Chapter 112
Epitope-Specific Regulation of Antibody Responses

TAKESHI TOKUHISA AND LEONORE A. HERZENBERG

Genetically identical animals immunized with a complex antigen produce antibodies to different subsets of the epitopes (determinants) on the antigen. Many of the cells and cell interactions that regulate antibody production have been defined in great detail; however, although much is known about these cell interactions and the molecules that mediate them, the processes that control the characteristic individuality of antibody responses still remain in mystery.

The epitope-specific regulatory system described here offers a basis for the generation and maintenance of this variation. In addition, it provides a mechanism through which regulatory T cells can block/suppress antibody responses to all of the epitopes on a given antigen and an explanation for many of the phenomenological findings that were central to immunoregulation studies of the 1970s and 1980s. Finally, and perhaps most important from a practical standpoint, the mechanisms involved in epitope-specific regulation play a key role in determining responsiveness to natural and genetically engineered vaccines. In fact, failure to heed the lessons learned from this system can result in the use of methods that induce long-term suppression, rather than responsiveness, in a given immunization.

The discussion that follows is not intended to give a detailed account of the mechanisms involved in epitope-specific regulation or the evidence that underlies the elucidation of these mechanisms. Although this evidence was published some time ago (ca. 1980), it was largely located in journals like *Nature* and the *Journal of Experimental Medicine* and is still available in most good libraries.

Furthermore, it and evidence from its antecedent studies were definitively reviewed in the first volume of *Annual Reviews of Immunology* [1], which is perhaps the best source of serious reading on the subject.

The purpose of our discussion here, which presents the epitope-specific system in a historical context, is to provide an entry into the overall system and underscore its importance in the overall regulation of immune responses. In essence, we hope to remind modern immunologists that myriad of the cells, molecules, and molecular interactions currently being defined operate in intact animals, in a complex regulatory environment that does not necessarily obey the simple rules that contemporary reductionist views project.

Helper T cells and memory B cells

The understanding that T cell help is required to stimulate B cells to produce high affinity IgG antibody responses derives from studies using hapten-carrier conjugates as antigens. The use of these antigens allows the presentation of epitopes (immunogenically recognizable structural determinants) in "mix and match" configurations that permit analysis of what various cell types recognize.

The conjugates are constructed by covalently coupling of haptens such as dinitrophenyl (DNP), which are not immunogenic when independently presented, to immunogenic "carrier" proteins such as keyhole limpet hemocyanin (KLH) or chicken IgG (CGG). In this form, the hapten becomes highly immunogenic. Thus, hapten-carrier conjugates typically stimulate production of strong primary and secondary anti-hapten responses in addition to stimulating production of antibodies to native epitopes on the carrier molecule.

In addition to being immunogenic in intact animals, hapten-carrier conjugates stimulate high-affinity IgG anti-hapten secondary responses in lethally-irradiated animals reconstituted with spleen cells from hapten-carrier primed animals when the same conjugate is used to prime the spleen cell donors and stimulate the adoptive recipients. This demonstration of anti-hapten responses in adoptive recipients paved the way for the dissection of the immune response and the recognition and characterization of helper T cells and memory B cells.

The phenotypes and functional activities of these cells were defined by data from co-transfers of lymphocyte subpopulations from mice primed with carrier proteins and mice primed with a hapten on a different carrier protein. In these studies, secondary IgG anti-hapten antibody responses were produced when 1) B cells were transferred from donors primed with the hapten (such as DNP) on any functional carrier molecule (such as DNP-CGG or DNP-KLH); 2) CD4-bearing T cells were transferred from mice immunized with a carrier molecule (such as KLH); and, 3) recipients were immunized with the hapten (DNP) on the carrier molecule used to prime the T cells (DNP-KLH, in this case).

These conclusions from adoptive transfer studies were naturally extrapolated to antibody responses in intact animal. Thus, in the terminology of the time, a secondary antibody response to a hapten-carrier conjugate was considered to be produced by memory B cells that recognize the hapten, and to be regulated by helper T cells that recognize elements in the carrier moiety of the conjugate.

The rise and fall of carrier-specific suppressor T cells

Shortly after helper T cells were shown to regulate antibody responses by B cells, the simplicity of the two-cell antibody response model was disrupted by data showing that carrier priming could also induce cells that suppress, rather than help, antibody production. These "suppressor" T cells, which express CD8 rather than CD4 surface molecules, were shown to drastically impair IgG anti-hapten responses when co-transferred or co-cultured with hapten-primed B cells and helper T cells primed to the same carrier.

At the time these studies were conducted, the anti-hapten response was universally accepted as an index of antibody responses to all of the epitopes on the hapten-carrier conjugate.

Therefore, the suppressor T cells were thought to be regulating all antibody responses to the hapten-carrier conjugate and the carrier-specific helper T cells were taken to be their logical target.

This interpretation was shown to be incorrect by data from later studies in which antibody responses to the hapten and to other epitopes on the carrier molecule were independently measured. These more extensive studies surprisingly showed that the carrier-specific suppressor T cells only suppressed (induced suppression for) responses to the hapten on the priming carrier. *Responses to other epitopes on the carrier molecule were not impaired.* Thus, the measurement of antibody responses to the entire carrier/hapten-carrier molecule established two critical points. First, that the immune system independently regulates antibody production to individual epitopes on an antigenic molecule; and second, that carrier-specific suppressor T cells initiate regulatory interactions whose specificity is otherwise defined.

These findings, which have far-reaching implications for basic studies of immunoregulatory mechanisms, were largely eclipsed by the emergence of molecular methods for cloning T cell receptors and other important molecules in the immune system. Similarly, the development of methods for growing helper T cells in long term cultures, for cloning these cells, and for testing their activity *in vitro* led immunological research away from whole animal studies. In any event, cell transfer methodology and cellular immunology as a whole rapidly became history and the principal focus of basic immunology shifted from complex hapten-carrier immunoregulatory studies to the isolation of genes coding for immunoregulatory molecules and the mechanisms that govern the interaction of these molecules.

Suppressor T cells were the most obvious casualty of this revolution. The inability to grow carrier-specific suppressor T cells and/or the failure to clone the genes for receptors on these cells fueled doubts as to their existence. Furthermore, the complexity of their regulatory interactions, perhaps exacerbated by the above-mentioned confusion concerning the specificity of the suppression that they mediate/induce, made them highly unattractive for study in the murine systems in which they were initially defined.

However, although extremist views tended to discount the validity of the earlier suppressor T cell studies, continued work has brought this earlier data into a modern context [2, 3], particularly in the arena of human T cell studies [4]. In addition, the evidence implicating carrier-specific suppressor T cells in the induction of epitope-specific suggests that when the mechanisms that regulate antibody production in intact animals are finally understood, the activity of these cells will be recognized as a central component of the overall regulatory system.

Epitope-specific regulation of antibody responses

Today's molecular world has identified a variety or receptors and mechanisms used by T cells to recognize antigenic (carrier) molecules and help B cells to produce antibodies to epitopes (natural or artificial haptens) on antigenic molecules. Most of the work required to define these processes and cell interactions has been conducted *in vitro* and satisfactorily explains the *in vitro* behavior of T and B cells. Furthermore, data from these studies are consistent with the current ruling paradigm, which sees B cells, helper T cells and antigen presenting cells as essentially the sole elements involved in determining the extent of antibody production in response to a given stimulus.

This paradigm, however, predicts that antibody responses to a hapten presented on a carrier protein would either be augmented or unaffected in animals that were first primed with the carrier protein (before being immunized with the hapten-carrier conjugate). Thus it does not account for extensive data from earlier studies showing that this carrier/hapten-carrier immunization sequence consistently induces minimal anti-hapten responses, well below those induced by simply immunizing with the hapten-carrier alone. In addition, it does not account for data discussed above, showing that anti-hapten responses are specifically suppressed when carrier-specific suppressor T cells are co-transferred with helper T cells and hapten-primed B cells.

Our studies on epitope-specific regulation were essentially triggered by results from a series of experiments in which we measured both anti-hapten and anti-carrier responses to haptens and carriers presented in a mix and match situation similar to that mentioned above. These findings led us to reopen the entire question of the mechanisms that regulate responses to individual epitopes on proteins and eventually to recognize that helper T cells and carrier-specific regulation in general represents only one component of the regulatory system that controls antibody responses in intact animals.

In essence, we showed that priming with a carrier molecule (such as KLH) prior to immunizing with a hapten on the carrier molecule (such as DNP-KLH) results in the induction of *specific, long-term* suppression for IgG antibody responses to the hapten! This curious suppression drastically decreases both the amount and affinity of the IgG antibody to the hapten but allows normal IgG secondary responses to the epitopes presented initially on the carrier molecule (i.e., the intrinsic KLH epitopes). Furthermore, once induced, it suppresses responses to the hapten presented on any protein carrier molecule without interfering with primary or secondary responses to other epitopes on the second carrier.

On the opposite side, we showed that once an IgG response is initiated, the epitope-specific system "supports" that response. Thus, for example, initial immunization with DNP on one carrier molecule induces a strong antibody response to DNP and prevents the subsequent induction of suppression when animals are immunized with a second carrier and then with DNP on the second carrier. Thus epitope-specific regulation adds a kind of inertia to the immune system, since a response in motion tends to stay in motion whereas a response at rest (suppressed) tends to stay at rest.

Mechanisms that could mediate this complex regulatory pattern are discussed in our published papers and reviews [5–11]. Later sections of this Handbook present a somewhat more extensive view of the characteristics of this regulatory system; however, a discussion of cells and mechanisms that potentially mediate epitope-specific regulation is beyond the scope of our presentation here.

Functions of epitope-specific regulation in real life

The epitope-specific system clearly did not evolve to regulate responses to haptens conjugated to immunogenic protein molecules and presented in a bizarre immunization sequence in which animals are immunized to the carrier molecule before the hapten is introduced. In fact, it most likely did not evolve to produce antibodies to haptens like DNP, which induce strong, high-affinity IgG responses in virtually all immunized animals (except those immunized with the carrier/hapten-carrier sequence). Thus the

data obtained with these hapten-carrier immunizations apparently reflect more subtle processes that occur normally and provide the animal with a more efficient or better regulated immune system.

The characteristics of antibody responses to typical protein antigens such as those used in phylogenic studies actually provide a good example of how the epitope-specific system may regulate normal immune function. When a group of inbred mice is immunized with bovine serum albumin (BSA), virtually all mice produce antibodies that react with the immunogen. However, when the antibodies produced by individual mice are tested against a panel of serum albumins from a variety of species, clear response patterns emerge. For example: some mice might produce antibodies that recognize albumin epitopes shared by goat and sheep but not horse; others might produce antibodies that recognize epitopes shared by all three species; and still others might produce antibodies that recognize epitopes that are uniquely present on the bovine albumin used as immunogen.

Surprisingly, although response patterns vary dramatically among the mice in an immunized group, the pattern for an individual mouse rarely changes, even after multiple challenges with the immunizing antigen. This constancy is similar to the response fidelity observed in the "original antigenic sin" studies by Fazejas de St Groth and colleagues [12] some years ago. They showed that antibody responses produced to viral epitopes on first encounter with a virus tend to be excusively produced in later antibody responses to related viruses. Thus, the maintenance of responses to a subset of the epitopes on a complex antigen, and the failure to engage responses to additional epitopes on subsequent challenge(s) with the antigen, appears to be evolutionarily valuable and to have been installed as a common feature of the immune system.

Characteristics of epitope-specific suppression

Specificity

As indicated above, the epitope-specific system plays a key role in regulating IgG antibody responses to haptens and native epitopes on commonly used carrier molecules such as KLH (keyhole limpet hemocyanin) and CGG (chicken gamma globulin). It can be specifically induced to suppress primary and secondary IgG antibody responses to the dinitrophenyl hapten (DNP) without interfering with antibody responses to epitopes on the carrier molecule on which the DNP is presented. Furthermore, once induced, it specifically suppresses antibody responses to DNP presented on unrelated carrier molecules.

The magnitude of suppressed primary anti-DNP responses is usually about 30% of the normal primary response; however, the affinity of a suppressed response is about 10-fold lower than normal. Suppressed secondary anti-DNP responses are typically less than 10% of normal and have average affinities that are at least 100-fold below normal.

B cell memory

The epitope-specific system controls antibody production by controlling the expression of memory B cells. It does not appear to affect memory B cell development since suppressed animals that fail to mount even a primary level IgG anti-hapten antibody response *in situ* have substantial anti-hapten memory B cell populations that produce normal, high-affinity secondary anti-hapten antibody responses when supplemented with helper T cells from carrier-primed animals and transferred to adoptive recipients.

T cell help

Transfer studies show that epitope-specific suppression does not decrease or interfere with T helper activity in adoptive recipients. Furthermore, *in situ* response studies show that animals in which specific suppression is induced for a particular hapten does not interfere with T helper activity for epitopes on the carrier on which the epitope is presented, either initially or in later immunizations. Thus, the mechanism responsible for suppression must operate independent of carrier-specific helper T cell function.

Persistance/reversability

Once induced for a given epitope, suppression tends to be maintained despite repeated re-immunization with typical, small "boosting" doses of soluble antigen. In one experiment, IgG anti-DNP responses in KLH/DNP-KLH immunized mice remained suppressed although the mice were restimulated once or twice a month for nearly one year with aqueous DNP-KLH (10 μg/dose) [Herzenberg and Hayakawa, unpublished].

The suppression can, however, be reversed by repeatedly stimulating suppressed mice with high ("priming") doses of antigen presented in insoluble form and/or with adjuvants (such as 100 μg of DNP-KLH on alum). The reversability of the suppression tends to differ according to the isotype commitment of the memory response being regulated (see below).

IgG isotype differences

The induction and maintenance of suppression varies in efficiency for individual isotype anti-hapten responses. IgM responses show essentially no evidence of suppression when induced by the carrier/hapten-carrier protocol. IgG2a, IgG2b, and IgG3 responses, in contrast, are easily suppressed and tend to resist escape from suppression. IgG1 responses are also readily suppressed; however, they tend to be more refractory to suppression than other IgG isotype responses in that suboptimal suppression-induction conditions induce suppression for these isotype responses much more readily than for IgG1 responses. Furthermore, IgG1 responses tend to escape from suppression more frequently than the other IgG isotype after a given number of restimulations with priming doses of the hapten on the same of different carrier molecules.

Induction

As indicated above, the carrier/hapten-carrier immunization protocol induces marked suppression for IgG anti-hapten antibody production but does not interfere with anti-carrier antibody responses or with the development of normal anti-hapten memory B-cell populations. The effector mechanism responsible for this suppression is epitope-specific in that it can be induced to specifically suppress responses to particular epitopes on a (carrier) protein. The induction mechanism, however, is carrier-specific in that the induction of suppression requires presentation of the hapten on a carrier to which the animal has already been primed.

Studies in a variety of systems have shown that epitope-specific suppression is induced whenever "new" epitopes are presented on proteins to which the animal has been primed. The "new" epitope can be DNP or another artificial hapten (such as NP) presented

on a carrier protein to which the animal was primed weeks or months earlier. Alternatively, it can be a protein or peptide epitope coupled to a carrier protein and similarly presented to a carrier-primed animal; or, as immunogenic studies discussed above suggest, it can be a native epitope of a protein if the carrier-priming aspects of immunization to the protein are completed before the native epitope succeeds in stimulating antibody production. Finally, it can be DNP or other epitopes on proteins to which animals are genetically unresponsive (such as TGAL, a synthetic amino acid co-polymer, in TGAL non-responder mice).

Virtually all conditions under which animals are immunized with the carrier/hapten-carrier immunization sequence result in the induction of typical epitope-specific suppression. For example, suppression for anti-DNP responses is induced when animals are primed with aqueous KLH, KLH on alum, or KLH on alum plus complete Freunds adjuvant (CFA), and stimulated subsequently with either aqueous DNP-KLH or DNP-KLH on alum at low or high dose levels. Surprisingly, however, suppression induction fails completely when animals are primed with KLH plus *Bordetella pertussis* (10^9 heat killed organisms).

Induction mechanism

The mechanism of epitope-suppression induction is largely unknown; however, a variety of studies implicate a carrier-specific $CD8^+$ T cell in this process. Furthermore, these studies suggest that the time at which this cell becomes functional relative to the time at which antibody responses to epitopes on the carrier molecule are initiated determines the epitopes to which responses will be suppressed.

The suppression-inducing T cell appears to become activated several days after animals are primed and to persist thereafter in a quiescent but readily re-activatable state. When activated, it apparently "recognizes" the (carrier) protein and "presents" haptens on it in such a manner that IgG responses to it will be suppressed. However, this presentation fails if antibody responses to the epitope are already in progress. Thus, immunization appears to be a race between the induction of antibody production to individual protein epitopes and the activation of T cells that induce the epitope-specific system to suppress those responses.

We have suggested that the T cells responsible for suppression induction are actually what have been referred to in the older literature as carrier-specific suppressor T cells. This function is quite distinct from the function proposed for carrier-specific suppressor T cells, which were thought to be effector T cells that operate by removing carrier-specific help. However, by repeating the earlier studies with additional controls to distinguish between removal of T cell help and the induction of epitope-specific suppression, we showed most of the known functional and developmental properties of the putative carrier-specific suppressor T cells are explained by the induction of epitope-specific suppression.

The evidence supporting the above conclusion, together with evidence on which most of the findings discussed here are based, is summarized and discussed at length in our article on epitope-suppression in the *Annual Reviews of Immunology* [1]. References to the original reports on epitope-specific regulation will also be found in this article.

Acknowledgments

This work is supported in part by NIH grants AI-34762 and LM-04836 to L. A. Herzenberg.

References

1. Herzenberg LA, Tokuhisa T, Hayakawa K: Epitope-specific regulation, in *Annual Reviews of Immunology* Vol. 1, edited by Paul WE, Fathman CG, Metzger H, Annual Reviews Inc., 1983, pp. 609–632
2. Taniguchi M, Saito T, Tada T: Antigen-specific suppressive factor produced by a transplantable I-J bearing T cell hybridoma. *Nature* 278:555–557, 1979
3. Yamauchi K, Green DR, Eardley DD, Murphy DB, Gershon RK: Immunoregulatory circuits that modulate responsiveness to suppressor cell signals. Failure of B10 mice to respond to suppressor factors can be overcome by quenching the contrasuppressor circuit. *J Exp Med* 153:1547–1561, 1981
4. Hirayama K, Matsushita S, Kikuchi I, Iuchi M, Ohata N, Sasazuki T: HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature* 327:426–430, 1987
5. Herzenberg LA, Tokuhisa T, Herzenberg LA: Carrier-priming leads to hapten-specific suppression. *Nature* 285:664–667, 1980
6. Herzenberg LA, Tokuhisa T, Herzenberg LA: Carrier-specific induction of Hapten-specific suppression. *Immunol Today* 2:40–46, 1981
7. Herzenberg LA, Tokuhisa T, Hayakawa K, Herzenberg LA: Lack of immune response gene control for induction of epitope-specific suppression by TGAL antigen. *Nature* 295:329–332, 1982
8. Herzenberg LA, Tokuhisa T: Epitope-specific regulation: I. Carrier-specific induction of suppression for IgG anti-hapten antibody responses. *J Exp Med* 155:1730–1740, 1982
9. Herzenberg LA, Tokuhisa T, Park DR, Herzenberg LA: Epitope-specific regulation: II. A bistable Igh-restricted regulatory mechanism control to immunologic memory. *J Exp Med* 155:1741–1753, 1982
10. Herzenberg LA, Tokuhisa T, Herzenberg LA: Epitope-specific regulation: III. Induction of allotype-restricted suppression for IgG antibody responses to individual epitopes on complex antigens. *Eur J Immunol* 12:814–818, 1982
11. Herzenberg LA, Hayakawa K, Hardy RR, Tokuhisa T, Oi VT, Herzenberg LA: Molecular, cellular and systemic mechanisms for regulating IgCH expression. *Immunol Rev* 67:5–31, 1982
12. Fazekas de St Groth B, Webster RG: Disquisitions on original antigenic sin. I. Evidence in man. *J Exp Med* 124:331–345, 1966