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Anti-idiotypic antibodies and the induction of specific tumor immunity

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Abstract

Immunization with anti-idiotypic antibodies is a strategy which, with variable success, can be used to elicit or amplify antigen-specific immune response. This article discusses the manipulation of specific idiotypes in anti-tumor immunity, emphasizing the appropriate consideration of genetic restriction, the choice of idiotype specificity, and the route of immunization. Two independent pathways are outlined: One uses anti-idiotypic antibodies to select and amplify tumor-specific T and B cells via their preexisting antigen-specific receptors, and the other uses anti-idiotypes as primary internal image immunogens to elicit immune recognition of determinants shared by the anti-idiotype and by tumor-associated antigens. Both pathways can be manipulated in attempts to favor the generation of anti-tumor effector cells and minimize the elicitation of suppression.

Anti-idiotypic immunization can be utilized to induce therapeutic immune reactivity in hosts lacking effective direct anti-tumor responses. By stimulating 'silent', or normally suppressed, T and B cell clones, appropriate immunization strategies can circumvent immune regulatory pathways associated with suppressor cells and factors derived from such cells. In these studies, adequate characterization of antitumor idiotype and anti-idiotype specificities is key to the experimental approach to tumor therapy using antibodies. The importance of individual host genetic variation in the specificity and scope of immune response to anti-idiotypic immunoglobulins is unknown, and remains an important potential barrier to therapeutic management.

Abbreviations: DTH — Delayed-Type Hypersensitivity, Id — Idiotype, Id+ — Idiotype positive, i.v. — intravenous, MCA — Methylcholanthrene, MHC — Major Histocompatibility Complex, s.c. — subcutaneous, T_H — T helper cell, T_S — T suppressor cell

Introduction

Immunization with anti-idiotypic antibodies (Ab2) can induce the formation of anti-anti-idiotypic immunoglobulins, some of which have the same antigen specificity as the antibody (Ab1) used to derive the anti-idiotype. This creates a powerful paradigm for manipulation of immune responses by offering a mechanism for generating and amplifying antigen-specific recognition in the immune system.

In this article we shall discuss evidence for an immune response to tumors, which involves idiotype-specific recognition of tumor antigen, and we shall consider strategies for manipulating this recognition towards achieving therapeutic benefit.

General concepts

The use of anti-idiotypic antibodies to induce immune responses to tumors can be viewed as two separate issues. First, such antibodies may be used to select or amplify a pre-existing antitumor repertoire, that is, to recruit, via idiotypic selection, T and/or B cells with specificity for tumor antigen. Second, anti-idiotypic antibodies can be employed as 'internal images' of antigens to induce a primary immune response which is anti-anti-idiotypic, and a portion of which is directed against the nominal tumor antigen. In the latter case, the immune specificity will be against anti-idiotope, rather than antigen.

By using anti-idiotypic antibodies to induce immunity, T and B cells may be selected that are different from those which participate in a naturally occurring antitumor response, either as a result of upregulation of an immune response that is normally suppressed or by the de novo induction of a response. Of significant therapeutic importance is the potential to induce effective antitumor reactivity in hosts that are otherwise incapable of mounting such reactivity.

Idiotypic interactions initiated by antigenic stimuli

In order to discuss therapeutic manipulations of the idiotypic recognition of tumor antigens, we need to know about the nature of the naturally occurring idiotypic response to a growing syngeneic tumor. We shall first consider the nature of the idiotypic repertoire in response to stimulation with any antigen.

Four possible idiotypic and anti-idiotypic responses that can be induced by antigens are illustrated in Fig. 1. Fig. 1a shows a sequential progression of induced complementary specificities. This is the 'standard' type of idiotypic cascade [1–5], in which immunization with antigen leads to a population of immunoglobulins which carry distinct idiotypes, the sum of which is known as the idiotype or Ab1 response. The presence of the Ab1 then induces an anti-idiotypic response, characterized by a heterogeneous population of antibodies,

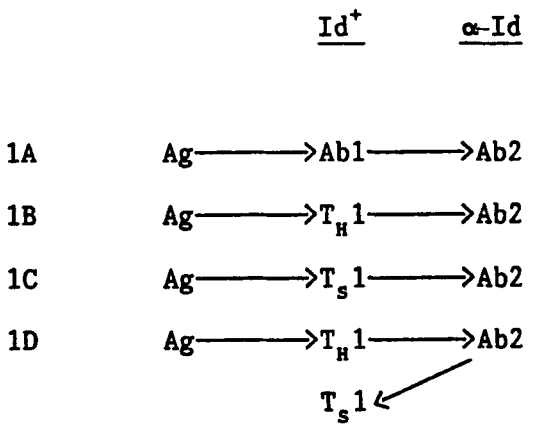

Fig. 1. Four types of idiotypic and anti-idiotypic responses initiated by antigenic stimuli. The possible role of T_H or T_S in substituting for Ab1 in the stimulation of α-Id is illustrated in panel 1D, in which Id^+ T_S arise under the influence of Ab2.

known as Ab2, which have specificity for the various idiotopes in the Ab1 population. Directionality of the antigen-elicited response is implied by the arrows in Fig. 1. The induction of Ab2 by Ab1 is independent of antigen. This has been demonstrated by experiments in which immunization with Ab1, in the absence of antigen, induces an Ab2 population similar to that arising in response to Ab1 after these have been induced by immunization with antigen [3, 5, 6].

Support for this model (Fig. 1a) in tumor immunity comes from several types of experiments. The role of specific antigen in the induction of tumor immunity has been documented over the past 30 years, particularly for sarcomas induced in mice or rats by chemical carcinogens, ultraviolet irradiation, or DNA viruses. Tumor-associated antigens include transplantation antigens that can induce rejection of tumor cells transplanted to syngeneic hosts, as well as various differentiation (oncofetal) antigens, some of which also elicit immunological reactivity in the tumor-bearing host (reviewed in [7]).

The existence of an Ab2 population in response to animal tumors has been demonstrated in several systems and is discussed in detail below. For example, in experiments performed with methylcholanthrene (MCA)-induced sarcomas in mice [9–11], polyclonal antibodies, which appear to be anti-

idiotypic, have been detected after multiple immunization with tumor antigen. These antibodies bind to effector cells and idiotypic factors (see below) and are capable of eliciting tumor-specific immunity in the absence of tumor antigen [9]. A monoclonal antibody with similar anti-idiotypic specificity has been described that primes for DTH reactivity in an allotype restricted fashion [9].

There is, on the other hand, a remarkable lack of evidence for the presence of Ab1 in response to tumor-specific transplantation antigens, which are of greatest interest in view of their ability to induce tumor rejection. Thus, the model drawn in Fig. 1a may have a gaping hole in the center of the postulated sequential cascade. In mice with MCA-induced sarcomas, one of the animal models most commonly investigated, a tumor inoculum elicits a rapid and vigorous antibody response. However, these antibodies are almost exclusively directed against retroviral and cell-associated antigens, which are different from the individually unique tumor-specific transplantation antigens [8].

The induction of idiotype-positive tumor-specific reactivity in the cellular compartment of tumor-bearing mice, on the other hand, has been well established. Both idiotype-positive T helper cells (T_H) and suppressor T cells (T_S), arising in response to tumor antigen, have been described [10, 11]. Monoclonal Ab2 have been used to positively select a population of T_H cells from mice immunized to transplanted MCA-induced sarcomas. These T_H cells were capable of eliciting antitumor DTH and also of inhibiting the outgrowth of transplanted tumor cells [11].

These observations suggest that an idiotypic cascade leading to the generation of Ab2 may proceed according to the pathway shown in Fig. 1B. Tumor antigen recognition induces antigen-specific T_H cells, which bear particular idiotypes, presumably on their antigen receptor molecules. These T cells in tumor immunity idiotopes stimulate the generation of an anti-idiotypic immunoglobulin response in the form of a population of Ab2.

What are the implications of a model in which the idiotypic cascade proceeds through alternate antibody and T cell components? In an analysis of idiotopes associated with the response to MCA-induced mouse sarcomas or carcinomas [11–13], immunization with monoclonal Ab2 failed to generate Ab3 with Ab1-like specificity, whereas antitumor T_H were easily induced. This observation, coupled with the finding of idiotype-positive T_H in the naturally occurring antitumor response, supports a model in which there is a direct regulatory interaction between Id+ T cells and anti-idiotypic B cells (and Ab2) [14–16]. *In vivo*, the Id+ T cells would provide the stimulus for production of anti-idiotype.

The apparent lack of Id+ B cell recognition may reflect a defect in the genetic capacity to generate antitumor idiotopes on immunoglobulin molecules; alternatively, the regulatory state in the tumor-bearing host may effectively suppress Id+ Ab1. As we will discuss when considering the use of anti-idiotypic immunization (see below), this apparent defect may be circumvented therapeutically in a host that does not normally develop Id+ antibody responses.

In addition to idiotypic interactions that result in a T_H response leading to tumor rejection, idiotypic responses are documented for the suppressor cell compartment [17]. As diagrammed in Fig. 1C, exposure to tumor antigen (or antigen-antibody complexes) leads to the generation of antigen-specific suppressor T cells (T_S). These have been shown to function as inducer cells in several well-studied tumor systems, triggering and amplifying tumor antigen-specific suppression [18]. The T_S are likely to carry idiotypic markers, since both polyclonal and monoclonal T_S derived suppressor factors, as well as suppressive serum factors from tumor-bearing mice, could be bound to and eluted from affinity absorbents bearing Ab2-like antibodies [10, 11, 19]. If Id+ T_S are directly induced by antigen stimulations, as in the model shown in Fig. 1C, it is possible that these cells, like the T_H in Fig. 1B, serve as stimuli for the generation of anti-idiotypic Ab2, thus substituting for the apparent lack of Ab1.

In a model in which idiotypic T_S are generated subsequent to stimulation by antigen, an alternate pathway should also be considered. As diagrammed in Fig. 1D, Id+ T_S may arise as a consequence of anti-idiotypic stimulation, and would substitute

for an Ab3-like compartment. Some recent observations suggest that this model has major implications for therapy with anti-idiotypes, since these might preferentially activate T_S. In an analysis of IgH-restricted T cell responses to the hapten azobenzenearsonate, the nature of immunoglobulin idiotopes was found to determine the development of T_S idiotopes [20, 21]. T cells developing in IgH congenic mice acquired the idiotypic repertoire of the host, and treatment of neonatal animals with antibodies to the μ immunoglobulin chain abolished the establishment of a normal repertoire of functional T cell idiotypes. Thus, the immunoglobulin compartment could determine the development of complementary T cell recognition elements.

It appears that the idiotypic pathway depicted in Fig. 1D also exists in the context of tumor antigen recognition. Evidence supporting this view comes from studies on mice that had either sarcomas or bladder carcinomas induced with MCA. In both of these cases, a single dominant T cell idiotope was found to be prevalent in the suppressor response mediated by both T_S and soluble factors [11, 22]. That is, instead of a heterogeneous mixture of idiotypes, some shared 'public' idiotopes predominate. This was documented by removing suppressor factors, using affinity absorbents made from either monoclonal or polyclonal anti-idiotypic antibodies. The findings indicate that most of the apparent idiotypic specificities in the suppressor response to any particular tumor antigen are shared.

In model systems, the presence of 'public' idiotopes has been attributed to regulatory idiotopes important for network interactions [23]. The presence of such putative regulatory idiotopes in antitumor immunity may reflect either a genetic selection in the generation of idiotypic T_S, or an influence of immunoglobulin selection in the generation of the T_S repertoire. If the latter is the case, the induction of idiotypic T_S may include the pathway shown in Fig. 1D, in which the Ab2 response influences the nature of T_S.

Manipulation of idiotope expression by anti-idiotypic antibodies

There are essentially two approaches to using anti-idiotypic antibodies to manipulate the immune response to tumor antigens for therapeutic benefit. One is based on selecting and amplifying pre-existing antitumor idiotopes within the T and B cell repertoires, and the other entails the priming of a de novo response using an anti-idiotype that acts as an internal image of tumor antigen. We will begin by considering the first model, discussing therapeutic efforts based on a reversal of the pathways illustrated in Fig. 1, in which the Ab2 compartment is used as the initiator of idiotypic interactions. We will then discuss the use of the internal image type of Ab2 and how such Ab2 are best identified.

There are three pathways by which Ab2 can induce tumor antigen-specific immunity (Fig. 2). Fig. 2A shows that immunization with Ab2 can lead to the development of antigen specific T_H. This has been accomplished against infectious agents, and was found to give protection from disease [24–26]. Analogous findings have been made for several chemically and virally-induced tumors [27–30]. These experiments have utilized Ab2 arising as a result of an antigen-induced idiotypic cascade, as well as Ab2 induced by immunization with antigen-specific T cells.

An analogy can be drawn between the tumor-immune situation and what has been learned about immunity to infectious agents in neonatal animals. Neonatal mice do not normally develop antibodies against capsular polysaccharides of E. coli, which leaves them susceptible to infection [31]. Immunization with anti-idiotypic antibodies, however, induces protective immunity [31]. By analogy with findings made with haptens, this protection is probably caused by the ability of the anti-idiotypic antibodies to stimulate 'silent' clones; i.e., clones that are normally suppressed even in genetically competent individuals [32]. Thus, the anti-idiotypic antibodies appear to be able to 'reprogram' the immune system to generate antibodies that would not otherwise be made (Fig. 2B).

For Ab2 to select and stimulate both Id+ B and T cell clones, shared idiotopes between B and T cell

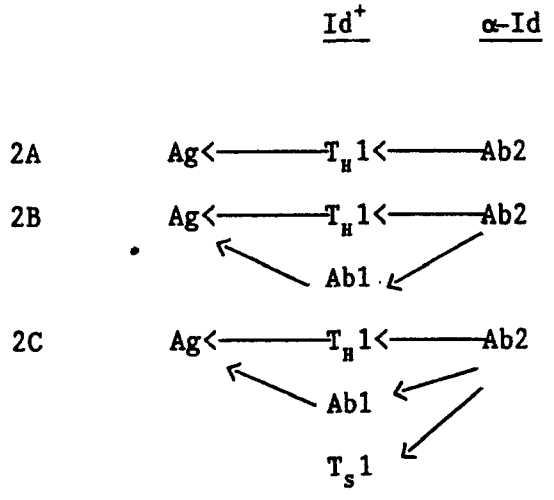

Fig. 2. Three pathways by which Ab2 can induce antitumor immunity. The elicitation of normally 'silent' Ab1 specificities is illustrated, as is the possible preferential triggering of Id^+ T_S (2C).

receptors must exist [1, 33, 34], corresponding to antigen-specific receptors that confer the same antigen specificity for both B and T cells. As discussed above, it is difficult to identify Id+ B cells that share idiotopes with tumor-immune T cells. This poses a problem in selecting appropriate Ab2 that can be used as immunogens for T cell immunity. Not only might B and T cell idiotopes differ, but different T cell subsets could potentially carry different idiotopes; an Ab2 population that arises in the course of a naturally occurring antitumor response might be directed at several different specificities that could select and stimulate quite diverse responses. One technique for circumventing this potential problem is to raise Ab2 experimentally by immunization with antigen-specific T cells [35]. In this case, the T cell receptor is used as the stimulatory idiotype so that the Ab2 population will be targeted specifically to the T cells of choice. Immunization with such an Ab2 would then be expected to select and stimulate T cell clones that share receptor idiotopes with the immunizing cells.

Although this approach to selecting specific Ab2 may seem impractical, recent advances in T cell cloning techniques suggest that this is a viable possibility for tumor therapy. Several investigators have reported successful cloning of tumor-infiltrating T lymphocytes recovered from tumor biopsies or surgical specimens. These lymphocytes, after culture *in vitro* with IL-2, are effective in eliciting antitumor responses when reinfused to the host [36]. Such cells should be ideal immunogens for raising Ab2 directed against the particular idiotopes associated with such antitumor clones. These Ab2 would then serve as anti-idiotypes that would potentially select and amplify T cells with similar antitumor specificity.

As discussed above, anti-idiotypic specificities in tumor-immunized mice appear to be complementary not only to T_H, but also to T_S and soluble suppressor factors [10, 11, 28–30]. The evidence that anti-idiotypic antibodies recognize a dominant public idiotope on T_S-derived factors suggests that there is a regulatory relationship between the Id+ cellular compartment and the Ab2 compartment [11, 14, 22]. It is then possible that the T_S compartment is preferentially activated by the administration of anti-idiotypic antibodies (as indicated in Fig. 2C). This may explain results in which animals immunized to certain pathogenic viruses by injection of anti-idiotypic antibodies can be more susceptible than controls to virus infection, rather than being protected from infection [37].

The concern that Ab2 immunization might activate suppressor cells is a theoretical obstacle to anti-idiotypic therapy for cancer. However, it may be possible to overcome this problem by proper manipulation of the idiotypic networks. Such manipulation revolves around three issues, which will be considered separately: The genetic restriction between anti-idiotype and the T cell compartment; the route of anti-idiotype administration; and the choice of idiotope specificity. Subsequently, we shall discuss the concept of internal image antibodies.

Genetic restriction

Two types of genetic restriction, MHC restriction and IgH restriction, present potential barriers to cellular recruitment by injected anti-idiotypes. MHC restriction confers an element of genetic control of T cell activation in an antigen-specific re-

sponse. To the extent that an anti-idiotype mimics antigen in the recognition process, it is logical to consider possible requirements for histocompatibility. Indeed, examples have been reported in which antigen-primed T cells are MHC restricted not only for their specific antigen recognition, but also for their ability to recognize cellbound anti-idiotype *in vitro* [38]. In other words, anti-idiotype stimulation of the T cells requires presentation in the appropriate MHC genetic context.

What are the implications of these findings for the *in vivo* administration of anti-idiotype? In a study using anti-idiotype priming to generate antiviral immunity, a genetically non-restricted response was observed [38]. This may have been due to the direct recruitment of T_H that subsequently activated other T cell compartments. Thus, this is not necessarily a barrier preventing T cell recruitment, even if the MHC restricting element for anti-Id presentation is different from that of nominal antigen presentation. With an appropriate route of administration (see below), anti-idiotypic antibodies should be introduced to the immune system by antigen-presenting cells in such a way that they may be 'seen' in the context of the right restriction elements. In fact, a host that is a genetic nonresponder to the nominal antigen may conceivably respond to challenge with anti-idiotype, depending on the mechanism maintaining the non-responsiveness. It thus appears that MHC restriction is of minor importance except when cell-bound anti-idiotype is used, and that anti-idiotype administration should seek to optimize the presentation of the Ab2 immunogen to the host immune system.

The issue of IgH restriction, i.e., the necessity for genetic matching of allotypic markers associated with immunoglobulin genes, is of greater concern. Since variable region antibody genes are linked to constant region genes, the genetic potential for specific idiotypic determinants is linked to Ig allotypic markers. As extensively documented by studying immunity to model antigens, IgH restriction governs many steps in the idiotypic cascade [17, 34, 39]. In essence, it acts as a permissive barrier that requires the presence of appropriate V genes and linked allotypic markers for idiotype-anti-idiotype recognition.

Immunization with anti-idiotypic antibodies has been used to recruit T_H that elicit tumor-specific DTH in mice, and this was shown to occur in an IgH restricted fashion [9]. In these experiments, an anti-idiotypic antibody was raised in BALB/c mice and was used to immunize mice of the same strain, as well as of strains CAL.20 and CB.20 (which are congenic with BALB/c for IgH genes). Tumor-specific DTH reactions were only obseved in the BALB/c mice, implying that only the presence of the BALB/c (IgH-1a)allotype permitted communication between Ab2 and the T_H compartment.

As discussed previously in the context of antiviral immunity, a strict IgH restriction of the immune response to Ab2 probably reflects requirements for direct recognition of network V genes; this has been referred to as a 'true idiotypic' interaction [40]. This requirement poses stringent limitations on the type of antibody that can be used as an anti-idiotype immunogen, since it would need to elicit complementary V genes in the host. Thus IgH restriction represents one of the probable causes of failure of immunization with anti-idiotype, since many experimentally derived anti-idiotypic antibodies are not IgH-matched with the host.

When is IgH-matching for anti-idiotypic administration unnecessary? Anti-idiotypic antibodies that act as internal images of the nominal antigen can substitute for this immunization with antigen. Since such de novo immunization is not based on specific selection of network V genes, internal image immunogens are generally not IgH restricted [40] (internal image Ab2 are discussed in the Section Internal image antibodies, below).

Route of immunization

As when immunizing with antigen, it is likely that the route of immunization with anti-idiotype will influence the nature of the immune response. One of the model systems in which this has been studied is based on immunization to reovirus. Reovirus-specific immunity could be established following immunization against idiotypic determinants, and DTH, cytolytic T cells, and antigen-binding antibodies have been observed. If soluble Ab2 was

used as the immunogen, only the DTH response was seen, while immunization with cell-associated anti-idiotype, in the form of a hybridoma-producing Ab2, also induced cytolytic T cells [38]. When Ab2 is used as an internal-image immunogen, as in the reovirus system, it essentially substitutes for antigen in the initial priming; many of the immune manipulations that facilitate antigen responsiveness also augment immunity to Ab2. For instance, several reports have documented success with anti-Id immunization when the Ab2 is coupled to an immunogenic carrier, such as LPS, or cross-linked with glutaraldehyde [41]. Allotypic determinants on the Ab2 molecule itself can also apparently be used. In the reovirus system, a vigorous Ab3 response was seen when the immunization with Ab2 crossed allotypic barriers, that is, when the host was IgH mismatched [38]. This suggests that Ig allotypic determinants on the anti-idiotype acted as helper determinants towards augmenting the immune responses.

Most experimental attempts to induce immunity with anti-idiotypic antibodies have utilized either subcutaneous (s.c.) or intramuscular injection of the antibodies in the presence of various adjuvants.

In the case of nominal antigen immunization, administration of a haptenated protein s.c. with adjuvant yields a vigorous T_H response, while the same antigen administered intravenously (i.v.) preferentially induces T_S, suggesting that i.v. administration bypasses the pathways for the normal T_H presentation and efficiently activates T_S [42]. There are two situations in which soluble Ab2 may occur i.v. and play a potential role in the induction of T_S. First, as part of a naturally occurring idiotypic cascade, Ab2 may direct and program the generation of specific T_S, as discussed above (Fig. 1D). Second, circulating Ab2 may occur as an iatrogenic complication when monoclonal antitumor antibodies are used therapeutically and some of the host response is anti-idiotypic [43]. A reasonable therapeutic concern is that such a response might lead to T_S induction by the Ab2 that are IgH compatible with the T_S, since both arise in the same host. Direct experimental evidence for this is lacking, however, and the opposite situation has been reported by Koprowski *et al.* [44], in which an anti-idiotypic response to injected mouse antitumor antibodies was apparently accompanied by a therapeutic effect in the patient.

There is also evidence (Yeh *et al.*, manuscript in preparation) that Ab2 can effectively inhibit the ability of an Ab1 to mediate antibody-dependent cellular cytotoxicity or kill tumor cells in the presence of complement. Route of Ab2 administration, as well as doses used, are likely to have an important impact on the kind of response induced.

Idiotope specificity

Complex antigens, including tumor antigens, contain multiple epitopes. The immune recognition which they evoke, therefore, contains many idiotopes that are determined not only by the heterogeneity of the epitopes, but also by the heterogeneity of the V genes selected among Ig and TcR genes in the host. These idiotopes are largely defined by the anti-idiotopes that they induce. Thus private idiotopes elicit Ab2 responses that are unique for a particular Ab1, public idiotopes elicit Ab2 to a specificityshared by many Ab1, and Ab2 of the internal image type are induced against antigen-binding structures on Ab1 that are complementary to the antigen [3, 45, 46].

Most investigations on idiotypic interactions have been performed in mice which were immune to haptens and in which public idiotopes were recognized. In such a system, a dominant Ab1 response with a shared public idiotope elicited a strong anti-Id response in mice of IgH-compatible strains. If anti-Id was given i.v. in neonates, or if anti-Id T_S were transferred, the public Ab1 idiotope was suppressed, and this was shown to cause the expression of alternate idiotopes on antigen-specific Ab1 [47].

When immunization with anti-Id is applied to tumor therapy, a similar shift in the idiotypic repertoire is likely to occur. Observations of idiotypic shift have, indeed, been made in the therapy of B cell lymphomas. These lymphomas carry Id+ immunoglobulins on their surface, which serve as the targets of administered anti-Id antibodies; that is, the lymphoma is used as an Ab1+ target, and a

xenogeneic Ab2 is given [48–51]. Several therapeutic responses have been documented. Tumor 'escape' is common, however, and occurs via an idiotypic shift that alters the Id+ immunoglobulin target and is presumably due to somatic mutation [50, 51]. B lymphomas treated with antibodies to surface idiotypes may not be accurate models for therapy with antibodies to idiotypes expressed on host lymphocytes. Nevertheless, the findings reflect one of the problems of therapy with anti-id, namely that there are shifts of idiotypic specificities within the Ab1 compartment. Although little is known about mechanisms for shifting idiotypic specificities in the T cell compartment, anti-Id induced selection for alternate T_H and T_S clones can be presumed to occur.

A practical reason for considering the role of idiotope selection is its dependence on the Ab2 used as immunogen. In a system with a dominant public idiotope on both T_H and T_S cells, anti-idiotypic antibodies may prime for T_H, if administered s.c. with adjuvant, or they may interact with soluble T_S factors. Studies on MCA-induced sarcomas in mice illustrate this (as outlined above).

In a system lacking a dominant public idiotope, or when no Ab1 is identified, the adaptability of the idiotope selection process offers some encouraging alternatives. Since administration of Ab2 can select for alternate idiotypic responses, antibodies to idiotopes that do not occur naturally in the tumor-bearing host might be chosen to direct an antitumor response. In other words, it may be possible to immunize with an Ab2 that selects a T_H repertoire which cannot be selected by exposure to tumor antigen. An attractive approach to this goal is to immunize with a monoclonal, internal-image Ab2 raised against a xenogeneic antitumor antibody. Many of the existing monoclonal antitumor antibodies are, indeed, specific for antigens that may not even be immunogenic in the tumor-bearing host [52]. The section Therapy with anti-idiotypic antibodies raised to tumor markers (below) summarizes recent experience in the use of Ab2 as immunogens, in which the Ab2 is directed to such antitumor Ab1.

Internal image antibodies

Ab2 immunization leads to Ab3 elicitation not only through specific V gene network interactions, but also by virtue of internal-image mimicry [3, 45]. That is, when the anti-idiotype represents the conformational mirror-image of the antigen, it may substitute for nominal antigen and elicit an Ab1like response [40]. Such anti-idiotypic antibodies, therefore, are desirable immunogens for tumor therapy in IgH-mismatched hosts. This is an important point, since the experimental generation, screening, andcharacterization of internal image anti-idiotypes differs from the approach for raising V-gene complementary 'true anti-idiotypes'.

While most polyclonal anti-idiotypic antibody preparations should theoretically contain both internal image and true anti-idiotypes, the desire to use monoclonal anti-idiotypes will lead to the need for more careful selection of the appropriate Ab2. Internal image anti-idiotypes compete *in vitro* with antigen for binding to idiotype-positive Ab1 and prime *in vivo* for Ab3 that mimic Ab1, and this priming occurs in an IgH unrestricted fashion [40].

The experimental verification that any particular Ab2 is an internal image type of anti-idiotype hinges on its ability to mimic the conformational characteristics that define recognition of the antigen. Thus, in addition to the inhibition of Id+ binding to antigen, internal image Ab2 substitute for antigen in terms of immune recognition: Ab2 may stimulate antigen-specific clones *in vitro* in the absence of antigen, or Ab2+ cells may serve as a target for antigen-specific CTL [33]. Since the Ab2 is substituting for antigen conformation, the Ab2 may be 'presented' to T cells in these assays in the context of MHC molecules, and therefore the response will appear to be MHC restricted, just as for antigen-specific response [33].

Studies of a variety of experimental models indicate that many naturally occurring Ab2 populations contain few internal image anti-idiotypes, whereas xenogeneic anti-Id antibodies more frequently succeed as internal image immunogens [40]. Still untested is the important possibility that internal image immunogens fail to stimulate suppressor cells, while at the same time inducing TH.

This supposition is based on the notion, outlined in Figs. ID and 2C, that regulatory idiotopes that are represented by the expression of dominant V genes determine the communication between Ab2 and T_S, and that some anti-idiotypic antibodies may be selected that lack this particular idiotope yet retain the internal-image characteristics priming for T_H.

Internal image antibodies with tumor antigen activity deserve serious consideration for use as tumor 'vaccines' towards induction of specific tumor immunity; for example, in patients whose primary neoplasms have been removed but who are at risk for development of metastases. In those cases where the tumor antigen is a protein, antigen prepared by recombinant DNA technology [53] may be the best vaccine to use, perhaps after modifications such as preparing constructs between the gene coding for the tumorantigen and a vaccinia virus gene. However, in those cases when the antigenicity resides in the carbohydrate part, or when the tumor antigen molecules have not been entirely characterized, it may be, practically, much simpler to employ anti-idiotypic antibodies. Whether such antibodies offer additional therapeutic advantages (as compared to antigen) remains to be investigated. (For further discussion of Ab2 in tumor therapy, see Section Therapy with anti-idiotypic antibodies raised to tumor markers below).

In view of the importance of internal image Ab2, better techniques for obtaining and characterizing such antibodies are needed. The ability of internal image Ab2 to compete with antigen for binding to Ab1 (and vice versa) is an integral part of their behavior. However, Ab2 that do not function as internal images may still compete, due to steric hindrance (and perhaps other mechanisms as well). We must now investigate the ability of an Ab2 to induce an immune response over IgH (and MHC) barriers in experimental animals as part of their characterization as potential internal images.

The distinction between internal-image anti-Id and 'true' anti-Id occasionally becomes blurred and difficult to ascertain. For example, in a recent study of monoclonal anti-idiotypes raised against a murine bladder carcinoma antigen-associated monoclonal antibody, immunization with the anti-idiotypes elicited a vigorous Ab3 response that lacked any detectable antigen-binding Ab1 [13]. The Ab3 raised against a particular anti-Id were apparently directed against 'private' specificities associated with that anti-idiotype. Although the anti-idiotypic antibodies were shown to inhibit antigen binding by the Ab1, this was presumed to be due to steric inhibition, and these data were interpreted to mean that the Ab2 were not internal image antibodies. Surprisingly, however, these same monoclonal Ab2 were able to prime mice for antitumor responses and to bind to tumor-specific T cell suppressor factors [12, 22]. Since the anti-idiotypic monoclonal antibodies in these experiments were raised in mice, and were directed against xenogeneic antitumor monoclonal antibodies raised in rats, the antitumor response elicited in mice by Ab2 immunization would not be expected to be based on specific V gene selection, and could instead be attributed to some internal image Ab2.

A second example comes from experiments analysing idiotypic responses to TMV-associated antigens, in which mice were immunized with rabbit anti-idiotypic antibodies that were specific for 'private' rabbit idiotopes [54]. Surprisingly, these mice made anti-TMV antibodies that were idiotypically cross-reactive with the rabbit idiotopes. This study directly demonstrated that the stimulatory anti-idiotype was not an 'internal image' specificity, yet it nevertheless elicited antigen-specific information in the face of apparent V gene incompatibility.

Therapy with anti-idiotypic antibodies raised to tumor markers

A wide variety of cell surface antigens have sufficient selectivity for tumor to serve as tumor-associated markers [7]. Antibodies (Ab1) have been used to characterize the relative specificity of such antigens, and to characterize them from the molecular point of view. Some of the antigens are unique for individual tumors [55], some are encoded by viral genomes that are involved in the neoplastic transformation [56], and still others are shared by many tumors [52]. Most of the antigens are differentiation ('oncofetal') antigens and fall into the last category.

In the light of hindsight, it seems likely that the treatment of rats with so-called unblocking antitumor antibodies, as pioneered by Bansal and Sjögren in the early 1970s, involved anti-idiotypic antibodies [57]. In this case, rats with transplanted or primary polyoma virus-induced tumors were treated with antisera that had been prepared in rats or rabbits by hyperimmunization with polyoma tumors and that had been absorbed with normal rat tissues. Striking *in vivo* responses were observed in the treated animals, including regressions of some primary kidney tumors and prevention of lung metastases [57]. These responses were probably the effect of anti-idiotypic antibodies, rather than of antibodies binding to tumor cells. The effects were not seen immediately after treatment, but rather after a latency period, as if they involved a modification of the host response to tumor, and they were accompanied by a decrease of circulating ('blocking') factors, which were detected in tumor-bearing rats by their ability to inhibit *in vitro* killing of tumor cells by immune lymphocytes [58]. The protocol used to generate unblocking antibodies was subsequently used to obtain Ab2 in a different tumor system [9, 19].

The experience with immunization against tumor markers using antibodies known to be anti-idiotypic (and not antitumor) is so far quite limited. One of the first such studies was done with human melanoma. Most melanomas are characterized by a relatively high density of the glycoprotein p97 ('melanotransferrin') at their cell surface [59]. p97 has been used as a marker for detection of tumor cells in histological sections, as well as for *in vivo* diagnosis of melanoma by nuclear imaging [60], and it may also serve as a therapeutic target [61]. Anti-idiotypic antibodies were raised in rabbits against a murine monoclonal antibody specific for p97 [62]. They could inhibit the binding between antibody and p97, and they elicited Ab1-like Ab3 in mice. A cellular immune response, detected as a DTH reaction, was elicited in mice primed with the anti-idiotype s.c. in adjuvant and challenged with human melanoma cells [62]. This response presumably involved the specific activation of anti-idiotypic T_H; since a xenogeneic anti-idiotype could be used, IgH determinants did not restrict it. Thus, the antitumor reactivity probably arose by antigenic mimicry, utilizing a direct pathway such as that outlined in Fig. 3.

Findings extending the observations made in the p97 system have been reported by Herlyn *et al.* [63]. These authors made a goat anti-idiotypic antiserum to a mouse monoclonal antibody which defined an oncofetal antigen expressed on many carcinomas from the digestive system. By using this antiserum, both polyclonal and monoclonal Ab1-like Ab3 could be induced.

Similar findings have been reported in an autologous murine tumor system, utilizing the T antigen of SV40-induced mouse sarcomas as the tumor target [27]. In this study, xenogeneic anti-idiotypic antibodies were raised against two murine monoclonal antibodies that had specificity for the SV40 T antigen. Although priming with the anti-idiotypes failed to induce Ab1-like antibodies to the SV40 T antigen, it led to increased survival times in mice inoculated with syngeneic SV40-induced sarcomas. In some cases, visible tumor growth was prevented in the treated mice. The cellular events responsible for this effect were not identified, but they presumably included activation of T_H for DTH-like responses and/or recruitment of cytolytic T lymphocytes (CTL). In a separate study, anti-idiotypic antibodies primed rats for a cytotoxic T cell response *in vitro* against a syngeneic tumor [28].

A detailed analysis of the idiotope specificity associated with immunity to a murine carcinoma was recently performed using mouse monoclonal anti-idiotypes made against a rat monoclonal antibody that recognizes a glycoprotein (gp170) associated with mouse bladder carcinomas [13]. Priming with Ab2 induced DTH reactivity *in vivo*, and anti-gp170 reactive Thy 1^+, Lyt1^+ lymphocytes were detected *in vitro* using a leukocyte adherence inhibition assay [12]. However, no antigen-binding Ab3 could be detected in Ab2-treated mice, although the Ab2 was syngeneic to the host [13]. These data are consistent with the model, shown in Fig. 3, in which separate pathways of Ab2 immunization are involved in T_H activation and the induction of an Ab1-like Ab3 response. Perhaps the T_H necessary for the production of Ab1-like antibodies differ from the T_H causing DTH. Alternatively, the spe-

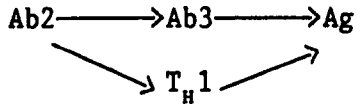

Fig. 3. Immunization with 'internal-image' α-Id to elicit anti-antigen responses.

cific V gene repertoire necessary for antigen-binding Ab3 is not expressed. In the analysis of SV40 T antigen immunity induced by anti-idiotype administration, as described above, a lack of antigen-binding Ab3 was also noted, in spite of an effective tumor protection [27]. In both of these cases, when anti-idiotype priming induced T_H, but not antigen-binding Ab3, the tumors were syngeneic to the host. The failure to elicit antigen-binding Ab3 is reminiscent of the lack of Ab1 in the naturally occurring antitumor response (see the Section General concepts, above), and it may reflect some fundamental characteristics of regulation of the antibody response to self antigens.

Treatment of a tumor-bearing animal with Ab2 may have several different effects. As already discussed, it may select and amplify T_H (or induce a *de novo* T_H response), leading to DTH, development of CTL, and, sometimes, to antigen-binding Ab3. An Ab2 may also inhibit suppressor cell activity and abrogate the activity of circulating Id+ suppressor factors. Evidence for this has come from studies done in mice with bladder carcinomas [22], showing that the ability of T_S to form suppressor factor *in vitro* could be abrogated by exposure to a monoclonal Ab2; as with other murine sarcomas [11], soluble suppressor factors could be removed by immunoadsorption with such Ab2.

Therapeutic benefit has also been reported in human patients with colon carcinoma in whom anti-idiotypic antibodies apparently arose in response to therapy with murine monoclonal antibodies [44]. In this study, treatment with antitumor monoclonal antibodies led to a beneficial antitumor effect; simultaneously, anti-idiotypic antibodies arose as an active immune response to the administered monoclonal antibodies. Although not yet analyzed, the antitumor effect may potentially be related to the anti-idiotypic antibodies, by any of the mechanisms listed above. Consistent with this model is the evidence that the time course and titer of anti-idiotype generation correlated with clinical response. This raises the possibility that monoclonal antitumor treatment may have a dual function, both directly in terms of antitumor reactivity and indirectly in terms of the elicitation of an anti-idiotypic response. Many issues, however, remain to be addressed, particularly the direct demonstration that idiotopes are shared between the administered monoclonal antibodies and the host immune system.

Conclusions

We have reviewed evidence that immunization with anti-idiotype can amplify pre-existing tumor-specific T cells and/or immunize against internal-image determinants shared by the anti-idiotype and the tumor antigen. Studies in animal systems indicate that the idiotypic response elicited by tumor antigen is predominantly cellular, with T_H and T_S components, while Ab1 are seen only infrequently. Hence, the Ab2 response seen after stimulation with antigen may recognize shared regulatory idiotopes associated with T_S cells and factors derived from such cells. Appropriate immunization with anti-idiotype can stimulate 'silent', that is, normally suppressed clones. Genetic restrictions at MHC and IgH loci, the route of anti-idiotypic immunization, as well as on the choice of idiotope specificity, all have an important impact on the therapeutic outcome.

Key unanswered questions

Several key questions remain unanswered, including the following: Will the use of xenogeneic internal-image anti-idiotypes as immunogens bypass normal restriction barriers associated with immunoglobulin V genes? What is the role of Ab3, if any, in the antitumor response elicited by anti-idiotypes? Should alternate immunization protocols be used to elicit T cells responsible for DTH compared to cytolysis? Will anti-idiotypes raised against T cell receptor-associated idiotopes prefer-

entially amplify $T_H$and not T_S? Do anti-idiotypes elicited by administration of monoclonal antitumor antibodies in patients play a therapeutic role?

The answers to these questions will determine the practical value of anti-idiotypes as tumor vaccines and of anti-idiotypic manipulations in the therapeutic management of tumor immunity. The experimental approach to resolving these issues must focus on characterizing the idiotope and anti-idiotope specificities. While many of these questions can be addressed in syngeneic animal tumor models, the specific issues of V gene repertoire induction in human patients in response to either anti-idiotype immunization or monoclonal antitumor idiotope administration is best addressed in the direct clinical setting. An additional complication, which cannot be resolved by studies in inbred animal strains, is the possibility that individual variation among different patients will alter the efficacy of any particular anti-idiotype used. At this time it is most appropriate to be cautious and curious about the therapeutic use of anti-idiotypes; it is not yet appropriate to be optimistic.

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