Idiotypes

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Idiotypes, the unique and characteristic determinants of an immunoglobulin or T cell receptor, can function as antigens. Anti-idiotypic responses, which include anti-idiotypic antibody-producing B cells and idiotype-specific T cells, form a network of immune cells that regulate their production.

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Introduction

The idiotypic network hypothesis, formulated by Niels Jerne in 1974, postulates that the immune response may be regulated by responses to idiotypes, unique determinants originally described on B cell, and now also on T-cell receptors. This thesis is based on the dual characteristics of the B- and T-cell receptors (BCR and TCR, respectively), such that they both react with an antigen through their antigen-binding sites, but may also be recognized by anti-idiotypic antibody and anti-idiotypic T cells. These interactions create a network of clones of B and T cells that express distinct idiotypic specificities that interact with each other to regulate the immune response. Data have accumulated supporting the existence of a functional idiotypic network in immune responses to haptens, and viral, bacterial, parasitic and tumour antigens. Furthermore, investigations in this area have contributed to the design of new vaccines and new therapeutic strategies for the treatment of various diseases such as B-cell lymphomas, and autoimmune disorders.

Structure and Expression of Idiotypes

The immune system consists of a large number of phenotypically and functionally distinct cells that interact with each other via specific receptors and effector molecules. When an antigen is recognized as foreign by the immune system, a series of events occurs, that leads to the induction of an immune response specific for the antigen. Similarly, it was noted that lymphocyte receptors such as the BCR or immunoglobulin (Ig) and the TCR could also induce an immune response under appropriate conditions. This observation led Jerne, in 1970, to propose that interactions between unique antigenic determinants on BCRs formed a functional network of communication, which he called the idiotypic network. His thesis was based on the dual nature of the antibody molecule, i.e. while antibody recognizes antigen through its combining site or paratope, it is also immunogenic, via its expression of idiotypic determinants, unique antigenic structures present in the variable region of the antibody. Originally, Jerne viewed the idiotypic network as a B-cell network in which interactions between lymphocytes required complementary V-region structures present on antibody molecules. This has been expanded to include T cells and their TCR, which are key to the production of immunoglobulin and regulation of the immune system.

The immunogenicity of idiotypes expressed on immunoglobulins was one of the first observations in support of the idiotypic network theory. Cross-species immunization demonstrated that antigenic determinants on the antibody could be xenotypic (between species), allotypic (between different strains of the same species), or idiotypic (specific for a given clone in an individual). Anti-idiotypic antibodies were shown to bind to idiotypes that were epitopes present in the variable regions of the receptors of both T and B lymphocytes, and each receptor carries a set of idiotopes that defines its unique idiotype. So the idiotype of each V region of a single immunoglobulin molecule may be comprised of as many as 15–20 idiotopes, which can be distinguished by monoclonal anti-idiotypic antibodies or defined by a specific and unique amino acid sequence. Both the binding specificity and the idiotype of an antibody are determined mainly through recombination events (VH, D and JH for heavy chain and VL and JL for light chain) that occur after exposure to a specific antigen. If variable regions of both the heavy and light chains contribute idiotypic determinants, the idiotype is likely to be a conformational determinant. On the other hand, individual idiotypes may be located either in the heavy or in the light chains and contained in the primary amino acid sequences of the variable regions. These idiotypes are more likely to be sequence-dependent, and linear antigenic determinants. The hypervariable regions (also known as complementarity determining regions or CDRs) are thought to be the primary immunogenic sites within the variable region, but any part of the variable region of immunoglobulin may contribute to the structure of an idiotype. In a number of systems, the CDR3 region has been shown to be the highest contributor; however, contributions by CDR1 and CDR2 are not uncommon. Synthetic peptides representative of CDR2 and CDR3 regions of antibodies like rheumatoid factor have been used to generate anti-idiotypic antibodies that bind to the original antibody. A similar approach has been used

to characterize idiotypes on anti-deoxyribonucleic acid (DNA) antibodies.

Several groups have described idiotype-specific T lymphocytes with helper or suppressor functions. Although, early experiments with polyclonal anti-idiotypic reagents suggested a sharing of idiotypic determinants between T and B cells specific for the same antigen, these initial observations were not confirmed with monoclonal antibodies. This was not surprising, since BCR bind to native epitopes present on antigens, while TCR bind to antigen only after it has been processed and presented as peptide fragments in association with major histocompatibility complex (MHC) molecules by antigen-presenting cells (APC). Idiotypic network interactions could play a role in the selection and maintenance of the T-cell repertoire. In addition, antibodies specific for TCR idiotypes could function as antigen and activate T cells. The nature of idiotopes present on TCRs has not been studied as thoroughly as immunoglobulin idiotopes. TCR of T cells, like immunoglobulin of B cells, is composed of two chains, α and β, either of which can bear idiotypic antigenic determinants that are defined by anti-idiotypic antibodies or by anti-idiotypic T cells. However, idiotypic determinants of TCRs are generally different from those of immunoglobulin receptor of B cells, due to the low degree of homology between V genes of immunoglobulins and those of the TCR.

Classification of Idiotypes

Idiotypes were initially defined by their reactivity with antisera to the variable region of receptor molecules, but now sequencing these regions has identified the molecular structures of idiotypic determinants. Conventional immunochemical classification of idiotypic determinants according to their location and their corresponding anti-idiotypic antibodies is shown in **Figure 1**. Antibody 1 (Ab1) is produced in response to an antigen. B-cell clones recognizing idiotopes on Ab1, referred to as Ab2, are a heterogeneous population displaying several specificities. Ab2α recognizes determinants that are located distant from the antigen-binding site or paratope, and thus are framework-associated idiotypes that may exhibit a regulatory effect on the production of antibody bearing the Ab1 idiotype. On the other hand, since the binding of Ab2β and Ab2γ to their corresponding idiotypes can be inhibited by the eliciting

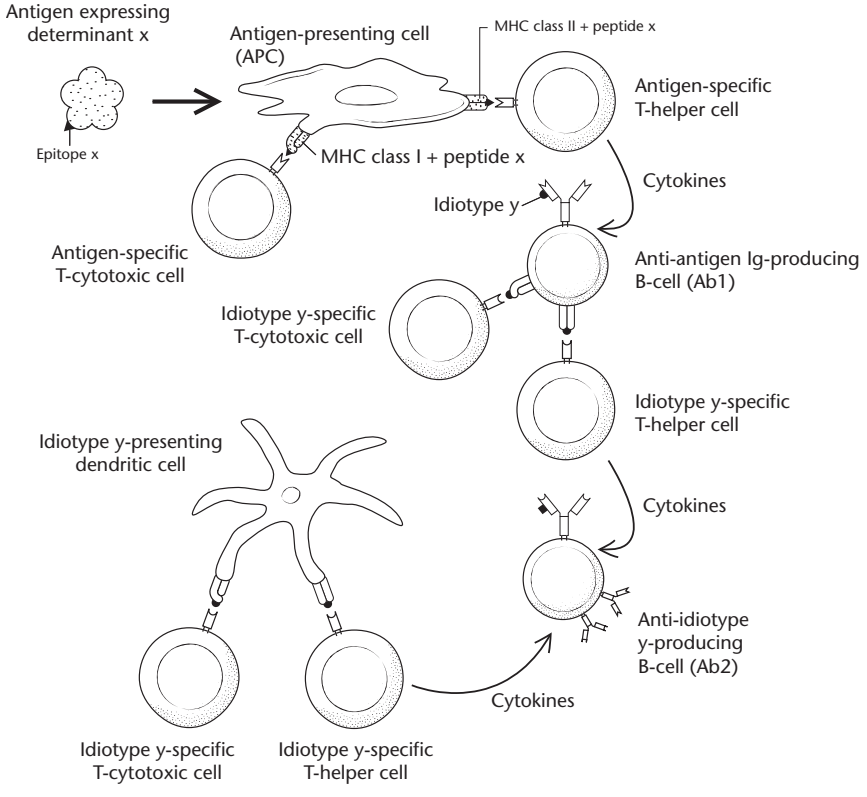

Figure 1 An antigenic epitope (x) is processed and presented by an antigen-presenting cell (APC) in association with Class II MHC to an antigen-specific T-helper cell or, in association with Class I MHC, to an antigen-specific cytotoxic T cell. Signals from the T-helper cell lead to the activation of B cells that produce anti-epitope x antibody (Ab1), which will express idiotype y. Anti-x producing B cells, as APCs, present idiotypic peptide y in association with Class II to idiotype y-reactive or specific T-helper cell and, in association with Class I, to idiotype y-specific cytotoxic T cells. Signals from the activated idiotype y-reactive T cells lead to the activation of anti-idiotope y-producing B cells (Ab2). Dendritic cells may also present idiotypic peptides to T cells.

antigen, these antibodies bind to determinants located in the paratope of Ab1 (Ab2β), or close to it, in the case of Ab2γ. The major difference between Ab2β and Ab2γ is that while Ab2β carries the internal image of the actual antigen, Ab2γ does not. Ab2β can fit in the paratope of an antigen receptor of B or T cells through idiotypic network determinants that mimic the three-dimensional structure of the antigen, and thereby activate Ab1 precursors reactive with foreign or self-antigens. They may also be responsible for the stimulation and maintenance of memory of T lymphocytes. In addition to those described above, Ab2ε or epibodies have also been reported. These anti-idiotypic antibodies have the ability to react with antigen, and also with the idiotope of an antibody reactive with the same antigen.

The location of idiotopes in the variable region as defined by anti-idiotypic antisera suggested that there was a relationship between the combining site for antigen and the expression of an idiotype. However, it was observed that the same idiotype could be shared by antibodies that bound to a variety of unrelated antigens, or by antibodies that reacted with different determinants on the same antigen. V genes from different VH or VL families may encode antibodies that share cross-reactive idiotypes (CRI). This indicates that various combinations of VH or VK families can produce the amino acid residues that interact with the same anti-idiotypic antibody that serologically defines a given idiotype. These 'shared' idiotypes may be defined as either public, CRI or IdX, or private idiotopes. Public idiotopes are expressed on BCR or TCR in all individuals of an inbred mouse strain, or individuals with different genetic backgrounds. Public idiotopes are the markers of random somatic mutations that occur in a single clone of T or B lymphocytes. CRI are markers of germline genes that are conserved during evolution and may be classified into three major groups: (1) idiotypes that are expressed in all inbred or recombinant inbred strains which share the same allotypes of the heavy or light chains, or CRI-linked to allotype; (2) idiotypes that are expressed on various inbred strains or on a major fraction of individuals of an outbred species independent of genetic allelism in the constant region, referred to as interstrain idiotypes; or (3) cross-reactive idiotypes shared by individuals of different species (interspecies idiotype). On the other hand, private idiotopes are confined to some individual antibodies, and likely result from a unique somatic recombination event, or as a consequence of somatic mutation. As might be predicted, the same idiotypic determinants can also be expressed on immunoglobulin of different isotypic or allotypic classes, due to the ability of any given VH or VL region to recombine with various constant region genes during clonal development or differentiation of B cells after antigenic stimulation.

Idiotypes may also be classified based on the extent of their expression in the immunoglobulin repertoire. They are considered to be major or dominant, if they are found on a large fraction of serum antibody, and the appearance of dominant idiotypes is related to selection of clones involved in the immune response to a specific antigen. Minor idiotypes are expressed on a relatively small fraction of antibodies produced during the response to a particular antigen. Some idiotypes are silent, and not normally detectable during an immune response, but can be elicited under certain conditions. Finally, shared idiotypes may also be found antibodies of unknown specificities, referred to as parallel sets.

Function of Idiotypes

Functionally, idiotypes can be classified according to whether the levels of anti-idiotypic antibodies to the idiotype vary during the immune response. Idiotypes found on both antibodies that are specific for foreign and self-antigens are involved in the regulation of responses to antigen. These are called regulatory idiotypes. In general, there is a reciprocal fluctuation between Ab1 and Ab2 clones in immune responses, as well as in the remission and flare stages of diseases such as systemic lupus erythematosus (SLE). In the steady state, or immunological homeostasis, there is equilibrium between Ab1 and Ab2, in which the production of Ab1 is controlled by Ab2-reactive lymphocytes (**Figure 2**). When an organism encounters a foreign antigen or a self-antigen, in the case of the autoimmune diseases, equilibrium is shifted in favour of the expansion of Ab1 clones that respond to antigen. This leads to expansion of Ab2-producing clones and increased production of Ab2 Ab, which modulates the numbers and functions of Ab1-producing B cells, resulting in decreased Ab1.

Idiotype-specific T-cell clones that can recognize and respond to idiotypic determinants on B cells have been described in a number of systems, and include various functional types of T cells such as regulatory cells with helper or suppressor function or effector cells that mediate cytotoxic, delayed-type hypersensitivity or contact sensitivity reactions. These idiotype-reactive T cells are MHC-restricted and recognize idiotypic determinants in the form of peptide fragments in the context of MHC Class II molecules presented on APCs. Immunoglobulin that is expressed on the surface of a B cell can be processed and presented in association with MHC Class II molecules present on the surface of the same B cell. However, processing of the idiotype for presentation only takes place when B cells are activated through crosslinking of immunoglobulin receptors on the surface. The requirement for these two events may prevent chronic activation of idiotype-bearing B cells. Several groups have also described anti-idiotypic T cells that are specific for native immunoglobulin (likely a conformational idiotype), but this is inconsistent with the current paradigms for T-cell activation. However, this type of binding might be possible if a conformational idiotype was processed and presented to T cells in a manner that maintained its structure. Then,

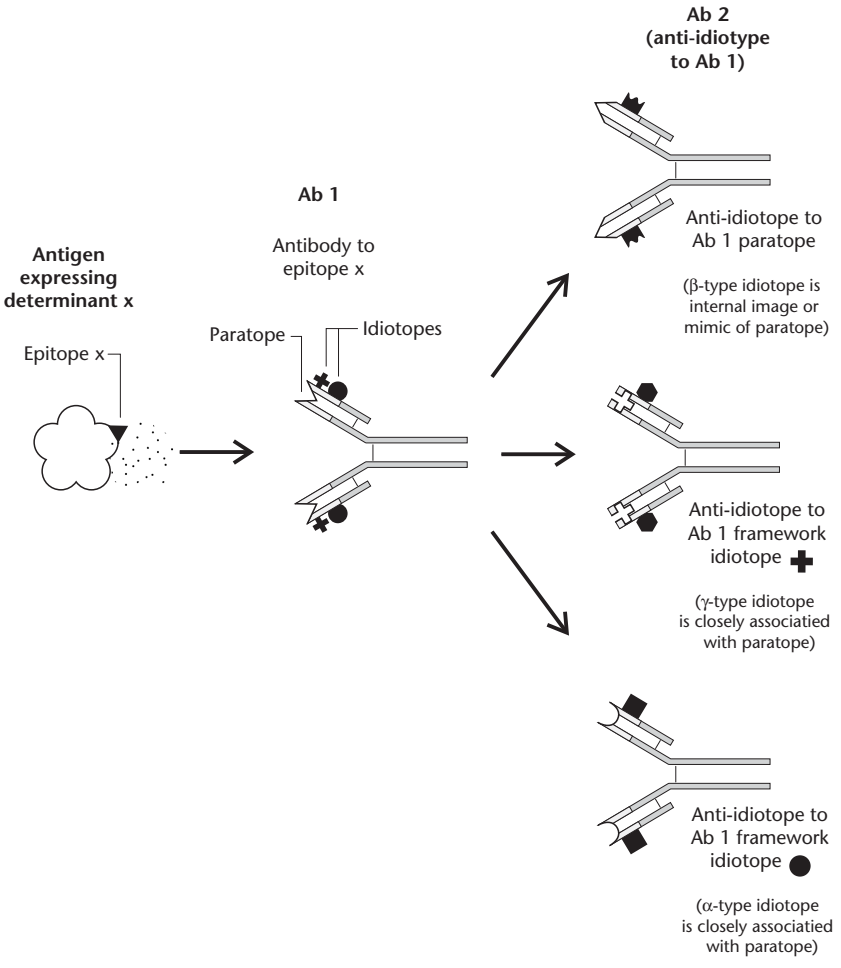

Figure 2 Idiotypic cascade. A foreign antigenic epitope (x) induces an immune response characterized by the production of Ab1 antibody, which is anti-x and contains several idiotopes, which can either be paratopic or nonparatopic (framework). Ab1 can elicit an anti-idiotypic response (Ab2), which can be of three types: (1) Ab2β, which is directed to the paratope of Ab1, and presents the internal image of antigen epitope x; (2) Ab2γ, which is directed to the paratope of Ab1 but does not carry the internal image; (3) Ab2α, which is directed to nonparatopic or framework idiotopes of Ab1.

in the presence of a second, antigen-specific conventional helper T cell, these cells could augment the production of idiotype-bearing antibody. Cloned lines of cells with these characteristics have been derived, and shown to require the presence of idiotype for their maturation and/or activation. In one case, TCR $\gamma\delta$ was expressed.

Idiotypic Network in Health and Disease

Because internal image idiotopes can serve as surrogates for foreign antigens, approaches using anti-idiotypic antibody as vaccines to bacterial, viral and parasitic antigens have been investigated. The first report of anti-idiotype-induced protection was from a study in which Balb/c mice that had been inoculated with a lethal dose of *Trypanosoma,* the causative agent of African sleeping sickness were protected from a subsequent infectious challenge by treatment with anti-idiotypic antibody. In humans, an anti-idiotype-based vaccine that mimicked hepatitis B virus surface antigen (HBsAg) (and thus was its internal image), has been tested. This anti-idiotype recognized a shared idiotype on antibodies against hepatitis B virus (HBV), and immunization of mice and rabbits with it generated Ab3 with anti-HBV activity. In another study, an anti-idiotypic fragment that mimicked the capsular polysaccharide antigen of *Group B Streptococcus* bacteria elicited an active protective humoral response in adult mice and a passive protective immunity in newborns.

In many autoimmune diseases, the autoantigen has yet to be defined, and a role for the idiotypic network in the induction of pathogenic antibodies in autoimmune diseases has been explored by a number of investigators. For example, SLE can be induced in nonautoimmune mice by immunization with native anti-DNA antibody expressing the 16/6 idiotype (Ab1), or peptides derived from its CDRs,

due to the production of a wide variety of autoantibodies that are found associated with SLE, including anti-DNA, anti-Sm, anti-Ro-SS-A and antihistone antibodies. These data suggest that SLE in humans could be induced in 'susceptible' individuals (due to genetic, immunological or hormonal factors) by a persistent antibody response to a 'trigger', such as an infectious agent, that may express antigenic determinants that are similar to a self-antigen. Production of these antibodies, now to both the foreign and self-antigens, which would express unique and perhaps cross-reactive idiotypes, would persist if their production was not regulated effectively via idiotypic network interactions. The reciprocal relationship between Ab1 and Ab2 anti-idiotypic antibodies during active disease versus remission, as well as functional differences in Ab2, provide support for the potential involvement of this mechanism in the pathogenetic process. This mechanism would also explain the beneficial effects of treatment with intravenous immunoglobulin (IVIg) for SLE nephritis. IVIg has been shown to have multiple mechanisms of action, including the modulation of the idiotypic network. Indeed, IVIg has been shown to contain anti-idiotypic activity to both anti-DNA and anticardiolipin antibodies, and IVIg treatment of experimental SLE and antiphospholipid syndrome led to decreased levels of pathogenic antibodies.

Immunotherapy for cancer is based on inducing an immune response against tumour cells. Therapeutic approaches for several different types of cancers based on the idiotypic network hypothesis have either vaccinated with antitumour Ab1 antibodies leading to the development of anti-idiotypic antibodies, Ab2, or administered anti-idiotypic antibodies, Ab2, which act as anti-idiotypic vaccines, and can functionally imitate tumour-associated antigens (TAA). One advantage of these approaches over immunotherapy that vaccinates with TAA is that the latter is limited, in part, by the relatively low immunogenicity of tumour antigens. Further, anti-idiotypic antibodies can mimic either protein or nonprotein antigens. In either case, whether Ab1 or Ab2 is used as the vaccine, the net result is that the host produces an anti-idiotypic response (Ab3) against the tumour that includes antibody-dependent cell-mediated cytotoxicity (ADCC), and other cell-mediated cytotoxic pathways, including cytotoxic T cells and natural killer (NK) cells. Immunization with antibodies against TAA that elicit internal image antibodies would result in the expansion of clones of cells that are involved in antitumour immunity. The efficacy of this approach has shown for several different cancers. Patients with surgically resected colon cancer generated a vigorous anticarcinoembryonic antigen (anti-CEA) response after immunization with CeaVac, an anti-idiotypic antibody that is an internal image of CEA. Tumour-specific mechanisms that were induced included ADCC, idiotype- and antigen-specific CD4 helper T-cell responses that contributed to clinical improvement in some patients. In another study, patients with ovarian cancer who were treated with a murine monoclonal anti-idiotypic antibody (Ab2), designated as ACA125, developed antitumour responses and slowed progression of the disease, with a low rate of side effects. Therapeutic approaches exploiting idiotypic network interactions have also been effective in the treatment of malignant melanoma. Patients treated with an anti-idiotypic antibody that mimicked the disialoganglioside GD2 developed Ab3 antibodies that reacted specifically with tumour cells, as determined by flow cytometry, and in some cases, ADCC. Trials with this vaccine now include patients with small cell lung cancer. Still, despite these successes, active immunization with antigen may still be preferable to anti-idiotype therapy. One reason is that an antigen-induced response would be polyclonal and target many tumour antigens. Further, there are additional concerns when immunoglobulins are used as therapeutic agents that include the generation of strong antibody responses, particularly if murine anti-idiotype antibodies are used; however, 'humanization' of mouse antibodies and the use of recombinant single chain antibodies would obviate this. Clearly, the efficacy of therapies that have used approaches exploiting idiotypic network interactions thus far support further effort towards the development of idiotypic and anti-idiotypic vaccines, and the continuation of clinical trials that test their efficacy, alone and in combination with other regimens to enhance the antitumour response.

Further Reading

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