Strange Connections in the Unspecific Parallel Set

George K. Lewis, Peter V. Hornbeck

Department of Microbiology and Immunology, University of California at San Francisco, Calif., USA

Introduction

Is the immune system as specific as an immune response? Immune responses have been investigated with the best available physicochemical methods since the time of *Arrhenius* [1], and by all accounts, they have been judged to be specific. On this basis, why pose the opening question? Is it not safe to assume a priori that the immune system is no less specific than the response it produces? Apparently not! A different view of the immune system is emerging in which the perturbation of a lymphocyte circuit by a specific antigen is signalled to other lymphocyte circuits which have no specificity for the exciting antigen. In order to have specific immune responses under these circumstances, the parallel circuits (e.g., the circuits having no specificity for the priming antigen) should give only transient positive responses if they are allowed to respond at all. Since positive responses in the parallel circuits should be dampened, these circuits must be connected in some way with the primary circuit (e.g., the one stimulated by antigen). Mechanistically, lymphocyte circuits of dissimilar specificity can be coupled with one another by having their receptors, factors, or antibodies

share idiotypy. In this view, the immune system is an aggregate of idiotypic families each containing individual members which are specific for different epitopes, but which are also commonly bonded by shared idiotypy. Our aim in this review is to briefly summarize our recent findings supporting the existence of idiotypic families whose members are not only specific for different antigens but which are also connected at the regulatory level as a consequence of their shared idiotypy.

Shared Idiotypy between Antibodies of Dissimilar Specificity

Shared idiotypy between antibodies of dissimilar specificity is not a new concept. It was first reported by *Oudin and Cazenave* [2] in 1971 and later was incorporated by *Jerne* [3] into his network theory as the unspecific parallel set. The Oudin-Cazenave enigma [1] has been confirmed several times both for shared idiotypy between antibodies against different epitopes on the same antigen [2, 4-6], and for shared idiotypy between antibodies of known specificity and antibodies of unknown specificity [2, 7-12].

Our studies began with the classical anti-azobenzenearsonate response of A/J mice [12] where we developed a unique rat monoclonal anti-idiotypic antibody (AD8) which recognizes a new idiotypic (CRI^{AD8}). CRI^{AD8} is coexpressed with the classical cross-reactive idiotypic (CRI^a) [13] on about 80% of the CRI^a-bearing anti-ABA antibodies from sera of A/J mice hyperimmunized against ABA-conjugated keyhole limpet hemocyanin (ABA-KLH). The major properties of CRI^{AD8} are summarized in table I. What makes CRI^{AD8} unique is that it exists on non-ABA-binding molecules (ABA⁻) in sera from normal A/J mice. Similarly, *Wysocki and Sato* [10] have recently reported the expression of another idiotype, CRI^{5Cl}, found on both ABA⁺ and ABA⁻ antibodies in A/J mice; however, only CRI^{AD8} is found in the sera of unprimed mice. The dissociation of CRI^{AD8} from CRI^{5Cl} is further supported by a hybridoma antibody, 5H5, which we developed by priming an A/J mouse with AD8. Protein 5H5 strongly expresses CRI^{AD8} but not CRI^{5Cl}, and only weakly reacts with a standard rabbit antibody against CRI^a. These data strongly support the existence of a new idiotypic family, CRI^{AD8}, which contains at least two sets of antibodies. One set is found endogenously in normal A/J mice, does not bind ABA, and is CRI^{5Cl}-negative. By contrast, the other set is found in mice immunized with ABA-KLH, and binds ABA and is usually CRI^{5Cl}-positive.

Our findings support *Oudin and Cazenave's* original report that idiotypes can be shared between antibodies of dissimilar specificity. At this point, the question of biological relevance becomes important. If CRI^{AD8} sharing between ABA⁺ and ABA⁻ antibodies is not biologically relevant, then responses of the ABA⁻ set will not be connected with those of the ABA⁺ set and vice versa. This construct of the immune system would yield specific immune responses without resorting to regulation through unspecific parallel sets. On the other hand, the finding of shared idiotypes between antibodies against different epitopes of the same antigen molecule [2, 4-6] strongly argues that co-regulation of different B cell clones through unspecific parallel

Table I. Immunochemical properties of CRI^{AD8}

1	CRI ^{AD8} is expressed only in mouse strains which are CRI ^a (+)
2	CRI ^{AD8} is coexpressed with CRI ^a on about 80% of the CRI ^a (+) antibodies found in hyperimmune anti-ABA-KLH antisera
3	The binding of AD8 to CRI ^{AD8} is variably inhibited by free hapten depending on the type of hapten and the source of CRI ^{AD8}
4	Full expression of CRI ^{AD8} requires both heavy and light chains; however, heavy chains alone partially express the idiotypic, light chains do not
5	After immunization with ABA-KLH, CRI ^{AD8} is found largely on ABA ⁺ antibodies of all subclasses
6	In normal A/J sera, CRI ^{AD8} is found at approximately 10 µg/ml on ABA ⁻ antibodies predominantly belonging to the IgG _{2a} and IgG ₃ subclasses

sets is biologically important. In the next section, we will summarize our findings for the CRI^{AD8} idiotypic family which further support the importance of unspecific parallel sets in idiotypic regulation.

Induction of Idiotypic Suppression via the Unspecific Parallel Set

Our finding of endogenous ABA⁻ CRI^{AD8} suggests that A/J mice are primed in situ for this response. To date, we have been unable to find the endogenous antigen responsible for priming this response. Nevertheless, despite our lack of knowledge about the specificity of these ABA⁻ antibodies, it is still possible to selectively prime these members of the CRI^{AD8} family. Immunizing A/J mice with a single dose of AD8 elicits a 5- to 20-

fold increase in the ABA⁻ members of the CRI^{AD8} family by 7 days postinoculation. By contrast, this manipulation primes the ABA⁺ members of the CRI^{AD8} family only marginally if they are primed at all.

Since CRI^{AD8} fulfills the immunochemical definition of an unspecific parallel set for the ABA⁻ and ABA⁺ members of this family, and since we empirically found that priming with a single dose of the anti-idiotypic AD8 selectively stimulates the ABA⁻ population, we were in a position to determine whether priming the ABA⁻ set affected subsequent responses in the ABA⁺ set. If the ABA⁺ responses were affected by priming with AD8, it would be likely that regulation is occurring via the unspecific parallel set. Such an experiment is shown in table II.

In this experiment, normal A/J mice were pre-treated either with varying doses of anti-idiotypic AD8 or with 20 µg of normal rat IgG; 7 days later, the mice were bled and then immunized with ABA-KLH. The mice were boosted 3 weeks later and bled 7 days after the boost. The titers of anti-ABA antibodies and of CRI^{AD8} were determined for all groups, both pre-ABA-KLH and post-ABA-KLH. The anti-ABA titers were nil for the pre-ABA-KLH group (data not shown); by contrast, all of the post-ABA-KLH groups made significant anti-ABA responses regardless of whether they had received anti-idiotypic antibody or normal rat immunoglobulin. Furthermore, no difference in the anti-ABA titers was found between the group of animals receiving normal rat IgG and those groups receiving varying doses of anti-idiotypic. On the other hand, in the group of mice receiving 20 µg of AD8, there was a significant reduction (ca 80%) of CRI^{AD8} after priming and boosting with ABA-KLH when compared to the control group receiving 20 µg of normal rat IgG and ABA-KLH. Interestingly, mice

Table II. Identical concentrations of anti-idiotypic AD8 stimulate the production of ABA⁻ CRI^{AD8} and prime for suppression of CRI^{AD8} after immunization with ABA-KLH

Group	n	Pretreatment ¹	CRI ^{AD8} content ²	
			before ABAKLH ³	after ABAKLH ⁴
A	6	1.1 µg AD8	179 (1.69)	21,460 (1.60)
B	6	3.3 µg AD8	87 (1.53)	13,022 (1.68)
C	6	10 µg AD8	182 (2.00)	9,651 (2.58)
D	6	20 µg AD8	993 (1.54)	3,685 (2.51)
E	6	20 µg nRatIg	100 (1.65)	16,482 (1.74)
F	6	-	n.d.	14,981 (1.72)

¹ Injected intraperitoneally in PBS.

² Determined by Elisa using plates coated with a CRI^{AD8}(+) protein R16.7. Values expressed as geometrical means of serum dilutions at 50% inhibition of maximal OD₄₀₅, followed in parentheses by geometrical deviations.

³ Sera from animals which were bled 7 days after pretreatment. Students' t test analysis between the mRatIg control group and the AD8 experimental groups indicated that a significant increase in CRI^{AD8} content ($p < 0.0001$) occurred only in the mice receiving 20 µg AD8.

⁴ Sera from animals which were immunized with 150 µg ABAKLH/CFA 7 days after pretreatment, boosted with 150 µg ABAKLH/IFA 3 weeks later, and bled 7 days after boost. Significant suppression in the AD8-treated groups relative to the nRatIg control group appeared only at 20 µg AD8 ($p < 0.0033$).

receiving 10 μ g or less of AD8 did not make significant titers of CRI^{AD8} on day 7 prior to priming with ABA-KLH; these mice also failed to generate significant suppression of CRI^{AD8} after priming with ABA-KLH.

In other studies, we have shown that in the mice receiving 20 μ g AD8 and after priming and boosting with ABA-KLH, most of the residual CRI^{AD8} was ABA⁺. This suggests that priming with the appropriate dose of anti-idiotypic transiently boosts the already primed ABA⁻ members of the CRI^{AD8} family, and that this boosting is imprinted onto the subsequent ability to prime the ABA⁺ members of the CRI^{AD8} family with ABA-KLH. However, priming of the ABA⁺ members of the CRI^{AD8} family is not prohibited since the magnitude of their response rises to a value slightly (but significantly) above the pre-ABA-KLH values for CRI^{AD8} (table II).

Within the limits of correlative data, these experiments suggest a causal relationship between stimulation of the ABA⁻ members of the CRI^{AD8} family and the subsequent ability of the ABA⁺ members of this family to respond to ABA-KLH. Most importantly, these observations fulfill the criterion set forth above for the identification of regulatory interactions via the unspecific parallel set.

Concluding Remarks

Our studies lend further credence to the original proposal of *Dzierzak and Janeway* [9] that the biologically relevant form of the MOPC-460 idiotype (CRI⁴⁶⁰) is one which does not bind DNP and which is found in normal serum. Our experiments substitute CRI^{AD8} for CRI⁴⁶⁰; nevertheless, our findings agree well with theirs. Additionally, we show that the selective manipulation of the ABA⁻ members of the CRI^{AD8} family apparently affects the subsequent responsiveness of the ABA⁺ members of this idiotype family.

This finding raises several additional questions. First, what is the endogenous stimulus which primes the ABA⁻ set? Three immediate choices are stimulation by enteric flora, stimulation by food antigens, and stimulation by materials used for housing the mice. While we have no firm evidence excluding any of these possibilities, we are also entertaining the notion that the endogenous levels of ABA⁻ CRI^{AD8} (+) antibodies are due to the activation of autoreactive members of the CRI^{AD8} family. Hopefully, by carefully choosing the antigen source, and by using our panel of ABA⁻ CRI^{AD8} hybridomas, we can decide among these possibilities.

Second, the finding of endogenously circulating idiotypes in other systems [9, 14] raises the possibility that idiotype dominance in *academically chosen* antihapten responses might be caused by idiotype sharing with endogenous responses. In our example, it is possible that the ABA⁻ and ABA⁺ members of the CRI^{AD8} family might use different V_H genes and that the shared expression of CRI^{AD8} is due to combinatorial pairing with appropriate light chains. On the other hand, evidence is accumulating that CRI^{AD8} can be found on purified heavy chains [15] of CRI^a (+) anti-ABA antibodies; therefore, it is possible that the same germ line V_H gene is used by both the ABA⁻ and the ABA⁺ members of the CRI^{AD8} family. Use of this germline V_H gene would ensure membership in the CRI^{AD8} family. The antigen-binding specificity of each individual family member would be determined by the choices of D gene segments, J_H gene segments, or both. Evidence supporting this model has been reported by *Gefter et al.* [16] where it was shown that the choice of J_H segment determines whether a common V_H gene will code for an ABA⁺ or an ABA⁻ antibody. Idiotype expression and

Fig. 1. Schematic representation of the CRIAD⁸ idiotype family after Jerne [3]. The arrows indicate pathways of regulation.

antigen-binding specificity could be further tempered by the choice of light chain. Regardless of our ignorance of the molecular basis for membership in the CRIAD⁸ family, our findings prompt the questions of how many idiotype families are there, and how many members can belong to one of these families?

Finally, in this discussion the focus has been on CRIAD⁸ expression at the antibody level. Does the CRIAD⁸ family also include T cells? Although the definitive answer is not yet available, all of our attempts to induce and suppress delayed-type hypersensitivity with AD8 have failed. Similar findings have been obtained for a small number of ABA-specific T cell clones. While these negative experiments are inconclusive, they stand in the face of our ready manipulation of the antibody response with AD8. It may be that priming with AD8 perturbs CRIAD⁸ (+) B cells which then elicit an anti-CRIAD⁸ T cell

response. This hypothesis is currently being tested.

In closing, our findings support the idea of viewing the immune system from the standpoint of idiotypic families. These findings also suggest that within an idiotypic family, lymphocyte circuits which recognize different antigens are connected at the regulatory level by unspecific parallel sets. Finally, because of hybridoma technology and the paradigm of immune regulation, it is now possible to begin naming Jerne's network elements (fig. 1) and to study their interactions at both the molecular and cellular levels.

Acknowledgements

This work was supported by NIH grant AI 17090. We thank Drs. L. Wysocki and A. Nisonoff for hybridoma proteins and Dr. Roberia Kamin for reading the manuscript. We wish to thank Dr. J.W. Goodman for advice and encouragement during these studies.

References

- 1 Arrhenius, S.: *Die Immunochemie* (Macmillan, New York 1907).
- 2 Oudin, J.; Cazenave, P.A.: Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. *Proc. natn. Acad. Sci. USA* 68: 2616–2620 (1971).
- 3 Jerne, N.K.: Towards a network theory of the immune system. *Ann. Immunol., Paris* 125C: 373–389 (1974).
- 4 Ju, S.T.; Benacerraf, B.; Dorf, M.: Genetic control of a shared idiootype among antibodies directed to distinct specificities. *J. exp. Med.* 152: 170–183 (1980).
- 5 Metzgar, D.; Miller, A.; Sercarz, E.: Sharing of an idiotypic marker by monoclonal antibodies specific for distinct regions of hen lysozyme. *Nature, Lond.* 287: 540–542 (1980).
- 6 Kohno, Y.; Berkower, I.; Minna, J.; Berzofsky, J.A.: Idiotypes of anti-myoglobin antibodies. Shared idiotypes among monoclonal antibodies to distinct determinants of sperm whale myoglobin. *J. Immun.* 128: 1742–1869 (1982).
- 7 Enghofer, E.; Glaudemans, C.P.J.; Bosma, M.J.: Immunoglobulins with different specificities have similar idiotypes. *Mol. Immunol.* 16: 1103–1110 (1979).
- 8 Urbain, J.; Collignon, C.; Franssen, J.D.; Mariame, B.; Leo, O.; Urbain-Vasenten, G.; Van de Walle, P.; Wikler, M.; Wuilmart, C.: Idiotypic networks and self-recognition in the immune system. *Ann. Immunol., Paris* 130C: 281–291 (1979).
- 9 Dzierzak, E.A.; Janeway, C.A.: Expression of an idiootype (Id-460) during in vivo anti-dinitrophenyl antibody responses. III. Detection of Id-460 in normal serum that does not bind dinitrophenyl. *J. exp. Med.* 154: 1442–1454 (1981).
- 10 Wysocki, L.J.; Sato, V.L.: The strain A anti-*p*-azobenzene-arsonate major cross-reactive idiotypic family includes members with no reactivity toward *p*-azophenyl arsonate. *Eur. J. Immunol.* 11: 832–839 (1981).
- 11 Miller, G.; Nadler, P.I.; Asano, Y.; Hodes, R.J.; Sachs, D.H.: Induction of idiootype-bearing nucleic acid specific helper cells by in vivo treatment with anti-idiootype. *J. exp. Med.* 154: 24–34 (1981).
- 12 Eichmann, K.; Coutinho, A.; Melchers, F.: Absolute frequencies of lipopolysaccharide-reactive B cells producing A5A idiootype in unprimed, streptococcal A carbohydrate primed, anti-A5A idiootype sensitized and anti-A5A idiootype suppressed A/J mice. *J. exp. Med.* 146: 1436–1449 (1977).
- 13 Pawlak, L.L.; Nisonoff, A.: Distribution of a cross-reactive idiootype specificity in inbred strains of mice. *J. exp. Med.* 137: 1442 (1973).
- 14 Lieberman, R.M.; Potter, M.; Mushinski, E.B.; Humphrey, W.; Rudikoff, S.: Genetics of a new Ig V_H (T15 idiootype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. exp. Med.* 139: 983–1001 (1974).
- 15 Unpublished observations.
- 16 Gefter, M.; Riblet, R.; Siekevitz, M.: The genetic basis of the 'Ars' idiootype. *Hybridoma* 1: 170 (1982).

George K. Lewis,
Department of Microbiology and Immunology,
University of California at San Francisco,
San Francisco, CA 94143 (USA)