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Images of Biologically Active Structures in the Immune System

Their Use in Biology and Medicine

Edited by
H. Koprowski and F. Melchers

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Preface

The diversity of antigen-binding structures of antibody molecules is so vast that every conceivable antigen can be bound by an antibody molecule within the immune system. This is true even for the antigen binding sites of antibodies called idiotypes, which are bound by complementary binding sites of other antibodies called anti-idiotypes. Thus, anti-idiotypes are structural homologues of antigens. These idiotypic-anti-idiotypic interactions constitute a network within the immune system. Since one lymphocyte produces only one type of antibody molecule, this network is in fact a network of cells. We expect that the network is functional: the appearance of antigen will disturb the equilibrium of the network at the point where it competes with the anti-idiotypic lymphocyte for binding to the idiotypic lymphocyte. It has been known for quite some time that anti-idiotypic antibody can be used to prime the immune system for memory to an antigen that it has never seen. This phenomenon is now being explored for possible use in immunization against viruses, bacteria, parasites and tumors as well as for the modulation of autoimmunity. The ability of anti-idiotypes to mimic, both antigenically and functionally, the corresponding biologically active molecules seen by an idiotypic antibody was first demonstrated for the hormone insulin and is now being observed in many other systems. The papers assembled in this volume bring the reader to the cutting edge of the potential practical applications of the network theory of the immune system. They relate the search for molecules and their biological functions and for the regulation of the immune response itself in autoimmunity and vaccination.

Summer 1985 HILARY KOPROWSKI, FRITZ MELCHERS

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1 Introduction

The term idiotype (Id) or idiotypic determinant was originally proposed by OUDIN and MICHEL (1963) to designate antigenic determinants unique to a small set of antibody molecules. An Id defines the variable (V) region of the antibody molecule and often serves as a V region phenotypic marker. Because the V region of the antibody molecule also contains the antigen-binding region, the area of the antibody that makes contact with the antigen has been referred to as the paratope. It becomes important to remember that even within a single antibody molecule, different areas within the V region are capable of combining with different antigens. Thus, antigen binding to antibody requires sufficient complementarity between the antigen and antibody molecule to generate the attractive forces necessary for this reaction to occur. This complementarity or fitting of structural conformations plays a dual role in both antigen binding to an antibody molecule and antigen mimicry by potential anti-antibodies that recognize the conformation induced by the V region of an antibody molecule.

In early studies, antisera were generated against homogenous immunoglobulins, such as myeloma or Bence Jones proteins. After the appropriate adsorp-

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tions, these antisera reacted only with the myeloma protein used as the immunogen and not with other myelomas or conventional antibodies. Only rarely were exceptions to this antigenic individuality noted in these early studies. The first reports of antigenic individuality were based on a study of human myeloma proteins (LOHSS et al. 1953; SLATER et al. 1955) and extended to a series of conventional antibodies in humans (KUNKEL et al. 1963) and rabbits (OUDIN and MICHEL 1963). In each of these instances, the antisera generated against either the myeloma proteins or conventional antibodies recognized only the immunizing antibody. Cross-reactions with other myeloma proteins or antibodies were not detected. Thus, each antiserum detected unique or private determinants present on the V region of the immunogen. A term coined for the immunogenic potential of individual areas or determinants on the surface of the antigen-binding region that encompasses a single antibody molecule has been idiotope. Idiotope, by definition, implies monoclonality, and the unique collection of idiotopes on each antibody molecule is referred to as an Id. The Id is often defined by an antiidiotypic antibody (anti-Id or Ab2), whereby the Id can behave as an antigen and induce the production of antibodies against itself. It is noteworthy that the paratope may or may not be the same site as that recognized by the anti-Id. Therefore, the idiotope and paratope may represent distinct regions within the V region of an antibody molecule; the paratope being the area that binds antigen and the idiotope being the site that binds anti-Id.

The early studies on Id defined private or unique IdI determinants; however, shared Id that were common to more than one antibody preparation and that were found in numerous individuals have been termed by different investigators as public, shared, cross-reactive, or IdX determinants. Shared Id have been observed in a wide variety of species, with antibodies generated to numerous antigens. The detection of shared Id that are expressed on antibodies to a given antigen in different species (interspecies) suggested that these V region genes were conserved through evolution and most likely represent germ-line gene products. A distinction between shared and private Id is that shared Id appear to represent V region phenotypic markers and tend to be inherited, whereas private Id are not.

2 Anti-Id Modulation and Id Networks

The idea that the immune response to an antigen can be regulated via an Id-anti-Id network was first proposed by JERNE (1974). Id, located on or close to the antigen-binding site of both antibody molecules and lymphocyte antigen receptors, represents components of this network. Numerous studies have documented the manipulation of the immune response by injection of anti-Id. In particular, antigen exposure following injection of anti-Id resulted in either suppression of the Id-positive antigen-binding molecules (COSENZA and KOHLER 1972; EICHMAN 1974; HART et al. 1972) or increased Id expression and antigen-binding activity (BLUESTONE et al. 1981; CAZENAVE 1977; KELSOE et al. 1980; SACHS et al. 1981; TRENKNER and RIBLET 1975).

The generation of auto-anti-Id (reviewed by RODKEY 1980) provided further evidence that immune regulation involves a series of Id networks. Characterization of anti-anti-Id (Ab3) reagents revealed that in some instances these antisera were not able to bind the antigen used in the induction of the Id. However, within the framework of the network proposal, Ab3 can theoretically be produced with the specificity to bind to the original antigen by mimicking the Id. Thus, the anti-anti-Id has a structure or conformational fit that allows it to bind to the Id expressed on the surface of the anti-Id (Ab2). Alternatively, the Id of Ab1 itself has a structural conformation which allows the anti-Id favorable attractive forces for binding. Thus, the conformation of the anti-anti-Id (Ab3) should represent the mirror or internal image of the original Id of Ab1 and have the capacity to bind antigen. Several kinds of Ab3 molecules can be produced that differ in the ability to bind the original inducing antigen. Ab3 with antigen-binding capacity are induced by an Ab2 molecule, which has a structural conformation, such that it represents the internal image of the antigen.

In keeping with the concept that Id networks can regulate the immune response, T and B lymphocytes have been shown to share similar Id receptors (reviewed by BINZ and WIGZELL 1977 and KRAWINKEL et al. 1976). The generation of Id-specific suppressor T cells after administration of anti-Id in numerous murine antibody systems provided experimental evidence for cell regulation via Id networks (BONA and PAUL 1979; DOHI and NISONOFF 1979; EICHMANN 1975; YAMAMOTO et al. 1979). In addition, anti-Id generation of Id-specific helper T cells is also well documented (GLEASON et al. 1981; INADA et al. 1982; MILLER et al. 1981; SACHS et al. 1981; WOODLAND and CANTOR 1978). These data indicate that Id networks operate in regulation of the immune response to a given antigen.

3 Anti-Id Modulation of Viral Antigen Systems

The focus here will be on Id networks associated with hepatitis B virus infections, and these studies will be described in detail later in the chapter. However, much work has recently be done using anti-Id to modulate numerous viral antigen systems and a brief description of this work will be given below.

The first studies involving anti-Id for analysis of viral antigen systems were performed using tobacco mosaic virus (TMV) capsid protein (URBAIN et al. 1980). A single anti-Id, produced in a rabbit, against antibody to TMV detected a shared Id present on anti-TMV produced in rabbits, mice, horses, goats, and chickens immunized with TMV. In addition, this anti-Id induced the formation of anti-TMV when injected into mice without subsequent antigen challenge. The anti-Id represented the internal image of TMV and was capable of inducing an antibody response without ever injecting the antigen. Although TMV does not represent either a human or animal pathogen, these studies opened the door for others to study Id networks associated with viral pathogens. In addi-

tion, these studies implicated anti-Id as possible vaccines for viral infections via antigen mimicry.

Anti-Id have also been produced to characterize the hemagglutinin receptor for reovirus on both T lymphocytes and susceptible host cells (ERTL et al. 1982; NEPOM et al. 1982). Subsequent work indicated that anti-Id preparations could be used to block reovirus infection by competitively binding to cellular receptors (KAUFFMAN et al. 1983; NOSEWORTHY et al. 1983). The vaccine potential of a monoclonal anti-Id was demonstrated by its ability to induce cellular immunity to reovirus in BALB/c mice (SHARPE et al. 1984).

Anti-Id has also been demonstrated to induce neutralizing antibodies to rabies virus glycoproteins without subsequent antigen challenge (REAGAN et al. 1983). These studies again suggest the potential for an anti-Id based vaccine utilizing an anti-Id that may represent the internal image of a rabies virus glycoprotein important in inducing protective immunity.

Recently, a mouse monoclonal anti-Id was generated against a Sendai virus-specific helper T cell clone (ERTL and FINBERG 1984). This anti-Id was capable of inducing in mice an anti-Sendai virus immune response in vivo and further indicated that a T cell-defined anti-Id was capable of stimulating both B and T cell immunity which was induced by the anti-Id appeared to be less genetically restricted to the murine major histocompatability complex than was Sendai virus antigen-specific T cell immunity. More importantly, injection of anti-Id without subsequent antigen exposure produced immunity that was capable of protecting mice against a lethal infection with Sendai virus.

To date, the only viral antigen system where anti-Id has produced deleterious effects to the immune system has been with herpes simplex virus (HSV). The injection of anti-Id prior to a challenge with a 50% lethal dose of HSV resulted in a decrease in the survival time of mice (KENNEDY et al. 1984a). It was concluded from this study that the immune response to HSV in mice can be modulated through Id-anti-Id networks, which result in an increase in the pathogenicity of HSV infections. Although this study does implicate potential deleterious effects of anti-Id in modulating the immune response to infectious agents, numerous other studies (see above) have indicated the potential of anti-Id, that mimics viral antigens, as virus-free based vaccines.

4 Hepatitis B Virus

Hepatitis B virus (HBV) infects 175 million people yearly and represents a major worldwide health problem. The presence of HBV is often associated with the development of chronic liver disease and primary hepatocellular carcinoma (MAUPAS and MELNICK 1981). Fortunately, the majority of infected individuals recover completely, with the virus being eliminated and hepatic injury resolved. However, a chronic carrier state may occur in 5%–10% of HBV infections, whereby infectious HBV persists. In most patients who develop a chronic carrier disease, biochemical and histologic abnormalities are minimal to absent, but in others, hepatocellular injury may progress and lead to cirrhosis

and carcinoma. It has been demonstrated that antibodies against hepatitis B surface antigen (anti-HBs), the surface or envelope material of HBV, are protective against infections, whereas antibody to the core antigen (HBcAg) has no protective effect. Serologically, hepatitis B surface antigen (HBsAg) contains within its structure a group-specific determinant(s) termed *a* and two sets of mutually exclusive allelic subtype determinants, *d* or *y* or *w* or *r*. Combinations of the *a* determinant with the various subtype determinants result in four major serotypes associated with HBsAg: *adw*, *ayw*, *adr*, and *ayr*. The group-specific *a* determinant(s) has been shown to induce protective antibodies against HBV (SZMUNESS et al. 1980).

At the present time, a vaccine for HBV is available and produced from the plasma of persons chronically infected with HBV by purifying HBsAg and treating the particles with disinfecting agents. Although this vaccine, licensed by Merck, Sharpe, and Dohme, has been proven safe and effective, its high cost and potentially limited availability preclude its use in developing countries, where HBV constitutes a major health problem. Thus, alternative approaches for an HBV vaccine are actively being pursued. The two major approaches under investigation for the preparation of a well-defined HBsAg-specific vaccine utilize recombinant DNA or synthetic peptide technology. A third possibility for an HBV vaccine lies within the confines of an anti-Id preparation that represents the internal image of HBsAg. Our laboratory has been actively pursuing the latter possibility.

5 Id Networks in HBV Infection: Characteristics of a Common Human Anti-HBs Id

Our impetus to study Id associated with antibodies to HBsAg (anti-HBs) came from the knowledge that anti-HBs, but not antibodies to the nucleocapsid core antigen, was protective against hepatitis B virus (HBV) infection. We initially generated four rabbit anti-Id antisera against affinity-purified anti-HBs from two different, naturally infected individuals. Each of the four anti-Id antisera detected a common anti-HBs Id (KENNEDY and DREESMAN 1983). A single anti-Id preparation was selected for further study. The common human Id was detected in purified anti-HBs from three individuals and also in anti-HBs positive sera obtained from six hemophilic patients. More recently, we have detected this Id in anti-HBs sera from 30 of 32 individuals who were either naturally infected with HBV or immunized with the HBsAg vaccine (KENNEDY et al., unpublished results). It is not known whether the two individuals who were negative for the anti-HBs Id failed to express that particular Id or expressed it at levels too low to be detected by the Id-anti-Id reaction. We are in the process of obtaining larger amounts of serum from these two individuals in order to affinity-purify the anti-HBs and test these preparations for inhibition of the Id-anti-Id reaction.

The ability of both HBsAg and a virus-derived HBsAg native polypeptide to inhibit the Id-anti-Id reaction suggested that the anti-HBs Id was associated

with or was near the antibody-combining site. Id was detected because the anti-Id antisera did not interact effectively with IgG preparations from (a) the Id donor after removal of anti-HBs, (b) a pool of human sera negative for anti-HBs, and (c) an individual with a high level of antibody to herpes simplex virus. Attesting further to the Id specificity was the inability of the anti-Id to bind either HBsAg or the native HBsAg-derived polypeptide. These data indicated that we were detecting a common human anti-HBs Id associated with the antibody-combining site (KENNEDY and DREESMAN 1983).

Further characterization of the common Id revealed that it was induced by the group *a* determinant, because three HBsAg preparations purified from three pools of human plasma positive for HBsAg *adw*, *ayw*, or *adr* subtypes inhibited the Id-anti-Id reaction equally on a weight basis. We also tested the ability of HBsAg-derived polypeptides to inhibit the Id-anti-Id reaction and found that sodium dodecyl sulfate-denatured HBsAg viral polypeptides virtually lost their capacity to inhibit compared with a native polypeptide preparation. In addition, reduction of the disulfide bonds and alkylation of free thiol groups destroyed the ability of the native HBsAg-derived polypeptide to inhibit the Id-anti-Id reaction. These data suggested that the common anti-HBs Id was directed against a conformation-dependent group-specific *a* epitope (KENNEDY et al. 1982). Again, it is of note that the *a* determinant(s) of HBsAg induces protective immunity against HBV infection.

We have tested the ability of a cyclic synthetic peptide (peptide 1, containing amino acid residues 122–137 and being analogous to the major polypeptide, P25, of HBsAg) to inhibit the common Id-anti-Id reaction. On a molar basis, this peptide was 10^3-fold less efficient than intact HBsAg in inhibiting the Id-anti-Id reaction. The inability of peptide 1 to compete equally on a molar basis with HBsAg indicates that this peptide does not represent the complete *a* determinant that is recognized by the Id-anti-Id reaction and moreover suggests that other amino acid sequences are also important in defining the complete *a* epitope. However, inhibition of the Id-anti-Id reaction by peptide 1 suggested that this sequence is related to antigenic determinants responsible for eliciting a population of human anti-HBs expressing a common Id. The importance of conformation of the epitope was again tested by reducing the disulfide bond in peptide 1 and alkylating the free thiol groups. This treatment destroyed the ability of peptide 1 to inhibit the Id-anti-Id reaction (KENNEDY et al. 1983b). It could be argued that inhibition of the Id-anti-Id reaction with relatively large HBsAg particles was due to nonspecific steric hindrance that potentially could have resulted. However, the ability to inhibit the Id-anti-Id reaction with this relatively small molecule (mol. wt. 1704) strongly supports the above conclusion that the anti-Id recognized the antibody-combining site.

In addition, an interspecies Id cross-reaction was detected on anti-HBs produced in rabbits, guinea pigs, swine, goats, chimpanzees, and BALB/c mice immunized with HBsAg. Expression of the Id in sera from these other species was intrinsically associated with anti-HBs positive molecules. We also determined that anti-HBs from chickens successfully immunized with HBsAg failed to express the common Id (KENNEDY et al. 1983c). It was confirmed by adsorption studies that the interspecies Id was associated with anti-HBs. We also

Table 1. Expression of the interspecies Id by monoclonal anti-HBs that recognizes distinct determinants

Monoclonal antibody	Percentage inhibition	
	Cyclic peptide[a, b] 122–137	Id-anti-Id reaction[c]
A-2	36–88	24
A-4	31–64	25
A-7	0	0
A-12	53–67	26
A-16	0	0

[a] The cyclic peptide was utilized to inhibit the binding of the monoclonal anti-HBs preparations to intact HBsAg particles

[b] The inhibition values obtained using various inhibitor concentrations of the cyclic peptide, ranging from 500 ng to 10 μg, are shown

[c] The monoclonal anti-HBs preparation was used to inhibit the Id-anti-Id reaction

selected a series of mouse monoclonal anti-HBs preparations which differed in their ability to bind to cyclic peptide 1 and tested these anti-HBs preparations for the expression of the interspecies Id. Previous studies had suggested that the expression of the interspecies Id was determined by the specificity of a given monoclonal anti-HBs preparation for cyclic peptide 1 (IONESCU-MATIU et al. 1983). As shown in Table 1, the Id-anti-Id reaction appeared to recognize an anti-HBs response that was induced by determinants associated with cyclic peptide 1. Monoclonal anti-HBs preparations that either failed to bind cyclic peptide 1 or whose binding to intact HBsAg was not inhibited by cyclic peptide 1 failed to inhibit the Id-anti-Id reaction. Only those monoclonal anti-HBs preparations that were induced by determinants associated with cyclic peptide 1 expressed the interspecies Id. These findings indicate that the ability to respond to a distinct HBsAg epitope is highly conserved in mammalian species, but may not be shared by avian species. In addition, there appears to be a heterogeneity in the immune response to HBsAg, and the Id inhibitory capacity for the Id-anti-Id reaction does not appear to be based on total anti-HBs levels, but rather on the antibody response to the HBsAg epitopes associated with amino acid sequences 122–137. The Id-anti-Id reaction may represent a means by which the potential heterogeneity of the immune response to HBsAg can be measured.

6 Priming of the Anti-HBs Immune Response by Prior Injection of Anti-Id

The modulating effects of in vivo administration of anti-Id that recognized the common anti-HBs Id prior to antigenic challenge with HBsAg have been

Table 2. Priming of the anti-HBs response by prior injection of anti-Id[a]

First injection	Second injection	Mice (*n*)	Anti-HBs Response[b]	
			Range	Mean
Pre-IgG	Peptide 122–137	6	0–10	4
Anti-Id	Peptide 122–137	7	10–50	38
Pre-IgG	HBsAg	5	10–50	34
Anti-Id	HBsAg	10	1250–31250	9100
HBsAg	HBsAg	6	6250–156250	35620

[a] BALB/c mice received alum-precipitated antibodies (50 μg) or HBsAg (6 μg) on day 0, followed by either 6 μg HBsAg or 50 μg peptide on day 14; mice were bled on day 26

[b] Reciprocal of the anti-HBs endpoint titer, which has been described elsewhere (KENNEDY and DREESMAN 1984)

studied in mice at both the serum and cellular levels. The injection of anti-Id prior to HBsAg resulted in an increased number of spleen cells secreting IgM anti-HBs (KENNEDY et al. 1983a). Spleen cells also were induced to secrete anti-HBs anti-Id only without antigen exposure. Serologically, it was determined that anti-Id given in saline induced predominantly an IgM anti-HBs response, whereas alum precipitated anti-Id produced an IgG anti-HBs response when administered prior to HBsAg (KENNEDY and DREESMAN 1984). Similarly, anti-Id were found to enhance the anti-HBs response to cyclic synthetic peptide 1 (KENNEDY et al. 1984c). The injection of anti-Id prior to immunization with cyclic peptide 122–137 increased the anti-HBs response compared with groups of mice that received control antibodies prior to the peptide. Although the anti-Id in conjugation with the peptide produced low levels of anti-HBs compared with anti-Id and HBsAg, the anti-HBs titers in these mice were comparable with those obtained in mice receiving a single injection of HBsAg. It was noteworthy that a single injection of anti-Id failed to produce a detectable anti-HBs response and could only prime the antibody response to a subsequent challenge with antigen. Also, the anti-HBs response generated in mice receiving anti-Id and cyclic peptide 1 was associated with the group specific *a* determinant(s) of HBsAg. The data on anti-Id priming of the anti-HBs response are summarized in Table 2.

7 Anti-Id Bearing the Internal Image of HBsAg

In a previous study, we demonstrated that BALB/c mice receiving anti-Id alone generated IgG anti-HBs secreting spleen cells (KENNEDY et al. 1983a). These data indicated that anti-Id alone could induce anti-HBs response. Mice that received two injections of anti-Id without subsequent HBsAg injection produced an anti-HBs response that was found to express the interspecies idiotype. In addition, the anti-Id induced anti-HBs recognized the group-specific *a* determinant(s) of HBsAg (KENNEDY et al. 1984b). The injection of anti-Id alone pro-

duced an antibody response that appeared to serologically reflect the internal image of HBsAg. That is, the anti-HBs expressed the interspecies Id and recognized the group-specific *a* determinant. Alternatively, the potential role of anti-Id as vaccines for HBV infection was demonstrated by the fact that anti-Id immunization alone induced an anti-HBs response that shares an Id expressed in a human anti-HBs response which resulted from a natural HBV infection. This anti-HBs response protects these individuals from subsequent re-infection with HBV. Also, the specificity of the anti-HBs response produced by anti-Id injection was directed against a determinant(s) on HBsAg that is responsible for inducing protective immunity. Whether or not the anti-Id represents a possible vaccine for HBV must await further testing, since only chimpanzees and humans can be infected with human HBV. We are in the process of testing this possibility by anti-Id induction of anti-HBs in chimpanzees.

8 Does the Anti-Id Induced Anti-HBs Represent an Anti-Anti-Id Response?

The fact that the anti-Id preparation appears to represent the internal image of HBsAg raised the question of whether the anti-HBs response produced by anti-Id injection represented the induction of anti-anti-Id (Ab3). In order to answer this question, we selected two affinity-purified anti-Id preparations produced in different rabbits which appeared to represent the internal image of HBsAg by their capacity to induce anti-HBs in mice. The two rabbits used in the anti-Id generation were rested for a period of 14 months following their last injection of human anti-HBs Id. These rabbits were then immunized with

Table 3. Induction of an anti-anti-Id response with HBsAg binding activity in rabbits[a]

Rabbit	Antiserum obtained after:	Anti-HBs titer[b]
#1	Pre-immune	<2[c]
	Primary injection	<2
	Secondary injection	10
	Tertiary injection	50
	Quaternary injection	250
#2	Pre-immune	<2
	Primary injection	<2
	Secondary injection	50
	Tertiary injection	6250
	Quaternary injection	31250

[a] Each rabbit received 500 μg of its respective anti-Id preparation as an alum precipitate in complete Freund's adjuvant at monthly intervals. Serum was obtained prior to each immunization

[b] Reciprocal of endpoint anti-HBs titer using a solid-phase radioimmunoassay with HBsAg coated wells and ^{125}I-labeled goat antirabbit-γ-globulin. Each serum was diluted in fivefold increments using 10% normal goat serum as a dilutent

[c] No anti-HBs activity at a 1:2 dilution of serum