matched for allotypes a1, a2, a3, b4, b5, b6, c7, c21, e14, d11, and d12 to induce anti-idiotypic antibodies (Ab-2). Specifically purified Ab-2 was injected into allotype-matched recipients to make antiserum specific for the idiotypic epitopes of Ab-2. These anti-(anti-idiotypic) antisera were termed Ab-3. The Ab-3 preparation was not reactive with the micrococcal carbohydrate antigen. The rabbits that produced Ab-3 were then immunized with *M. lysodeikticus* vaccine. This anticarbohydrate antibody (Ab-1′) bore idiotypes similar to those of Ab-1.

In additional studies Wikler et al. (157) used both *M. lysodeikticus* and tobacco mosaic virus (TMV) as antigens to elicit Ab-1 molecules specific for each. Anti-idiotypic antibodies (Ab-2) were made for each of the Ab-1 preparations. The Ab-2 preparations were purified and injected into rabbits matched for allotypes a1, a2, a3, b4, b5, b6, c21, e14, d11, and d12 recipients

to raise anti-idiotypic antibodies (termed Ab-3) specific for idiotypes of Ab-2 molecules. Antigen (either *M. lysodeikticus* or TMV) was then injected into the Ab-3 rabbits to elicit Ab-1'. The Ab-3 antibodies were purified and used in recipient rabbits to elicit Ab-4. Thus, Ab-4 was specific for the idiotypes of Ab-3. As in the previous study (152), Wikler et al. found that Ab-1 and Ab-1' shared idiotypic specificities. Their Ab-3 antiserum did not react with antigen (*M. lysodeikticus* or TMV), but it did share idiotypes with Ab-1 and Ab-1'. The Ab-4 antibodies (specific for idiotypes of Ab-3) reacted specifically with Ab-1 and Ab-1'. The results suggested that Ab-1 and Ab-3 were idiotypically similar and that Ab-2 and Ab-4 bore the same idiotype or similar idiotypes.

The studies of complementarity of idiotype–anti-idiotype structures in outbred rabbits by Cazenave (29), Urbain et al. (152), and Wikler et al. (157) were consistent, finding that Ab-3 antisera did not react with antigen. This is explained by the heterogeneity of the antibodies at each step in the immunization "ladder." A more serious criticism of these studies concerns the lack of characterization of the Ab-1 used to elicit Ab-2, the Ab-2 used to elicit Ab-3, and the Ab-3 used to elicit Ab-4. In no instance was the immunogen characterized completely for isotype or allotype content. Indeed, Cazenave (29) typed the whole sera of donor and recipient rabbits only for *a* and *b* locus allotypes and not for *c*, *de*, *f*, *g*, and *n* locus allotypes. Urbain et al. (152) did not type for e15 or for *f*, *g*, and *n* locus allotypes, and Wikler et al. (157) did not type for b9, c7, or e15 or for *f*, *g*, and *n* locus markers. The presence of such markers on antibodies used as immunogens would elicit antiallotype antibodies in a mismatched recipient that could, without extensive controls (which were not included), be mininterpreted as being anti-idiotype antibodies. This problem of allotype matching does not occur in inbred systems in which syngeneic recipients are used.

Bona et al. (12) used the MOPC-460 system to study idiotype–anti-idiotype complementarity in an inbred mouse system. MOPC-460 is an IgA, κ-myeloma protein from BALB/c mice that binds trinitrophenyl (TNP) and dinitrophenyl ligands. MOPC-460 myeloma protein was used to elicit anti-MOPC-460 anti-idiotype antibodies. The anti-MOPC-460 idiotype antibodies were conjugated to keyhole limpet hemocyanin and injected into BALB/c recipients. The resulting antiserum (Ab-3) agglutinated sheep erythrocytes coated with Ab-2 (anti-MOPC-460 idiotype). Ab-3 antiserum was absorbed with TNP-keyhole limpet hemocyanin, and the reactivity for Ab-2 was not diminished. The results indicated that Ab-3 lacked anti-TNP activity. Bona et al. were not able to exclude the possibility that the Ab-3 molecules were MOPC-460 idiotype-bearing molecules that lacked anti-TNP activity.

Frischknecht et al. (58) used an alloantigen system in both rats and mice to show close complementarity between idiotype and anti-idiotype. In this system, a histocompatibility antigen was the target antigen, and anti-idiotypic antisera were made by injecting parental T-cells into F_1 animals. In vitro the anti-idiotypic antibodies induced selective proliferation and development of antigen-specific cytolytic T-cells. These T-cells, therefore, reacted specifically with cells bearing the appropriate histocompatibility antigen, which resulted from treatment with an anti-idiotypic antibody. In addition, the purified anti-idiotypic antibodies elicited high titers of specific alloantibodies when injected into syngeneic recipients in adjuvant, which showed a high degree of complementarity between idiotype and anti-idiotype.

Studies of the suppression of idiotype-positive T-effector cells in a delayed hypersensitivity reaction to the phenylarsonate epitope in A/J mice have shown (42, 144) that the relevant suppressor T-cells can be induced with anti-idiotype. Thus, in cell-mediated systems in addition to antibody systems, anti-idiotypic antibodies can functionally duplicate the structures of epitopes. This extends the concept of complementarity to the T-cell level, in addition to the B-cell or antibody level.

An elegant study by Ramseier (125) suggests extremely close complementarity between idiotype and anti-idiotype through several cycles. His experimental design used an alloantigen system as the target antigen. T-cell receptors specific for the alloantigen difference between strain A and strain CBA mice were used as idiotypes. The receptors were collected from culture supernatants of spleen cells that had been enriched for T-cells. These idiotype-bearing T-cell receptors were used to elicit anti-idiotypic antisera either by intraperitoneal injection of soluble T-cell receptors into F_1 adults or by intradermal injection of posttransplantation alloantiserum into F_1 adults. The receptors that were shed spontaneously by strain A T-cells were used to immunize A × CBA F_1 recipients. The resulting anti-idiotypic antiserum was then injected into strain A mice, where it induced serum activity indistinguishable from the activity of a conventionally raised alloantiserum. This serum displayed strain A anti-CBA activity at a titer similar to that of a posttransplantation antise-

rum with the same specificity. This serum was then injected into A × CBA F_1 mice. The serum induced production of F_1 anti-(A anti-CBA) serum which was indistinguishable from an F_1 anti-strain A T-cell receptor site specific for the CBA alloantigen. Similar data were obtained with the opposite strain combination (strain A responding to strain CBA alloantigens). The same results with both strain combinations (strain CBA responding to strain A alloantigens and strain A responding to strain CBA alloantigens) were obtained by starting antibody cycles with alloantigens instead of soluble T-cell receptors. In this study, Ramseier produced a complete antibody cycle by using soluble T-cell receptors to raise anti-idiotype, and he initiated four complete cycles by using an allograft to induce the first idiotypic antibody response. These two experiments are diagrammed in Fig. 2. They clearly showed a close complementarity in the structures of idiotype and anti-idiotype. The example of Ramseier is the most complete example of idiotype eliciting anti-idiotype and of the injection of anti-idiotype eliciting an anti-

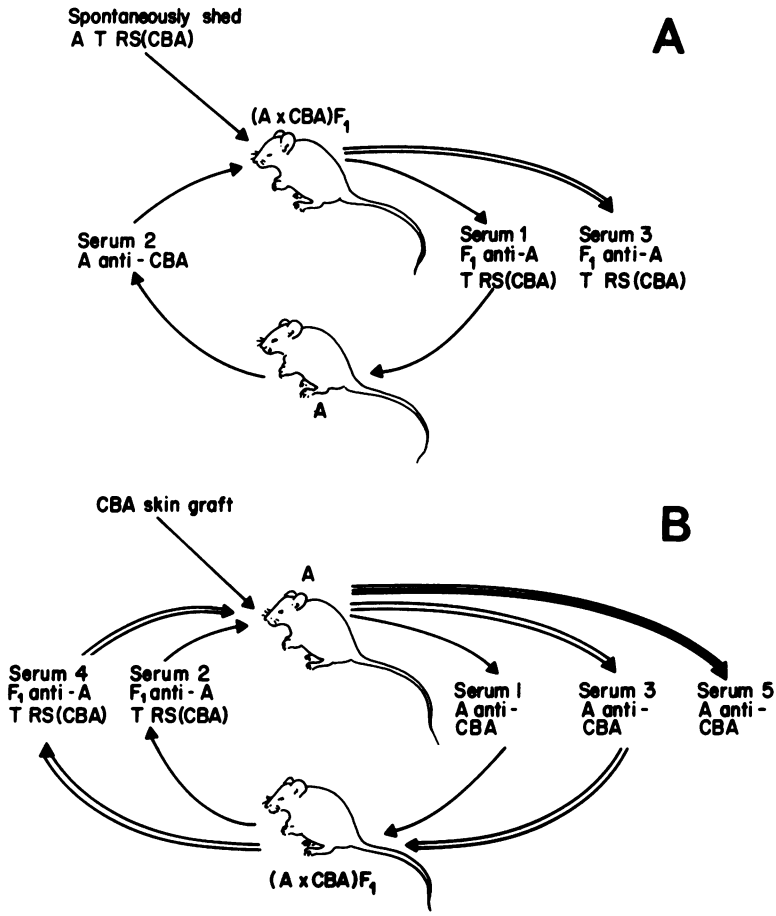

FIG. 2. *(A) Antibody cycle initiated with cell receptors (RS) shed from strain A T-cells. T-cell receptors shed from strain A lymphocytes with CBA alloantigen specificity were injected into an A × CBA F_1 hybrid. The resulting anti-idiotypic antiserum (serum 1) was injected into a strain A mouse, in which it elicited production of a serum activity (serum 2) indistinguishable from the activity of a conventional strain A anti-CBA antiserum. Serum 2 was then injected into A × CBA F_1 mice and elicited the production of F_1 anti-(A anti-CBA) serum, which had the same specificity as serum 1. (B) Two antibody cycles initiated with CBA skin allografts. A strain A mouse grafted with CBA skin produced serum 1 containing antibodies specific for CBA alloantigens. Injecting this serum into an A × CBA F_1 hybrid elicited an antiserum (serum 2) specific for the T-cell receptors of strain A that specifically bound CBA alloantigens. Injecting serum 2 into a strain A mouse elicited an antiserum specific for CBA alloantigens. Thus, this cycle was repeated for inducing serum 4 and serum 5. Serum 3 and serum 5 each reacted with CBA alloantigens. (From reference 125 and used with the permission of H. Ramseier and S. Karger AG, Basel.)*

(anti-idiotype) that appears to be indistinguishable from the first idiotype. Comparing the rabbit data (32, 152, 157) with the inbred mouse and rat data (12, 58, 125) may not be entirely valid because raising the different reagents in rabbits requires that a different outbred individual be injected at each step, which increases the heterogeneity of the reagents.

Trenkner and Riblet (151) studied the S-107 mouse myeloma idiotype that binds the PC epitope of pneumococci. Their in vitro studies showed that anti-idiotypic antibodies induced the formation of anti-PC antibodies with the S-107 idiotype. They showed that helper T-cells were necessary for this response. In their studies, a rabbit anti-idiotype antiserum was fully effective in stimulation, suggesting that complementarity in idiotype–anti-idiotype reactions can extend across species lines.

Studies by Eichmann and Rajewsky (52) and by Black et al. (8) showed close complementarity of idiotype and anti-idiotype in an inbred mouse system. Antibodies from A/J mice specific for group A streptococcal carbohydrate were used to elicit anti-idiotypic antibodies in guinea pigs. The guinea pig anti-idiotypic antibodies were separated into IgG1 and IgG2 populations. The IgG1 fraction stimulated anti-group A antibody production, but the IgG2 fraction suppressed synthesis. In addition, B- and T-cells with the same antigen-binding specificity shared idiotypic determinants, had the same idiotypic polymorphism, and displayed similar degrees of heterogeneity in binding antigen and anti-idiotype. Once again, it was shown that complementarity of idiotype–anti-idiotype interactions can extend across species lines.

T-Cell Idiotypes

Thymus-derived lymphocytes, or T-cells, are distinguished operationally from antibody-producing bone marrow-derived lymphocytes, or B-cells, by the lack of detectable immunoglobulin molecules on the T-cell membrane. T-cells clearly have a specific receptor on the membrane surface that allows them to recognize and respond appropriately to an antigenic stimulus. Several reports have shown that several of the known functional types of T-cells have idiotypic structures on their receptors that can be recognized by anti-idiotypic antibodies, which clearly brings the whole T-cell system into the network regulation concept.

It is clear that T-cell receptors share some structural properties with immunoglobulin binding sites. For example, Krawinkel et al. (101) showed that isolated T-cell receptors bind epitope with similar affinity and fine specificity. Their data suggested that the T-cell receptors carry the entire immunoglobulin heavy-chain V-region, which suggests that T-cells may bear idiotypic determinants on their receptors and could be subject to idiotype network regulation.

Binz and Wigzell (6, 7) demonstrated that T-cell receptors and IgG antibody molecules specific for the same alloantigen have similar or identical idiotypes. Cosenza et al. (36) showed that helper T-cells bear the same idiotype as do the responding B-cells in the response of BALB/c mice to PC. Rajewsky and Eichmann (124), Janeway et al. (79), and Bottomly et al. (19) have also shown idiotype markers on helper T-cells in other systems.

The T-cell compartment contains at least two sets of T-cells, suppressor T-cells and helper T-cells. Suppressor T-cells directly suppress the action of other lymphocytes (i.e., antibody synthesis by B-cells or delayed hypersensitivity reactions of effector T-cells). Lewis and Goodman (106) were the first to report that suppressor T-cells share idiotypes with antibodies of the same specificity. They used the A/J mouse system of an idiotype associated with antiarsonate antibodies. T-cells incubated first with anti-idiotype were not able to bind antigens. In a second assay, T-cells were reacted with anti-idiotype, washed, and incubated with fluorescent protein A. These assays showed clearly that suppressor T-cells can express idiotype. Studies by Weinberger et al. (154) also showed that suppressor T-cells that suppress antibody synthesis bear idiotype on their receptors. Anti-idiotypic antiserum plus complement effectively eliminated the ability of these cells to suppress idiotype synthesis by B-cells. One possible mechanism for the suppression of B-cells is that the suppressor cells interact with the idiotype-bearing target B-cells via an antigen bridge or an antigen-containing factor bridge. Another possibility is that the idiotype-bearing suppressor T-cells induce another cell population (anti-idiotypic suppressor T-cells) with specificity for the B-cell idiotype. Kim (91) demonstrated in another system the generation of idiotype-specific suppressor T-cells by culturing spleen cells with anti-idiotypic antibodies and antigen.

In two separate systems, Bona and Paul (15) and Owen et al. (121) demonstrated direct effects of suppressor T-cells specific for idiotype. The suppressor T-cells acted directly on idiotypic B-cells, suggesting that suppressor T-cells can have anti-idiotypic specificity in addition to idiotype specificity, as shown by Lewis and Goodman (106) and Weinberger et al. (154).

These data show that idiotypic determinants can be present on helper T-cells and on suppres-

sor T-cells. Furthermore, suppressor T-cells with functional anti-idiotypic specificities have been described. It seems that, in addition to the idiotypes and anti-idiotypes expressed by B-cells, the T-cell compartment must be considered a functional part of any regulatory network concept.

Idiotypes on B-Cell Mitogen Receptors

It has been suggested that a B-cell uses two distinct receptors to respond to antigen (40, 41). The immunoglobulin receptor recognizes epitopes, and the mitogen receptor controls triggering and growth and recognizes mitogenic structures on thymus-independent antigens or helper T-cell factors in response to thymus-dependent antigens. Using the thymus-independent antigen dextran B1355, Coutinho et al. (39) showed that antidextran antibodies in mice bear the J558 myeloma idiotype, a myeloma protein that binds dextran. Anti-idiotypic antibodies specific for J558 idiotype were mitogenic both for 10 to 20% of the spleen cells of a high-responder strain of mice, which had the J558 idiotype, and for a low-responder strain, which did not have the J558 idiotype. These results suggest that idiotype or idiotype-like structures are present on a subset of B-cells in the form of mitogen receptors. This could be explained if it is assumed that mitogen receptors are encoded by primordial germ line genes from which some immunoglobulin genes are derived. This explanation could be verified by deoxyribonucleic acid sequencing the J558 V-gene and the mitogen gene. It is not yet clear whether the idiotype-like structures on mitogen receptors can mediate suppression or stimulation of B-cells by the appropriate anti-idiotype antibodies.

Antibody Production in the Absence of Antigen Stimulation

A fundamental aspect of the network concept is that a steady state of mutual suppression exists between the idiotype and the anti-idiotype until an antigen arrives to upset the balance. This suggests that B-cells can be triggered to synthesize low levels of antibody in the absence of antigen. Until recently, this idea had no experimental support. Studies by Hetzelberger and Eichmann (70), Eichmann et al. (50), and Bona (10) lend significant support to the idea. These studies present evidence for a class of T-helper cells that are idiotype specific and not antigen specific. These T-helper cells were shown to activate B-cells in vitro in the absence of antigen. It is possible that these results merely reflected an in vitro artifact. However, if this stimulation mechanism proves functional in vivo, it may play an important role in the production of low concentrations of idiotypes, which assures the maintenance of a steady state in the absence of antigenic challenge. Mitogenic molecules on microorganisms (lipopolysaccharides) could also stimulate production of low levels of antibodies with diverse specificities and idiotypes in the absence of antigen.

Forni et al. (57) have shown that an injection of IgM antibodies induced by immunization of IgM hybridoma antibodies elicits the production of antibodies of the same specificity as the specificity of the injected IgM. Responses were induced with IgM antibodies specific for both thymus-dependent and thymus-independent antigens, but the response was not observed in athymic (nude) mice. These results suggest that this may represent the mechanism functioning after an initial primary response that generates waves of idiotypic antibodies, followed by anti-idiotypic antibodies.

ANTI-IDIOTYPE REGULATION

Anti-Idiotypic Antisera

The results obtained from idiotype suppression experiments depend critically on the specificity of the anti-idiotype reagent used, particularly in studies in which idiotype suppression is achieved by using an antiserum made in another animal. Some general guidelines for making anti-idiotypic antisera have been suggested, based on studies comparing specificities of antisera made in different ways.

Helman et al. (67) compared an anti-idiotypic antiserum made in an syngeneic animal with one made in a heterologous species. The idiotypes were the myeloma proteins MOPC-315 and XRPC-25, which are dinitrophenyl-binding plasmacytomas of BALB/c mice. Anti-idiotype antiserum was elicited in syngeneic BALB/c recipients by injecting intact protein, but Fv fragments of the proteins were not immunogenic in BALB/c mice. Fv fragments were immunogenic in a heterologous species (rabbit). Both the syngeneic and heterologous antisera were fractionated into anti-binding site and anti-nonbinding site specificities by absorption to an idiotype-Sepharose column and eluting the binding site-specific anti-idiotypic antibodies with dinitrophenyl-lysine, followed by eluting the anti-nonbinding site-specific anti-idiotypic antibodies with acetic acid. These authors found that the heterologous anti-idiotypic antibodies were more than 90% anti-nonbinding site specific, whereas the syngeneic anti-idiotypic antibodies were 60 to 70% anti-binding site specific. Claflin and Davie (34) isolated a heterologous anti-idi-

otypic antibody preparation by hapten elution from a Sepharose-idiotype conjugate. They found that an allogeneic anti-idiotype antiserum was only partially hapten inhibitable, but they gave no information concerning the relative amounts of anti-binding site- and anti-nonbinding-site-specific antibodies from the heterologous antiserum.

Köhler et al. (97) compared homologous and isologous anti-idiotypic antibodies specific for the BALB/c PC-binding myeloma protein HOPC-8. The homologous anti-idiotypic antiserum was made in A/He mice, and the isologous anti-idiotypic antiserum was made in BALB/c mice. Both kinds of antibodies were predominantly of the IgG1 class, and they had similar affinities for the PC-binding site of HOPC-8. Significant differences in specificities were not detected when homologous and isologous anti-idiotypic antisera made in the same species specific for a homogeneous idiotype were compared.

I (129) elicited an antibody response to the epitope *p*-aminophenyl-*N*-trimethylammonium chloride (TMA) coupled to keyhole limpet hemocyanin in rabbits. Isologous anti-idiotype was elicited by injecting anti-TMA antibodies into recipient rabbits matched for *a*, *b*, and *c* locus products. Autologous anti-idiotype was also made for each anti-TMA preparation by injecting anti-TMA back into the same rabbit that originally synthesized the antibody. When I compared the two anti-idiotypic antisera by radioimmunoassays, in all instances the isologous anti-idiotype antiserum recognized a larger percentage of the heterogeneous anti-TMA preparation than did the autologous anti-idiotypic antiserum. However, in each case the smaller percentage of molecules recognized by the autologous antiserum was contained within the larger percentage recognized by the isologous antiserum. This is equivalent to what was found when different isologous anti-idiotypic antisera specific for the same antibody preparation (73) were compared. The isologous and autologous antisera did not differ significantly in the apparent affinities of the two kinds of antibodies for the idiotypes, based on epitope inhibition assays. The isologous and autologous antisera each recognized the same set of idiotypes. However, isologous antisera recognized an additional set of idiotypes that was not recognized by autologous antisera. This difference may have been due to the disappearance of some, but not all, clonotypes in the autologous recipient at the time that the idiotype was injected. The autologous recipient may have been tolerant to some clonal products because of their persistence, whereas the isologous recipient may not have been tolerant to any of the idiotypes.

It is clear from these and other experiments that the specificity of the anti-idiotype reagent can vary both qualitatively and quantitatively, depending on the choice of recipient. One generality that can be drawn from these studies is that the only way to insure that a site-specific anti-idiotype preparation is being used is to use hapten-eluted anti-idiotypic preparations from immobilized idiotype. Eichmann (48) gives a more detailed treatment of anti-idiotype reagents.

Artificially Induced Anti-Idiotype Regulation

Numerous investigators have shown anti-idiotypic antibodies to be potent agents for suppression of synthesis of antibody with the idiotype. Anti-idiotype suppression has been used extensively in inbred mouse strains in which the same sets of idiotypes are expressed on antibody molecules synthesized in response to a particular epitope.

Kuettner et al. (102) first described the idiotypic cross-reactions of antibodies from individual A/J mice specific for the phenylarsonate epitope when the epitope was coupled to keyhole limpet hemocyanin. Between 20 and 70% of the antibodies made by an individual A/J mouse are reactive with anti-idiotypic antiserum. Hart et al. (65) showed that injecting rabbit anti-idiotypic antibodies specific for the cross-reactive A/J anti-phenylarsonate antibodies suppressed almost completely subsequent production of antibodies with that idiotype. There was no suppression of antibodies to the keyhole limpet hemocyanin carrier molecule or of antibodies specific for phenylarsonate with a different idiotype in the suppressed mice. Hart et al. (64) also showed that such suppression was effective for an extended time, even when 9 weeks elapsed between the administration of anti-idiotypic antibodies and the first challenge with phenylarsonate. In suppressed mice, the antiarsonate antibodies that were synthesized did not share idiotypes with anti-phenylarsonate antibodies synthesized by other suppressed or even nonsuppressed A/J mice. Thus, strain A/J mice produce at least two populations of anti-phenylarsonate antibodies; one population shows intrastrain cross-reactions, the other is unique to each mouse.

Pawlak et al. (123) showed that memory cells are also susceptible to the suppressive effects of anti-idiotypic antibodies. Mice were primed with phenylarsonate and given anti-idiotypic antibodies 2 weeks later. A secondary challenge of phenylarsonate was given on day 28, and then sera were assayed for idiotype 7 days after the secondary challenge. The cross-reactive idiotype

was completely suppressed, but synthesis of anti-phenylarsonate antibodies lacking the idiotype and synthesis of antibodies to the carrier molecule keyhole limpet hemocyanin were not suppressed. In contrast, Owen and Nisonoff (120) showed memory cells resistant to the suppressive effects of idiotype-specific suppressor T-cells. These contrasting results, from experiments in which the same systems were used, suggest that anti-idiotypic antibodies and idiotype-specific suppressor T-cells exert their effects on different target cells. Janeway et al. (78) and Tada et al. (148) have proposed the existence of two types of helper T-cells, one specific for antigen and one specific for immunoglobulin receptors. Hetzelberger and Eichmann (70) and Eichmann et al. (50) presented evidence that both antigen-specific helper T-cells and idiotype-specific helper T-cells participate in antibody responses. Another explanation of the results described above is that a high concentration of anti-idiotypic antibodies might directly inactivate primed B-cells, whereas idiotype-specific suppressor T-cells cannot act directly on idiotypic B-cells.

Ju et al. (86) studied the idiotypes of the anti-phenylarsonate antibodies that are synthesized in suppressed mice. Of four such private idiotypes studied, two were not found in 181 anti-phenylarsonate antisera made in A/J mice, one was found at extremely low concentrations in 3 of the 181 sera, and one was found at low concentrations in 28% of the sera tested. When Pawlak et al. (122) attempted to induce suppression with the $F(ab')_2$ fragments of the anti-idiotypic antibodies, these fragments injected into adult mice failed to induce suppression. This suggested an important role for the Fc fragment of anti-idiotypic antibodies in mediating idiotypic suppression.

Studies by Eichmann (43) showed that strain A/J mice synthesized high serum levels of antibodies with limited heterogeneity specific for group A streptococcal carbohydrate. Anti-idiotypic antisera were made in A/J mice or in guinea pigs specific for the A5A anti-streptococcal carbohydrate idiotype. The anti-idiotypic antisera reacted with antibodies specific for group A carbohydrate from all A/J mice but not with anti-group A antibodies from two other strains. Extending these studies, Eichmann (44) showed that the A5A and A2C idiotypes were strain specific and were transmitted to A/J × BALB/cJ F_1 hybrid mice and that both idiotypes remained associated with the A/J heavy-chain C-region allotype in such hybrids. Eichmann (45) used the streptococcal system to study idiotype suppression. Idiotype production was eliminated by administering anti-idiotypic antibodies, and the isoelectric focusing patterns of idiotype-negative antibodies were unique to individuals, whereas the spectra of idiotype-positive antibodies were shared among different mice. Suppression was transient in mice treated with high and low doses of anti-idiotypic antibodies but was maintained for long periods in mice treated with intermediate doses. Suppression was mediated by IgG2 guinea pig anti-idiotypic antibodies, whereas IgG1 anti-idiotypic antibodies had an enhancing effect.

Extensive studies have been done with an idiotype in BALB/c mice that is associated with antibodies specific for the PC epitope of the R36A strain of pneumococci. Several reports document suppression of this idiotype by anti-idiotypic antibodies. Cosenza and Köhler (37, 38) showed that anti-idiotypic antibodies made in A/He mice could specifically inhibit plaque formation by cells synthesizing IgM anti-PC antibodies either in vivo or in vitro. The suppression was specific for the anti-PC idiotype TEPC-15. Strayer et al. (140, 142) showed that in vivo idiotype suppression in this system was transient in adults but suppression in neonatal mice was long lasting, with 8-month-old, neonatally suppressed mice giving only 4% of the response of nonsuppressed controls. Köhler et al. (95) showed that no idiotype-positive precursor cells could be detected by indirect immunofluorescent antibody assays in the spleens of neonatally suppressed mice. They interpreted this finding to mean that anti-idiotype antibodies specifically deplete the pool of clones of idiotype-positive antigen-sensitive cells. Köhler et al. (98) elicited heterologous (rabbit) anti-idiotypic antisera specific for three PC-binding myeloma proteins of BALB/c origin. Two of the antisera induced specific suppression in BALB/c mice, but the third, which was specific for an idiotype with a different specificity for PC, was not suppressive. The data showed that the target epitope for anti-idiotype suppression was the binding site of the anti-PC receptor on the lymphocyte.

Augustin and Cosenza (4) showed that neonatally suppressed BALB/c mice respond to injections of R36A pneumococci with TEPC-15-negative anti-PC antibodies. The antibodies bind PC but are completely devoid of the TEPC-15 idiotype. In showing the importance of the Fc fragment in suppressive anti-idiotypic antibodies, Köhler et al. (96) found that intact anti-idiotypic antibodies were most suppressive, $F(ab')_2$ fragments were less suppressive, and Fab' fragments were nonsuppressive. They suggested that the residual suppressive effects of $F(ab')_2$ fragments reflected contamination of the preparation by intact IgG molecules. They also

suggested that the requirement for the Fc fragment for anti-idiotype antibodies to be effective in idiotype suppression might have reflected the participation of complement in mediating the suppression. However, this possibility was very unlikely because they found that the intact anti-idiotype was suppressive in cultures containing heat-inactivated fetal calf serum and cobra venom factor.

Idiotype-specific regulation appears to be functional not only with respect to B-cell-mediated antibody responses but also in T-cell-mediated immune responses. A substantial amount of data suggests that in some cases T-effector cells are regulated by apparently anti-idiotypic suppressor T-cells. Some studies show that idiotype production by B-cells may be regulated by suppressor T-cells. This may be an alternate strategy that can be used by the immune system in addition to, or in place of, the direct antibody-mediated anti-idiotype suppression described above.

Dohi and Nisonoff (42) found that the synthesis of the cross-reactive idiotype made by A/J mice in response to the phenylarsonate epitope was suppressed by inoculation of A/J mice with syngeneic thymocytes coupled to anti-phenylarsonate antibodies. Mice recently challenged with phenylarsonate were refractive to this type of suppression. Allogeneic thymocytes from BALB/c and C57BL/10 mice were also effective carriers for the anti-phenylarsonate antibodies, which showed that H-2 restriction did not play a role in inducing the suppression. This suppression was mediated by suppressor T-cells, which demonstrated the feasibility of generating idiotype-specific suppressor T-cells that suppress idiotype production without either anti-idiotypic antibodies or the epitope.

In a study of the mechanism of idiotype-specific suppression of T-cell-mediated delayed hypersensitivity reactions, Yamamoto et al. (159) found that in BALB/c mice the delayed hypersensitivity responses specific for PC could be suppressed by passive transfer of anti-T15 anti-idiotypic antiserum. The transfer of anti-idiotypic antibodies inhibited the inductive phase, but not the effector phase, of the delayed hypersensitivity reactions. The results of Yamamoto et al. showed that treatment with anti-idiotype antiserum induced the activity of idiotype-specific suppressor T-cells and that these cells were highly efficient in suppressing both the inductive and the effector phases of delayed hypersensitivity reactions.

Sy et al. (143–145) studied the idiotype-specific regulatory mechanisms functioning in T-cell-mediated delayed hypersensitivity to the phenylarsonate epitope in A/J mice. Their data showed that doses of anti-idiotypic antibodies which were given after antigen stimulation induced functional suppressor T-cells that suppressed the delayed hypersensitivity responses. Further studies showed that suppressor T-cells capable of suppressing delayed hypersensitivity responses could also be induced by injecting the idiotype coupled to syngeneic spleen cells. Thus, in this system idiotypic suppression is mediated by suppressor T-cells that can be functionally triggered by either idiotype or anti-idiotype. These authors explained this apparent enigma by observing that suppressor T-cells and their factors bear idiotypic determinants (5, 61). The activation of idiotype-bearing suppressor T-cells with either epitope or anti-idiotypic antibodies and the release of a soluble suppressor T-cell factor (receptors?) which is probably complexed with antigen may stimulate the development of second-order regulatory suppressor T-cells, which would further limit the response. Sy et al. (146) showed that second-order suppressor T-cells which express anti-idiotype receptors are induced by idiotype-bearing I-J$^+$ suppressor T-cell factors. They suggested that these two sets of T-cells are mutually stimulatory. The second-order cells could bear either epitope receptors or anti-idiotypic receptors capable of binding idiotype. Therefore, idiotype suppression may be either direct or indirect via a second suppressor cell type, depending upon the initial stimulus.

These studies clearly demonstrate that auto-anti-idiotypic antibodies and idiotype-specific suppressor T-cells can effectively suppress either idiotype synthesis by B-cells or effector T-cell functions. Thus, in the future the network must be viewed as encompassing not only the synthesis of antibodies, but also the functioning of effector T-cells in cell-mediated immunity. In addition, the regulation apparently can be mediated by either antibodies or suppressor T-cells.

Naturally Occurring Anti-Idiotype Regulation

Autoregulation in outbred animals. In 1971, I began a series of studies aimed at elucidating the mechanisms of specific autoregulation of immune processes in normal outbred animals, choosing the rabbit as an experimental animal because it is an outbred animal with a well-characterized system of allotypic markers on its immunoglobulin molecules. These markers provide built-in controls on the purity and integrity of the reagents during each step in experimental manipulations. The *a* locus markers a1, a2, and a3 are particularly valuable, as numerous studies have shown that they are located in the heavy-chain V-region. The rabbit is the only experi-

mental animal available with these heavy-chain V-region markers.

The initial studies (128) were designed to determine whether it is possible for a normal outbred individual to mount an antibody response specific for its own previously synthesized antibody idiotypes. For 189 days rabbits were immunized with TMA coupled by diazotization to keyhole limpet hemocyanin and anti-TMA antiserum was collected. The immunizations were stopped, and the rabbits were rested for the next 432 days. During the rest period, anti-TMA antibodies from the sera collected from each rabbit were purified by affinity chromatography, and the $F(ab')_2$ fragments were prepared by pepsin digestion at pH 4.3 Each rabbit was then immunized with its own anti-TMA $F(ab')_2$ fragments on day 621 after the fragments were polymerized with glutaraldehyde, and sera were collected for the next 39 days. A radioimmunoassay was used to assay for auto-anti-idiotypic antibodies in the late (day 621 to 660) sera. Anti-TMA $F(ab')_2$ fragments were iodinated and mixed with late antiserum samples, and antigen-antibody complexes were precipitated with monospecific goat anti-rabbit IgG Fc antiserum (Table 1).

The data show that each rabbit made auto-anti-idiotypic antibodies which were specific for its own anti-TMA antibodies but nonreactive with the anti-TMA antibodies from other individuals. This reaction was characterized by the ability of free epitope to inhibit the reaction. Free TMA, *N,N*-dimethyl-*p*-phenylenediamine hydrochloride, and *p*-nitrobenzoic acid were used for inhibition (Table 2).

The homologous epitope TMA was the most effective inhibitor, whereas the structurally similar epitope *N,N*-dimethyl-*p*-phenylenediamine hydrochloride was inhibitory only at much higher molar excesses. The unrelated epitope *p*-nitrobenzoic acid was not inhibitory. Additional experiments showed that the reaction did not stem from a determinant created on the anti-TMA $F(ab')_2$ fragments by the purification, digestion, or polymerization procedures. This study was the first to report that a normal outbred animal possesses the genetic and biosynthetic capacity to mount auto-anti-idiotypic antibody responses specific for the individual's own, previously synthesized antibody idiotypes; this verified a fundamental prerequisite in the network concept, namely, that auto-anti-idiotypic antibodies can be synthesized under the right conditions.

TABLE 1. *Reactions of auto-anti-idiotypic antisera with ^{125}I-labeled $F(ab')_2$ fragments of anti-TMA antibodies*[a]

Antiserum	Reaction with ^{125}I-labeled $F(ab')_2$ fragment:[b]				
	B-18	B-19	B-21	B-22	B-26
Preinoculation[c]	0.8	1.3	1.5	2.3	2.3
Pooled normal rabbit serum	1.1	1.5	2.0	2.3	1.2
B-18 anti-B-18	17.0	2.3	0.6	2.3	2.7
B-19 anti-B-19	0.9	39.3	1.6	2.0	2.4
B-21 anti-B-21	0.6	3.4	40.7	1.3	2.3
B-22 anti-B-22	0.1	0.7	0.3	23.3	0.7
B-26 anti-B-26	0.2	1.6	0.1	0.3	23.3

[a] From reference 128 and used with the permission of The Rockefeller University Press.

[b] Percentage of labeled $F(ab')_2$ fragments bound in each reaction.

[c] Preinoculation (day 0) serum from each individual.

The data suggested that auto-anti-idiotypic antibodies recognized the same types of structures on the idiotypic antibody preparations as did isologous anti-idiotypic antibodies (129) and that auto-anti-idiotypic antibodies could be elicited regularly in normal outbred animals by using an appropriate immunization schedule. Confirmation of the regulatory and functional aspects of the network concept required that anto-anti-idiotypic antibodies be demonstrated to occur naturally, without artificial manipulation or re-immunization, after antibodies appear in response to immunization with antigen, and that the naturally occurring auto-anti-idiotypic antibodies be shown to have a demonstrable suppressive effect on the further synthesis of the antibodies bearing the idiotype.

Of several experimental designs used to try to identify natural auto-anti-idiotypic responses, one was successful in a rabbit that exhibited this type of response. The initial antibody response of this rabbit to the carbohydrate antigens of *M. lysodeikticus* was later modified by the natural production of auto-anti-idiotypic antibodies (27). The antibodies in this individual were detected because of simultaneously occurring rheumatoid antibodies.

Rabbit 102 was given three separate courses of intravenous injections of *M. lysodeikticus* vaccine. Substantial quantities of antimicrococcal antibodies were detected in the first-round antiserum. After an extended rest period, a second round of injections was started, and after 3 weeks serum from this animal showed the peculiar property of forming large amounts of precipitate when it was diluted 1:10 to 1:30 in neutral buffer. The presence of rheumatoid antibodies was shown in the second-round serum by passive hemagglutination assays. Rheumatoid antibodies were absent from the supernatant after dilution-induced precipitation of the serum. The washed precipitate consisted solely of IgG molecules, as shown by sodium dodecyl sulfate-poly-

TABLE 2. *Effects of free haptens on inhibition of the reaction of ^{125}I-labeled F(ab')$_2$ anti-TMA antibodies from rabbits B-19 and B-21 and homologous auto-anti-idiotypic antisera*[a]

Prepn[b]	Effect with the following final concn of free hapten:[c]				
	2.5×10^{-2} M[d]	2.5×10^{-2} M	7.7×10^{-4} M	2.5×10^{-4} M	7.7×10^{-5} M
Free TMA					
B-19 system[e]		36.1	40.7	47.0	57.2
B-21 system[e]		40.3	41.7		57.7
Free DMA					
B-19 system[e]	53.9	66.7		77.6	
B-21 system[e]	53.5	68.7		85.1	
Free PNBA					
B-19 system[e]	96.7	102.4		102.9	
B-21 system[e]	93.4	96.6		98.8	

[a] From reference 128 and used with the permission of The Rockefeller University Press.
[b] DMA, *N,N*-dimethyl-*p*-phenylenediamine hydrochloride; PNBA, *p*-nitrobenzoic acid.
[c] Percentage of the reaction obtained in the absence of inhibitor.
[d] Concentration of free hapten before goat anti-Fc was added.
[e] Refers to ^{125}I-labeled F(ab')$_2$ reaction with homologous auto-anti-idiotypic antisera.

acrylamide gel electrophoresis. The pH optimum for the dilution-induced precipitation procedure was between pH 7 and pH 8. Dilution-induced precipitation was done in numerous sugar solutions in an attempt to inhibit the precipitation of the interacting IgG molecules (Table 3).

The most effective inhibitors of the dilution-induced precipitation procedure were glucose and mannuronic acid; a combination of these two molecules produced maximum inhibition of precipitate formation. These same sugars did not inhibit the rheumatoid factor activity, as detected in the passive hemagglutination assay. The results indicated an active, specific involvement in the dilution-induced precipitation process of micrococcal carbohydrate-reactive antibodies, particularly those antibodies reactive with sugar ligands homologous with the immunodominant carbohydrate determinants of the micrococcal cell wall, glucose, and mannuronic acid (156). Several co-precipitation assays and inhibition radioimmunoassays showed the presence of antibodies in second-round sera which were reactive with antibodies specific for micrococcal carbohydrate from the first-round antisera. Other experiments demonstrated that the observed reactivity did not result from antigen fragment bridging of two different antimicrococcal antibodies. The presence of auto-anti-idiotypic antibodies was confirmed by affinity chromatography. Specifically purified antibodies from first-round antisera were coupled to Sepharose. After the rheumatoid antibodies were removed, second-round serum was passed through the column, and the eluted antibodies bound specifically to first-round antimicrococcal antibodies.

This study clearly demonstrated that auto-anti-idiotypic antibodies were present when antimicrococcal carbohydrate antibody clonotypes were redistributed. At 51 weeks after the second round, a third-round series of immunizations was given to the same rabbit. Sera from the first-, second-, and third-round immunizations were analyzed for clonotype distribution by using an analytical isoelectric focusing method, followed by radioactive antigen localization of isolated bands. Samples of two different first-, second-, and third-round sera were electrofocused, fixed in a gel, exposed to ^{125}I-labeled micrococcal carbohydrate, washed, dried, and exposed to X-ray film. The resulting autoradiograph (Fig. 3) shows substantial clonotype distribution differences among first-, second-, and

TABLE 3. *Effect of various inhibitors on dilution-induced precipitation of serum 102*[a]

Inhibitor(s)[b]	% Inhibition
Phosphate buffer	0
NaCl (0.3 M)	0
Glycine (0.25 M)	0
N-Acetylgalactosamine	5
D-Galactose	9
N-Acetylmuramic acid	10
D-Mannose	14
N-Acetylmannosamine	14
N-Acetylglucosamine	14
D-Glucose	18
D-Mannuronic acid	37
D-Glucose + 0.3 M NaCl	20–28
N-Acetylglucosamine + 0.3 M NaCl	22
D-Glucose + *N*-acetylglucosamine + 0.3 M NaCl	26–31
D-Glucose + D-mannuronic acid	47

[a] From reference 27 and used with the permission of The Rockefeller University Press.
[b] All inhibitors were prepared in 0.02 M phosphate buffer, pH 7.2. Carbohydrate solutions were prepared at concentrations of 0.28 M.

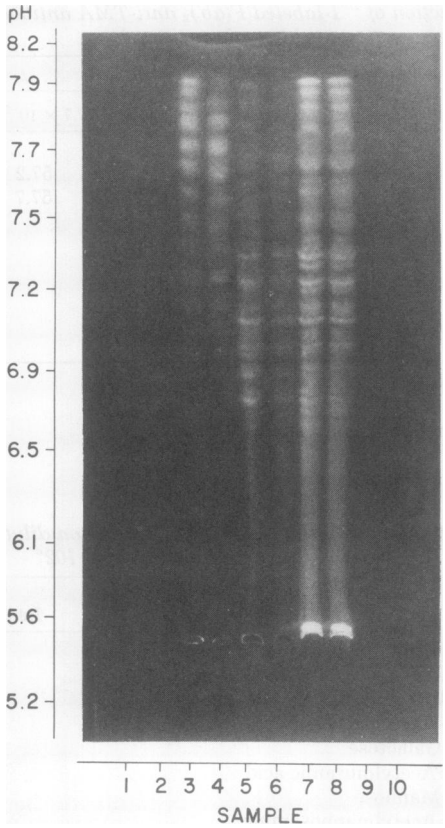

FIG. 3. *Autoradiogram of isoelectric focusing analysis of rabbit 102 antimicrococcal antisera. Samples 1 and 10, Pooled normal rabbit serum; samples 2 and 9, rabbit 102 serum, preinoculation; sample 3, serum from day 190 of first-round response; sample 4, serum from day 230 of first-round response; sample 5, serum from day 127 of second-round response; sample 6, serum from day 161 of second-round response; sample 7, serum from day 34 of third-round response; sample 8, serum from day 41 of third-round response. A 20-μl amount of each sample was applied to the plate. The pH gradient is shown at the left. The sample application point is indicated by an arrow. (From reference 27 and used with the permission of The Rockefeller University Press.)*

third-round sera. Samples 3 and 4 (first round) contained antimicrococcal antibody clonotypes migrating predominantly in the range from pH 7 to pH 8. The second-round response, in which the auto-anti-idiotypic antibodies were detected (samples 5 and 6), showed a dramatic decrease or absence of the antibodies in the range from pH 7 to pH 8 and an increase or first emergence of several distinct clonotypes in the range from pH 6.5 to pH 7.4. After the 51-week rest period, the third-round immunization schedule was begun, and sera from the peak of this third-round response (when auto-anti-idiotypic antibodies were no longer detectable and dilution-induced precipitation did not occur) showed (samples 7 and 8) the reappearance of the clonotypes in the range from pH 7 to pH 8 (which were characteristic of the first-round clonotypes), in addition to the clonotypes that were present in the second-round response. This suggests that the clonotypes that were absent in the second-round response were reexpressed in the third-round response in the absence of detectable auto-anti-idiotypic antibodies. The data for this rabbit strongly support the earlier findings of MacDonald and Nisonoff (107), who showed that idiotypes that were detected early in an immune response disappeared, only to be replaced by a new set of idiotypes specific for the same epitope. Recent unpublished data have shown that a fourth-round immunization of rabbit 102 with *M. lysodeikticus* elicited an anamnestic auto-anti-idiotype response. The quantity of auto-anti-idiotypic antibody was about threefold greater in the fourth round than in the second round.

The precise mechanism of suppression was not clear in this study. Several possibile mechanisms exist. The direct suppressive effects of auto-anti-idiotype on the idiotypic clones is the first possibility. These auto-anti-idiotype-producing cells may then be the equivalent of the suppressor B-cells that Stockinger et al. (139) described. Other investigators (96, 122) have shown that the Fc portion of anti-idiotypic antibody is necessary to suppress responses, suggesting that anti-idiotype suppression might be mediated by the interaction of anti-idiotype with the antigen receptor and with the Fc receptor, thereby cross-linking the two receptors on the lymphocyte surface. Our rabbit data suggest yet another possible mechanism, in which suppression may be mediated by Fc-specific rheumatoid factor. The auto-anti-idiotype binding to the idiotypic receptors on the lymphocyte membrane might then cause small aggregates to form and be further cross-linked with the Fc-specific rheumatoid factor into a complex large enough to cause capping and internalization of the complexes, thus eliminating the idiotypic antigen-specific receptors from the cell surface so that the cells cannot respond to antigen stimulation.

Tasiaux et al. (149) used TMV to elicit antibodies in rabbits in a study in which they related decreases in binding affinities during the response to the appearance of auto-anti-idiotypic antibodies. After rabbits were immunized with TMV, anti-TMV antibodies were purified, pepsin digested, labeled with fluorescein, and used to stain peripheral blood lymphocytes from the same rabbits after rest periods of 2 to 24 months. The results showed that 0.1 to 0.5% of such

lymphocytes were stained. Reinjection with TMV caused a sharp decrease in the number of stainable circulating cells, but the number increased again after 70 days and returned to initial levels after 100 days. A correlation between the presence of auto-anti-idiotype-bearing cells and decreases in affinity of anti-TMV antibodies was documented; 30% of the idiotype-positive cells could be labeled with anti-rabbit thymus cell serum, suggesting that both B- and T-cells participated in the regulation in this system.

Jackson and Mestecky (76) immunized rabbits with human serum albumin and human lactoferrin. Spleen and other lymphoid tissues were examined to search for cells which synthesized auto-anti-idiotypic antibodies. These cells were detected with IgG $F(ab')_2$ or Fab' fragments of specifically purified antibodies after fluorochrome labeling. Thus, the localization of labeled idiotype on auto-anti-idiotypic cells was used to quantitate the number of anti-idiotype-positive cells. The percentages of such cells ranged from 0.7 to 44% in different individuals. These data suggest that auto-anti-idiotype-producing cells may be a consistent feature of immune responses in normal outbred animals, which is a prediction of the network theory.

It should be noted that in neither of the experiments described above dealing with detection of idiotype-binding cells (76, 149) was the possibility of residual antigen fragments rigorously ruled out. Small antigen fragments or intact antigen bound to receptors on B-cells could bind fluorescent or iodinated antibody fragments and appear to be anti-idiotype cells. Extensive cross-assays of cells from one rabbit with antibodies from several other rabbits would rule this out.

Autoregulation in inbred animals. Several investigators have used the unique features of the inbred mouse and rat systems that are available to study the natural occurrence of auto-anti-idiotypic antibodies. The inbred mouse and rat systems offer the distinct advantage that treated or untreated cells from one animal can be transferred to another without the problem of rejection due to histocompatibility differences.

The TEPC-15 or T-15 system of anti-PC antibody responses in BALB/c mice has been used extensively as a model for studies of autoregulation of immune responses. Such responses are T-cell independent. Kluskens and Köhler (94) immunized BALB/c mice repeatedly over a 28-week period with pneumococcal vaccine. Plaque-forming cell and antibody responses specific for PC either stabilized or declined with time. Sera from the mice agglutinated sheep erythrocytes coated with the T-15 myeloma protein, and the activity increased as the plaque-forming cell response to PC declined. The responses of spleen cells from previously immunized mice were much greater than the responses of spleen cells from nonimmunized controls when the cells were stimulated in vitro and PC-specific plaque-forming cells were measured. Several control experiments showed that the serum agglutination of T-15-coated sheep erythrocytes did not result from polyvalent PC antigen in the serum. The reaction of late serum with T-15-coated sheep erythrocytes was inhibited most effectively with the T-15 protein; this reaction also could be inhibited with the PC-binding myeloma proteins MOPC-167 and McPC-603 at 62-fold-higher concentrations. Absorption of late sera on T-15–Sepharose columns eliminated the ability of the sera to suppress anti-PC plaque-forming cell responses in vitro.

Strayer and Köhler (141) used the T-15 system in studies of the responses of neonatal mice. The anti-PC response of adult BALB/c spleen cells was markedly suppressed by mixing various ratios (0 to 20%) of liver cells from 6-day-old BALB/c mice in the cultures. Responses to PC, horse erythrocytes, and sheep erythrocytes were suppressed nearly equally. The culture fluids from cultures of neonatal cells showed the same effect. Absorption of the culture media from cultures of neonatal cells on T-15–Sepharose columns eliminated the ability of the culture fluids to suppress anti-PC responses, but this treatment did not affect responses to other antigens. Time course experiments were used to demonstrate that the autoregulatory anti-T-15 idiotype antibodies were produced transiently approximately 14 days after the mice were born.

Cosenza (35) used the T-15 system to study anti-idiotypic cells. He immunized BALB/c mice with R36A pneumococci and detected plaque-forming cells specific for PC and for T-15 idiotype by coating indicator sheep erythrocytes with either pneumococcal polysaccharide C or T-15 myeloma protein. Spleen cells were then assayed daily for 12 days for both anti-PC plaques and anti-T-15 anti-idiotypic plaques. Anti-PC responses peaked on day 4 and then declined to day 12. Anti-T-15 plaques were first detected on day 6, with a peak response at day 8; this was followed by a rapid decline. The T-15-reactive plaques were inhibitable with the T-15 protein but not with several other myelomas tested.

Kelsoe and Cerny (88) used the technique of direct cell binding of ^{125}I-labeled T-15 protein to quantitate anti-T-15 cells and used plaque-forming cell assays to measure anti-PC responses and to study the reciprocal expansions of idiotype

and anti-idiotype in the T-15 system. Their results showed a reciprocal interrelationship in which peaks of anti-PC responses correlated with valleys of anti-T-15 responses and peaks of anti-T-15 responses correlated with valleys of anti-PC responses. Control studies of ^{125}I-labeled T-15 binding confirmed the specificity of the reaction. Kelsoe et al. (89) suggested that T-cells are necessary for cyclical idiotype and anti-idiotype responses because cycling was not observed in athymic (nude) mice. The persistence of antigen on anti-PC-specific B-cells that mediated binding of labeled T-15 was not rigorously excluded in this study.

Two studies have documented the natural occurrence of autoregulatory anti-idiotypic antibodies in an alloantigen system in inbred rats. In the first study McKearn et al. (111) used Lewis rats and Brown Norway (BN) rats, which differ at the major histocompatibility locus but have the same immunoglobulin allotype. Lewis anti-BN alloantiserum was absorbed onto BN fibrosarcoma cells, eluted, glutaraldehyde polymerized, and injected into Lewis × BN F_1 hybrid rats in complete Freund adjuvant. The resulting idiotype recognized in this system was termed Id-1. Lewis alloantisera elicited by giving antigen injections at monthly intervals had the Id-1 idiotype and reacted in immunodiffusion assays with sera containing Id-1. These sera were not reactive with normal Lewis serum. Hemagglutination assays showed that cycling of idiotype and anti-idiotypic antibodies occurred naturally in the same animals.

After studying the autoregulatory properties of autologous anti-idiotype in the rat system model of graft-versus-host disease, McKearn et al. (110) reported that newborn hybrid Lewis × BN F_1 animals injected with parental strain lymphoid cells developed fatal graft-versus-host disease, whereas adult hybrid animals receiving weight-adjusted doses of such cells did not develop clinically evident disease. The resistance of the adults was age dependent and radiosensitive. Resistance to graft-versus-host disease developed in 3- to 4-week old rats and could be abolished by sublethal total-body irradiation. McKearn et al. showed that adult Lewis × BN F_1 animals injected with normal Lewis spleen cells produced natural anti-idiotypic antibodies. This idiotype was associated with receptors of Lewis anti-BN antibodies but was not present on Lewis anti-dark agouti strain or BN anti-Lewis antibodies. Irradiated rats did not produce detectable natural anti-idiotype. The protective role of the autologous anti-idiotype was shown in a cell transfer experiment. Lewis × BN F_1 adults were given 400 rads of X-irradiation and either Lewis spleen cells (group a), Lewis spleen cells plus normal Lewis × BN F_1 cells (group b), or Lewis spleen cells plus spleen cells from Lewis × BN F_1 rats that had been given spleen cells from Lewis × BN F_1 rats that had resisted fatal graft-versus-host disease (group c). Group c was fully protected from the lethal effects of injected parental spleen cells, group b showed partial protection, but group a showed no protection. Thus, the autologous anti-idiotype is produced naturally in the inbred rat system and is capable of suppressing the immune response to alloantigens when alloantigens initiate antibody production.

Bona et al. (13) used the T-cell-independent antigen bacterial levan to detect cells secreting naturally occurring anti-idiotypic antibodies. BALB/c mice immunized with bacterial levan synthesize a family of cross-reactive idiotypes which are found on BALB/c myeloma proteins that bind inulin and levan. The myeloma protein EPC109 is an IgA κ-immunoglobulin that binds both inulin and levan. EPC109 coupled to sheep erythrocytes was used to assay for plaque-forming cells in the spleens of levan-immunized mice. Significant numbers of EPC109-reactive direct plaques were found, as well as a few indirect plaques that peaked at about 30 days after immunization. The frequency of anti-EPC109 plaque-forming cells correlated inversely with the fraction of antilevan and antiinulin plaque-forming cells that secreted EPC109 idiotype-bearing antibodies. These naturally produced anti-idiotypic antibodies were not shown to have regulatory effects in this system, but the evidence was highly suggestive.

In another system, Bona et al. (11, 12, 15) used three T-cell-independent TNP antigens and showed significant effects of anti-idiotypic antibodies in regulating immune responses. Anti-TNP antibodies synthesized by BALB/c mice in response to the thymus-independent antigens TNP-levan, TNP-*Nocardia* water-soluble mitogen, and TNP-dextran include a subpopulation of antibodies bearing the idiotype characteristic for the MOPC-460 myeloma protein. MOPC-460 is an IgA κ-immunoglobulin of BALB/c origin that binds dinitrophenyl and TNP epitopes. Treating spleen cells before in vitro stimulation with anti-Thy 1.2 and complement significantly increases the proportion of plaque-forming MOPC-460 idiotypic cells. Treatment with anti-Thy along with complement kills T-cells because T-cells are the only lymphocytes with the Thy antigen on their surfaces. T-cells from allotype-congenic C.B20 mice, which possess the IgC_H and IgV_H genes of C57BL/Ka mice on a BALB/c histocompatibility genetic background, do not suppress the MOPC-460 idiotype component of the response.

Furthermore, absorption experiments showed that the T-cells that suppressed the response were specific for the MOPC-460 idiotype but were not reactive with anti-MOPC-460 determinants. BALB/c mice first immunized with MOPC-460 to elicit anti-idiotype and subsequently immunized with TNP-*Nocardia* water-soluble mitogen failed to elicit detectable anti-TNP antibodies bearing the MOPC-460 idiotype; T-cells from the MOPC-460-immunized mice were 25 times more active in suppressing the responses of normal B-cells to TNP-*Nocardia* water-soluble mitogen. This experiment showed that idiotype-specific suppressor T-cells were present. A suppressor T-cell is a thymus-derived lymphocyte that can interact with another lymphocyte and suppress any subsequent action of the target lymphocyte. Therefore, in the idiotype system a suppressor T-cell with anti-idiotypic receptors might interact specifically with a B-cell (or another T-cell) with idiotypic receptors on it and suppress production of the idiotype by the B-cell or suppress effector functions of a T-cell with idiotypic receptors on it. Bona et al. also point out that direct elimination of the MOPC-460 idiotype-bearing precursors of anti-TNP antibody-secreting cells may result from the reaction of anti-MOPC-460 idiotype on the cells. In additional experiments, they immunized BALB/c mice with keyhole limpet hemocyanin conjugated to specifically purified BALB/c anti-MOPC-460 idiotype to make syngeneic anti-(anti-MOPC-460) idiotype. Such immunized BALB/c mice showed a twofold enhancement of the MOPC-460 idiotype response after immunization with TNP-*Nocardia* water-soluble mitogen and TNP-levan. This increase was correlated with an absence of MOPC-460 idiotype-specific suppressor T-cells. The most reasonable explanation for the absence of MOPC-460 idiotype suppressor T-cells in actively immunized mice is that the anti-(anti-MOPC-460) idiotype antibody that the mice produce reacts with and eliminates MOPC-460 idiotype-specific suppressor T-cells. Obviously, this implies that the receptors of these cells share idiotypic determinants with the receptors expressed by the anti-MOPC-460 idiotypic antibodies used for immunization. Bona and Paul (16) showed that the MOPC-460 idiotype-specific suppressor T-cells exert their effect on B-cells. This is in contrast to the allotype-specific suppressor T-cells, which exert their effect on helper T-cells (64).

In a study of the autoregulation of responses of BALB/c and AKR/J mice immunized with TNP-Ficoll, Schrater et al. (132) noted an unusually rapid decline in plaque-forming cells between days 4 and 7 after TNP-Ficoll injection. Plaque formation during that period was enhanced dramatically (41 to 445%) by adding free TNP epitope to the agar used in the plaque assay. Free dinitrophenyl epitope was less effective than TNP for this augmentation. Treatment of immune spleen cells with anti-mouse brain antiserum or anti-Thy 1.1 plus complement failed to eliminate the epitope augmentation, suggesting that this regulation was not mediated solely by T-cells. However, T-cells were shown to play an important secondary role. These authors suggested that the appearance of epitope-augmentable plaque-forming cells was mediated by a B-cell product that itself was under T-cell control. This suggestion was substantiated by the finding (133) that nude (athymic) AKR/J mice do not show a detectable auto-anti-idiotype response. The anti-TNP response was greater in athymic mice than in euthymic mice, it declined less precipitously, and it showed an increase rather than a decrease in affinity between days 4 and 7 after antigen injection. Schrater et al. concluded that the auto-anti-idiotypic antibody response is thymus dependent and that athymic mice lack the helper T-cells required to induce auto-anti-idiotypic antibodies. Goidl et al. (60) showed that serum from AKR/J mice taken 7 days after TNP-Ficoll was injected could inhibit plaque formation by spleen cells from AKR/J mice immunized with TNP-Ficoll 4 days previously. Epitope elution techniques were used to elute the serum factor from blocked spleen cells, and the eluted material was used to inhibit anti-TNP plaque-forming cell responses. Thus, in the thymus-independent antibody response to the TNP-Ficoll antigen in AKR/J mice, a T-dependent serum factor may naturally regulate a response by apparent masking of the idiotypic receptors on the anti-TNP-specific B-cells. A clear interpretation of these data is difficult because there was no immunochemical characterization of the putative anti-idiotypic antibodies other than absorption on an anti-mouse immunoglobulin column. The purity of the mouse immunoglobulin preparation was not documented. Therefore, it was not shown conclusively that the suppressive serum factor was antibody.

Sy et al. (147) studied a system in which delayed hypersensitivity reactions, presumably mediated by T-cells, could be regulated by auto-anti-idiotypic antibodies. Mice were sensitized with optimal doses of 2,4-dinitrofluorobenzene, and the subsequent delayed hypersensitivity reactions were measured by ear swelling. The rapid loss of immunity in this system at 9 to 15 days was mediated by a serum factor that blocked the ability of 2,4-dinitrofluorobenzene-immune lymph node cells to transfer immunity.

The factor was an immunoglobulin devoid of anti-dinitrophenyl activity, but it was antigen specific in its suppressive properties. The antibody blocked 2,4-dinitrofluorobenzene-immune lymph node cells but not trinitrochlorobenzene- or oxazalone-immune cells. The blocking effect of the serum was absorbed only by 2,4-dinitrofluorobenzene-immune lymph node cells (syngenic and allogenic), and pretreatment of the 2,4-dinitrofluorobenzene-immune lymph node cells with an auto-anti-idiotypic antiserum prepared against 2,4-dinitrofluorobenzene-immune lymph node cells blocked the absorption. Thus, the auto-anti-idiotypic antibodies specifically reactive with the T-cell receptor in this system can suppress the T-cell response to the antigen.

Fernandez and Möller (55) showed that sera from CBA and C57BL mice immunized 8 or more days earlier with dextran B-512 contain molecules that specifically suppress the development of plaque-forming cells specific for dextran. Both the thymus-independent and thymus-dependent forms of dextran induce the natural appearance of these factors. These data suggest that both T-dependent and T-independent antibody responses can induce natural auto-anti-idiotypic suppression. The suppressive factor in this study was assumed to be auto-anti-idiotype antibody by virtue of absorption experiments, but immunochemical evidence was not presented.

Triggering of autoregulatory antibody. Initial studies of anti-idiotypic antibody production (90, 119) indicated that injecting the antibody bound to the antigen was an extremely efficient way to elicit anti-idiotypic antibodies. Numerous reports have shown that allogeneic or xenogeneic anti-idiotypic antisera can be elicited with antibody alone and that autologous or isologous anti-idiotypic antibodies sometimes can be demonstrated only with adjuvant-assisted immunizations. Klaus (92) has shown that antigen-antibody complexes are extremely potent inducers of auto-anti-idiotypic antibodies. His results showed that microgram amounts of antigen-antibody complexes can induce auto-anti-idiotype synthesis in the absence of any added adjuvant. In addition, Klaus (93) suggested that antigen-antibody complexes generated auto-anti-idiotypic antibodies by providing a molecular environment for antigen-specific helper T-cells to cooperate effectively with anti-idiotypic B-cells to generate the response. These results suggest that antibodies, particularly if present in small quantities, may require antigen to form immunogenic antigen-antibody complexes to trigger auto-anti-idiotypic antibody production.

CONCLUDING REMARKS

In just a few years, the concept of a structural relationship between the binding sites within the immune system (called the idiotype or lymphocyte network) has received overwhelming experimental support. Regulatory interactions have been described for antibodies and for subsets of T-cells. It is clear that antibodies can elicit regulatory auto-anti-idiotypic antibodies in certain systems in which T-cells participate only as helper cells for auto-anti-idiotype production. Whether complement is strictly required for auto-anti-idiotypic antibody-mediated suppression is still not clear. Idiotype-specific or anti-idiotype-specific suppressor T-cells or both can regulate B-cell function or effector T-cell function or both in some systems. Antibodies can trigger suppressor T-cells that can act on effector T-cells. These interactions and other interactions (interactions with mitogens and the sharing of idiotypes by B-cell sets with different epitope specificities) suggest that the regulatory mechanisms may differ widely from one system to another. However, the data so far strongly suggest that these types of regulatory interactions may exist in all immunological systems. Different degrees of regulation in different systems may make the network components more or less difficult to demonstrate from one system to another.

One aspect of the immune system not yet mentioned may play an important role in the network. Macrophages have long been known for their role in antigen collecting, processing, and presentation and could play a major role by dampening or enhancing network effects. Studies to test this are lacking at this time.

A potential area for future studies of network modulation of immune responses is related to IgE-mediated immediate hypersensitivity responses. Auto-anti-idiotypic suppression of the specificities represented on IgE molecules might be a useful approach for eliminating, in a specific way, responses associated with clinically active hypersensitivities without affecting other responses. Blaser et al. (9) have successfully suppressed the synthesis of PC-specific IgE antibody in BALB/c mice by using isologous anti-T15 antiserum. Geczy et al. (59) have published similar results for a reaginic antibody response in guinea pigs.

Another aspect of immune responsiveness that could be affected profoundly by auto-anti-idiotypic antibodies is the general maternal-fetal interrelationship. If a pregnant female experiences an immune response which results in auto-anti-idiotype regulation and a sufficient concen-

tration of the auto-anti-idiotypic antibodies remains at a critical time during fetal development as IgG antibodies, these antibodies could be passed indiscriminately across the placenta to the fetus, along with other IgG molecules. Fetuses appear to be particularly sensitive to tolerance induction by epitopes presented to them. Maternal auto-anti-idiotypic antibodies might suppress identical or cross-reactive idiotypic B- or T-cell clones permanently. Indeed, Strayer et al. (140–142) and Köhler et al. (95) have shown that neonatal exposure to anti-idiotypic antibodies leads to permanent suppression. However, suppression by anti-idiotypic antibodies in adults is transient. This suppression could strongly affect the immune status and potential of the mature immune system of such an offspring. It is clear from the results of Adler and Noelle (3) and Hagen et al. (63) that offspring can effectively mount immune responses specific for epitopes on maternal immunoglobulin. The synthesis of antibodies specific for either maternal idiotypic antibodies or auto-anti-idiotypic antibodies could then set in motion a complete chain of network-mediated reactions in the total absence of the foreign epitope.

Antibodies involved in responses to tumor antigens may constitute another system in which the effects of network interactions play an important role. It is apparent that T-cells, macrophages, B-cells, and other cell types participate in immune responses to tumor antigens. The role of T-cells seems to be critical. Cytolytic T-cells can lyse tumor cells, and suppressor T-cells can limit effector T-cell responses. Furthermore, it is apparent that antibodies to tumor antigens can modulate or abolish many cell-mediated immune functions (158). Some of the effects of antitumor antibodies could be reversed or eliminated by synthesis of auto-anti-idiotypic antibodies by the individual carrying the tumor.

One possibility for auto-anti-idiotype antibody modulation of tumor immunity is in the system first described by Möller (113), in which antibodies cover tumor antigens and render tumor cells less susceptible or nonsusceptible to the action of immune cytolytic T-cells (66). Auto-anti-idiotype could reverse this system by eliminating the clones synthesizing the idiotype that recognizes and covers the tumor antigen.

A second possibility for interference by auto-anti-idiotypic antibodies, which was described by Sjogren et al. (138), is elimination of the effects of antigen-antibody complexes in blocking immune reactivity in tumor systems. If in antigen excess, these complexes could then block the receptors of tumor-specific cytolytic effector T-cells. If complexes exist in antibody excess, they could cover the cell-bound form of the antigen and sterically interfere with tumor antigen recognition by effector T-cells. With either possibility, the production of auto-anti-idiotypic antibodies and the subsequent elimination of the antitumor antibody would allow for more efficient T-cell lysis of the tumor cells.

On the other hand, auto-anti-idiotypic antibodies specific for the T-cell antigen receptors could, in effect, paralyze the effector T-cells. T-cells share idiotypes with immunoglobulins. The demonstration by Sjogren et al. of immune complexes in tumor systems, and the data of Klaus (92, 93) which showed that antigen-antibody complexes are excellent molecular forms for eliciting auto-anti-idiotype suggest that tumor antigen complexed with antitumor antibodies could effectively elicit auto-anti-idiotype antibodies specific for the antitumor antibodies. These could then suppress the function of the necessary cytolytic T-cells and, thus, allow tumor growth. This should be a research area that is technically feasible and potentially rewarding. Indeed, Flood et al. (56) have shown that animals immunized with tumor-stimulated lymphoblasts (to elicit an immune response to the antitumor structures on the lymphoblasts) develop anti-idiotypic cytolytic T-cells that eliminate tumor-specific lymphocytes. This is an example of elimination of a protective and presumably beneficial response via idiotype interactions.

Antigenic modulation, as described by Taylor et al. (150) and Kourilsky et al. (99), may be another area of tumor immunology in which auto-anti-idiotypic antibodies could play a major role. The concept of antigenic modulation concerns the temporary loss of tumor antigens via antigen-antibody interactions at the cell surface, followed by capping and ingestion of the complexes by pinocytosis. Thus, the cell surface may become devoid of tumor antigen, so that the effector cytolytic T-cells can find no suitable receptors to trigger cytolysis. Here again, the production of auto-anti-idiotypic antibodies could eliminate these antibodies and leave the tumor antigen intact and available for T-cell action.

Transplantation immunology is an area in which auto-anti-idiotypic modulation could be beneficial. The approach would be to interfere with the antibody or T-cell response to histocompatibility antigens by using an auto-anti-idiotype response. Hans Binz and Hans Wigzell have published numerous papers on this subject. Most of this work was not cited in this article because their work is so extensive that it constitutes a separate area by itself. Their studies (7) may lead to ways of inducing tolerance to his-

tocompatibility antigens for clinical purposes.

The experimental basis for the idiotype network seems well established, and natural occurrence of network-mediated regulation has been shown in several systems. It is not yet clear whether network regulation occurs as an integral part of all immune responses or only under defined conditions. For example, Braun et al. (23) studied the clonotype distribution of anti-group A streptococcal polysaccharide antibodies in acute rheumatic fever patients. Sera were studied from samples taken during 2 years. The same clonotype patterns were observed in individual patients even with reinfection and recurrence of rheumatic fever. These data could be interpreted as arguing against any effects of anti-idiotypic regulation because clonotype patterns were stable for long periods. One might also argue that no single clonotype reached threshold levels for anti-idiotype triggering in this system.

The evidence supports the existence and functional properties of an idiotype network. The introduction of the idiotypic network concept, followed rapidly by data supporting its natural functioning in a variety of inbred and normal outbred animal systems, should spur many investigators to include the network concept in future experimental designs.

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