Chapter 74
Overview: epitope-specific regulation

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The epitopes on a typical T-dependent antigen collectively stimulate production of a highly heterogeneous set of antibody molecules that vary with respect to constant region structure and combining-site specificity. Even the response to a single epitope in a single animal consists of several Ig heavy chain isotypes (e.g. IgM, IgG1, IgG2a) in association with a wide variety of idiotype and combining-site structures. Thus, as a rule, immunizations generate a vast number of different antibody molecules that can be subdivided along general lines (isotype, light chain type, combining-site specificity for individual epitopes, etc.) and classified into a myriad of small subsets when all of these properties are jointly taken into account.

Immunoregulatory studies have traditionally avoided addressing this extensive heterogeneity by adopting what might be termed 'isolationist' strategies, e.g. concentrating on the production of a particular isotype, allotype or idiotype in an antibody response; concentrating on genetically controlled responses to particular antigens or epitopes; concentrating on antibody responses to haptens on particular carriers (T dependent or T independent); and/or concentrating on carrier-specific mechanisms that exert an equal influence over the production of antibodies to all epitopes on an antigen.

These simplification strategies brought immunology through its formative years; however, at this point, more can be gained by treating individual regulatory mechanisms as components of an integrated system that selectively control the production of various kinds of antibodies. Once this concept is accepted, a wholly new perspective develops: previously unsuspected relationships among regulatory mechanisms begin to emerge; studies of one mechanism reveal key aspects of another; the integrated operation of two mechanisms accounts for findings that neither mechanism alone could explain; and many of the bizarre phenomena associated with *in vivo* antibody responses become understandable in terms of the operations of a highly organized regulatory system.

The author's experience with the epitope-specific regulatory system illustrates this approach.

The concept: integrated regulatory circuits and antibody production

Several years ago, the author published some theoretical papers [1,2] suggesting that antibody responses are controlled by sets of integrated regulatory circuits, each centred around a 'core circuit' that regulates the expression of a small number of memory B cells committed to producing very similar antibody molecules (same isotype, same allotype, similar idiotype, similar combining site). As proposed, this core circuit can be induced either to support or suppress antibody production by its covey of B cells but cannot be induced to support antibody production by some and suppress production by others. Thus it constitutes the basic unit of specificity in the system.

In this model, several 'auxillary circuits' (carrier-specific, allotype-specific, etc.) are linked to each core circuit. These sense conditions in the immunological environment calling for the production (or non-production) of particular subsets of antibody molecules and then pressure the core circuit to act accordingly. The state (suppression or support) to which an individual core circuit will be induced is determined by the net pressure from all of its auxillary circuits.

The core circuit proposed here is configured such that it will tend to maintain itself in its initially induced

state but will still be capable of shifting to the alternate state in response to a drastic change in the immunological environment. By and large, therefore, the operation of this circuit will perpetuate the response pattern determined by the conditions in the immunological environment when an epitope is first introduced. These characteristics, which define the core circuit as a bistable regulatory mechanism, introduce currently intriguing possibilities *vis à vis* the mechanisms responsible for immunologic memory and tolerance. It must be admitted, however, that the author was only dimly aware of these possibilities when she completed her work on the model and moved on to more concrete studies.

The findings: selective control of IgG responses to individual epitopes

Several years later, while investigating the selective regulation of memory B cell development and expression in allotype suppressed mice, the author made a series of observations that were difficult to reconcile with accepted dogma. These observations, however, were readily predictable from the bistable properties of the integrated circuits in the model she had proposed. Thus this model became a guide for real-life studies characterizing what we now call the 'epitope-specific' regulatory system.

Nearly all of the author's work in this area has been published and reviewed in detail quite recently [3–12]. In the interests of brevity, therefore, she presents a summary of her findings at the end of this article and a discussion here to highlight the major concepts developed in the course of these studies.

In essence, the author's initial studies with the epitope-specific system showed that primary immunization with a hapten–carrier conjugate induces equivalent IgG anti-hapten memory B cell populations in carrier primed mice and unprimed mice. Despite the presence of these memory cells, however, IgG anti-hapten antibody production fails markedly in the carrier primed mice. Thus, even when stimulated repeatedly with the hapten–carrier conjugate, these mice still tend to produce antibody responses to hapten that are substantially lower in magnitude and affinity than the primary IgG anti-hapten responses produced by control mice immunized initially with the hapten–carrier conjugate.

Subsequent studies showed that the 'carrier/hapten–carrier' immunization sequence induces a specific and persistent suppression for IgG antibody production to the 'new' epitope (hapten) belatedly introduced on the carrier molecule. Once induced, this suppression impairs antibody responses to the hapten presented on a variety of carrier molecules but does not interfere with antibody production to other epitopes on the carrier molecules. Therefore it is epitope-specific in the sense that it controls antibody production to individual epitopes on the carrier molecule.

In a more precise terminology, this mechanism should probably be described as combining-site specific since it selectively impairs production of a subset of anti-hapten antibodies that have relatively high combining affinities for the hapten. This specificity suggests a close similarity (or identity) between the mechanisms that mediate epitope-specific suppression and idiotype-specific suppression, i.e. both are capable of specifically regulating antibody production according to variable region structure. The specificity of the epitope-specific mechanism also approximates that defined for the core circuit in the model discussed above.

Selective isotype and allotype regulation

In general, IgG1 antibody responses are the most difficult to suppress initially, the most difficult to turn off once initiated and the first to escape suppression after repeated stimulation with hapten–carrier conjugates. IgG2a, IgG2b and IgG3 responses, in contrast, are more easily suppressed and more easily maintained under suppression. Each of the isotypes, however, is independently regulated since individual animals not infrequently produce one (or two) without the others. Furthermore, certain immunization and treatment protocols tend to selectively induce suppression for IgG1 responses (M. Waldor, K. Hayakawa, R.R. Hardy & L.A. Herzenberg, unpublished observations).

These findings suggest that the effector mechanism that mediates epitope-specific suppression is capable of recognizing and selectively controlling the expression on B cells committed to the production of antibodies that have different Ig heavy chain constant regions. Thus, in addition to being epitope specific (or combining-site specific), this effector mechanism might appropriately be described as Igh restricted.

Bistable regulation

Data from sequential immunization studies with carriers and hapten–carriers indicate that the mechanism that mediates epitope-specific regulation is bistable. Like the core circuits discussed earlier, it appears to have two mutually exclusive stable states (support and suppression), and generally tends to remain in its initially induced state. Thus suppression tends to be maintained despite repeated stimulation with hapten–carrier conjugates at dose levels sufficient to easily induce antibody production in non-suppressed ani-

mals; and support tends to be maintained despite immunization with the carrier/hapten–carrier sequence at dose levels sufficient to induce clear-cut suppression in non-immunized animals.

However, in keeping with the definition of a bistable system, both suppression and support can be eroded by strong stimulation towards the alternate state. Thus suppressed animals can initiate antibody production if given enough antigen in immunogenic form; and antibody-producing animals can terminate antibody production if exposed to a sufficiently strong suppression-induction stimulus.

The ability to maintain a stable state requires the ability to prevent the induction of the alternate state under conditions where it would normally have been induced. This means that support (once induced) prevents the induction of suppression and vice versa. As a practical matter, therefore, conditions that determine which state is induced first will, by and large, determine the state that will be maintained throughout subsequent immunizations. In other words, the conditions under which an animal initially encounters an epitope will define its immediate response and will strongly influence (but not absolutely determine) its future response to that epitope.

The induction of epitope-specific suppression

The induction of suppression for IgG anti-hapten antibody production in carrier/hapten–carrier immunized animals traces to the presence of functional carrier-specific suppressor T cells (CTs) in carrier primed animals. Direct isolation and transfer studies show that such CTs induce epitope-specific suppression for IgG antibody responses to haptens on the priming carrier (presumably by presenting hapten to the relevent cells in the epitope-specific suppression effector mechanism). Thus there is good reason to believe that CTs induced by carrier priming subsequently induce suppression for IgG anti-hapten antibody responses in carrier/hapten–carrier immunized animals.

The induction of this clear-cut suppression is possible because the hapten is introduced after CTs have matured to full function and before the animal has had the opportunity to induce stable anti-hapten antibody production. Introducing the hapten and establishing antibody production before CTs mature basically prevents suppression induction (or markedly reduces it). Thus, since functional CTs usually emerge a few days after animals are primed with a T-dependent antigen, there is reason to suspect that these well-known regulatory cells play a kind of scavenger role in animals producing normal primary responses to the antigen. That is, once mature, the CTs may serve to induce suppression for antibody production to priming antigen epitopes that have not yet succeeded in inducing stable support for antibody production.

Scavenging of this sort could account for the selective antibody responses produced to epitopes on protein antigens. The tendency for individual animals to respond to different epitopes on a single protein (a phenomenon well known to serologists and immunogeneticists) has previously been explained in terms of the dominance of individual B cell clones in the memory pool; however, assigning responsibility for this selectivity to an epitope-specific system is equally consistent with the data.

Induction of suppression for subsets of anti-epitope antibodies

The epitope-specific regulatory system can be induced to selectively suppress or support the production of distinctive subsets of antibody molecules to a given epitope. As indicated above, the effector mechanism is Igh-restricted in the sense that it selectively regulates production of individual isotype and allotype anti-epitope responses. Furthermore, it can selectively suppress or selectively support individual idiotype-marked antibody responses (L.A. Herzenberg & T. Tokuhisa, unpublished observations).

This selectivity is characteristic of the partially suppressed anti-hapten responses found in carrier/hapten–carrier immunized animals that were poorly suppressed initially or are escaping from suppression following repeated hapten–carrier stimulations. In addition, it characterizes responses to epitopes on priming antigens in several kinds of immunodefective mice and probably defines the patterns of isotype and allotype representation typical of antibody responses to different kinds of antigens and immunization protocols.

The mechanisms responsible for the induction of these various selective suppressions have yet to be clearly defined; however, most of the cases that the author has investigated can be explained by the failure to generate stable support for a particular segment of the antibody response before CTs mature into full function and induce suppression for that response segment. That is, in general, suppression appears to be induced for a particular subset of the antibodies normally produced in response to a given antigen whenever conditions in the regulatory environment prevent the antigen from rapidly stimulating production of those antibodies (i.e. before CTs mature).

For example, if animals are immunized during a period when they are temporarily unable to produce allotype-marked antibodies, they develop a long-term suppression that is specific for allotype-marked anti-

bodies to the epitopes on the priming antigen. Later, when they have recovered their overall ability to produce allotype-marked antibodies, they still retain the 'scars' of this earlier encounter in that they fail to produce allotype-marked antibodies to the priming antigen epitopes, even when these epitopes (haptens) are presented on a different carrier molecule. Thus a transient allotype suppression results in the induction of a restricted epitope-specific suppression that continues to impair production of the initially suppressed antibodies long after the initial suppression has dissipated.

Epitope-specific regulation and other regulatory systems

There is clearly a close functional relationship between carrier-specific and epitope-specific regulation. As already indicated, CTs induce the epitope-specific system to suppress antibody production. In addition, CTh (carrier-specific helper T cells) very likely induce the system to support antibody production. Thus the carrier-specific system can be viewed as the basic induction mechanism for epitope-specific regulation and the epitope-specific system as providing the basic effector mechanism for carrier-specific regulation.

Idiotype-specific regulation also fits easily into this construction. Idiotype-specific suppressor T cells suppress production of idiotype-bearing antibody molecules with particular variable region (combining-site) structures. Idiotype-specific helper T cells, in contrast, support production of such molecules. By definition, therefore, idiotype-specific systems can be viewed as individual regulatory modules that collectively comprise the effector mechanism of the epitope-specific system. In other words, epitope-specific suppression for anti-hapten antibody production appears to be a concerted idiotype-specific suppression induced to selectively impair production of anti-hapten antibodies.

The Igh (isotype/allotype) restrictions in epitope-specific regulation would appear to negate the direct involvement of idiotype-specific regulatory cells, since idiotype-specific regulation does not appear to be additionally specific for individual isotypes. However, judgement should be reserved on this point until idiotype-specific suppressor T cells are cloned and shown to regulate production of the idiotype on more than one isotype.

This overall hypothesis, too lengthy to defend fully here, also suggests that the induction of idiotype suppression may be a two-step process. That is, the injection of anti-idiotype antibodies, which temporarily compromises the ability to rapidly produce idiotype-bearing antibodies, may serve only to prepare the animal for suppression induction. The actual induction of suppression, in contrast, would occur when the compromised animal is immunized with an appropriate epitope (to which idiotype-bearing antibodies bind) and the suppression would be specific for those idiotype-bearing molecules that actually bind the inducing epitope.

According to this view, the immunization procedure used to determine whether suppression has been induced in an animal treated with anti-idiotype antibodies is responsible for inducing the stable idiotype-specific suppression that is observed. Furthermore, the fine specificity of this suppression is determined by the inducing antigen, i.e. the suppression induced will be specific for idiotype-bearing molecules that bind to the inducing epitope rather than for all idiotype-bearing molecules that can be produced. Thus our hypothesis accounts for the available facts: the induction of idiotype-specific suppression becomes a special case of the induction of epitope-specific suppression in which only a single module of the epitope-specific system is activated to suppress antibody production.

These ideas, which unite idiotype-specific, epitope-specific and carrier-specific regulation, also lay the groundwork for integrating other commonly studied regulatory systems into a comprehensive framework. Basically, all such systems can be viewed as operating through mechanisms that selectively influence the induction of stable support or suppression for antibody production to epitopes presented on priming antigens. That is, the specific regulation provided by these systems can be explained by their tendency to selectively impair the initiation of certain kinds of antibody production (and hence to favour the induction of epitope-specific suppression for those antibody responses).

Ir gene control mechanisms, also, need only prevent the rapid initiation of antibody production to epitopes on the target antigen. A short delay would be sufficient to allow CTs to mature to the point where they could induce long-term suppression for such antibody production to the epitopes on the antigen. Thus, as the author has shown, immunizing non-responder animals with a hapten coupled to the genetically controlled antigen results in the induction of epitope-specific suppression for antibody responses to the hapten (and presumably for antibody responses to the protein epitopes on the antigen as well). Similarly, as indicated earlier, immunizing allotype-suppressed animals induces stable, allotype-restricted, epitope-specific suppression that persists after the generalized allotype suppression dissappears.

The epitope-specific system as a whole, therefore, emerges as a modular (idiotype-specific) regulatory system that provides a common channel through

which many (all) of the known regulatory mechanisms operate to selectively control antibody production. The behaviour of the bistable modules in this system approximates the behaviour of the integrated regulatory circuits that the author proposed some time ago. Thus, in theory, the epitope-specific regulatory system provides a mechanism for taking into account all of the various pressures that favour and oppose the production of individual antibodies when defining the long-term response that an animal will tend to produce to a given set of epitopes.

Epitope-specific regulation, immunologic memory and immune tolerance

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 (carrier/hapten–carrier immunized) animals that fail to mount even a primary level IgG anti-hapten antibody response *in situ* have substantial anti-hapten memory B cell populations. In fact, when compared in adoptive (co-transfer) assays, the memory populations in these mice are usually indistinguishable from the memory populations found in non-suppressed (hapten-carrier primed) animals that produce normal *in situ* primary and secondary anti-hapten antibody responses.

Normal anti-hapten memory B cell populations can also be found when epitope-specific suppression is induced in other ways. For example, in some (or perhaps all) responses under Ir gene control, suppression for anti-hapten antibody responses is induced when non-responder animals are challenged with a hapten coupled to the antigen to which they cannot respond. These animals produce minimal IgG anti-hapten responses, even when immunized subsequently with the hapten on a carrier to which they respond normally. Nevertheless, their anti-hapten memory B cell populations are equivalent to the anti-hapten memory populations in Ir congenic mice that respond strongly to the initial stimulation (K. Hayakawa & L.A. Herzenberg, unpublished observations).

Similar results are again obtained when allotype suppression is responsible for the induction of epitope-specific suppression. Thus the author's experience with a variety of regulatory systems indicates that suppressed animals generally have fully competent (albeit unexpressed) memory populations. The induction of B cell memory, therefore, is necessary but not sufficient for the production of *in situ* IgG secondary (anamnestic) antibody responses, i.e. epitope-specific suppression frequently prevents such responses and consequently obscures the presence of otherwise normal memory B cell populations.

Carrier-specific helper T cells (CTh) can be similarly 'hidden' by the induction of epitope-specific suppression, particularly when anti-hapten responses are suppressed and the response to the hapten is taken as an index of CTh activity. In general, suppressed animals have normal levels of CTh activity, detectable either in adoptive assays or *in situ* (as the ability to help IgG responses to epitopes that accompany the hapten on a carrier molecule). Thus epitope-specific suppression can mimic CTh development or depletion defects; however, CTh activity in suppressed animals is usually present at normal levels when measured appropriately.

These findings introduce a new perspective on the mechanisms responsible for immunologic memory and acquired immunologic tolerance. In essence, the author demonstrated the presence of equivalent CTh and memory B cell populations in animals that show diametrically opposed antibody response patterns (memory or tolerance) when challenged with a previously encountered antigen. These findings largely exclude CTh and memory B as populations responsible for maintaining these response differences (which are determined by the conditions surrounding the initial antigenic stimulation and maintained thereafter with considerable fidelity). Therefore they suggest that immunologic memory and at least some forms of tolerance represent learned responses that are remembered and mediated by a system(s) evolved to provide consistent control for antibody production to epitopes encountered under a variety of conditions.

The epitope-specific system has much to recommend it for this role. It is selectively inducible to support or suppress production of individual components of an antibody response. Furthermore, once induced, it acts as a guardian of the *status quo* in that it perpetuates initial antibody responses and discourages the entrance of new responses into the pool. Thus it provides a basic mechanism capable of memorizing regulatory decisions made when an epitope is first introduced and then controlling subsequent responses to the epitope accordingly.

The epitope-specific system, therefore, appears to be the repository for a new kind of immunologic memory that records a regulatory programme to be followed in the initial and subsequent responses to a given epitope. The author views this 'regulatory memory' as responsible for: (1) maintaining what is classically called immunologic memory (by 'remembering' to support the expression of large numbers of high affinity clones); (2) maintaining immunologic tolerance (by 'remembering' to suppress the expression of most of the clones capable of participating in the response); and (3) maintaining the individuality of antibody responses (by selectively supporting or suppressing the

expression of different subsets of memory B cells in different animals). Thus both tolerance and classical memory (augmented antibody production) are seen as anamnestic responses that reflect regulatory memory programmes recorded with the epitope-specific system when the antigen was first introduced.

Methods and protocols: *in vivo veritas*

The author's work on this project largely represents a return to the kinds of sequential immunization studies that were in vogue years ago, about the time that hapten–carrier conjugates were first introduced as immunogens. These earlier studies were severely hampered by technical limitations and by the lack of direct knowledge about the cells and cell interactions of the immune system. Thus is is not surprising that they fell into disfavour when the introduction of cell culture and transfer methods opened a more direct access to the cells and mechanisms involved in antibody production.

The development of an accurate picture of how the immune system functions, however, clearly requires precise knowledge of how animals respond to different kinds of immunizations. Hypotheses that incorrectly predict the behaviour of the intact system must either be incomplete or wrong, no matter how rational they are or how consistent they may be with data from cellular studies. Thus, to evaluate current thought, we have either to rely on data from studies based on outdated methodologies and concepts or to commit ourselves to repeating parts of those studies with additional controls and response measurements so that the older data becomes interpretable in a modern context.

The use of this latter approach in re-examining previously known *in vivo* defects in antibody production revealed some of the key characteristics of the epitope-specific system. For example, to measure anti-hapten antibody responses, the author used newly developed radioimmune assays (RIA) that allowed her to determine the amount and affinity of the various allotype and isotype anti-hapten antibodies present in serum samples from individual mice [13]. These assays proved sensitive enough to accurately distinguish typical primary anti-hapten responses from lesser (suppressed) responses that are usually tenfold lower in affinity and fivefold lower in amount. Furthermore, they enable rapid and accurate measurements of primary and secondary IgG anti-carrier responses. Thus the author was able to determine whether a given anti-hapten response was suppressed and whether such suppression was accompanied by the suppression of antibody production to other epitopes on the carrier on which the hapten was presented.

The author also used a variety of hapten–carrier cell transfer and culture methods in these studies in order to evaluate the status of memory B cells, CTh and CTs populations in animals immunized in different ways. The inclusion of anti-carrier antibody response measurements in the CTh and CTs experiments helped to resolve several (previously unrecognized) ambiguities that hampered interpretation of earlier data on carrier-specific regulatory mechanisms. Furthermore, the examination of memory B cell populations in non-responding animals brought new insights into the distinctions between those regulatory mechanisms that control memory B cell development and those that control memory B cell expression. All of the author's findings in these studies were consistent with earlier observations; however, the additional information she gathered led to the development of substantially new concepts of how memory B cells and regulatory T cells work.

These concepts led in turn to the framing of the overall epitope-specific regulation hypothesis presented here, which accounts for many of the characteristics of *in situ* antibody responses and offers plausible explanations for a number of the more bizarre 'phenomena' associated with such responses. The author is certain that this hypothesis will need corrections and additions as more data accrue; however, at the moment it meets the basic test for a description of the immune system, i.e. the predictions it makes accord well with *in situ* (as well as *in vitro* and adoptive) response patterns.

Characteristics of the epitope-specific system (summary)

Several recently published reviews [2–6] and papers [7–12] describe the epitope-specific system in detail. Briefly summarized, these show the following characteristics.

Specificity

Epitope-specific suppression is mediated by a mechanism that selectively controls the production of IgG antibodies to individual epitopes on complex antigens: this mechanism can be induced to suppress IgG anti-hapten responses to a hapten–carrier conjugate without interfering with primary or secondary antibody responses to the carrier protein epitopes; once induced to suppress antibody production to a hapten, it will suppress such antibody production regardless of the carrier on which the hapten is presented; it can be induced to suppress antibody

production to epitopes on one of the proteins in a protein–protein conjugate without interfering with antibody production to epitopes on the other protein; and it can be (and generally is) induced to selectively suppress high affinity IgG anti-hapten antibody production without impairing production of low-affinity antibodies to the same hapten.

The suppression effector mechanism also appears to be Igh restricted in that it selectively regulates isotype and allotype representation in IgG antibody responses: it can be induced to suppress IgG2a, IgG2b and IgG3 anti-hapten antibody production without interfering with the production of IgG1 anti-hapten antibodies; it can be induced to suppress Igh-1b (IgG2a) allotype anti-DNP responses without interfering with Igh-1a anti-hapten responses; and it apparently maintains the isotype and allotype representation patterns characteristic of responses produced to epitopes on different kinds of carriers, e.g. proteins, erythrocytes.

Mechanism

The suppression effector mechanism blocks memory B cell expression (rather than development): normal anti-hapten memory B cell populations (demonstrable in adoptive assays) are present in severely suppressed animals; suppression for anti-hapten antibody production can be induced in animals currently producing a primary IgG anti-hapten response.

The effector mechanism does not interfere with carrier specific helper T cell (CTh) activity: CTh-supported primary and secondary antibody responses to other epitopes on carrier proteins proceed normally despite the suppression of antibody responses to haptens presented on the carrier.

The suppression is mediated by T cells: treatment with anti Thy-1 plus complement abrogates the ability to transfer a suppressed response with spleen cells from a suppressed animal.

The effector mechanism is bistable, i.e. it has two alternate regulatory states (suppression or support for antibody production) and tends to maintain itself in its initially induced state: by definition, the induction of suppression for anti-hapten antibody production specifically interferes with the subsequent stimulation of anti-hapten antibody production; similarly, the induction of anti-hapten antibody production specifically interferes with subsequent induction of suppression for anti-hapten antibody production; however, sufficient stimulation can induce a shift to the alternate state and thus either terminate an ongoing response or initiate a previously suppressed response.

Induction

Carrier priming results in the induction of epitope-specific suppression for antibody production to 'new' epitopes presented on the carrier molecule once carrier priming is complete: sequential immunizations with a carrier protein and a hapten conjugated to the immunizing carrier protein (carrier/hapten–carrier immunization) induce suppression for IgG anti-hapten antibody production; traditional carrier-specific suppressor T cells such as those that recognize keyhole limpet haemocyanin (KLH) are responsible for inducing this suppression (presumably by presenting the hapten in stimulatory fashion to an appropriate cell in suppression effector mechanism); thus the epitope-specific system is the effector mechanism that mediates carrier-specific suppression.

Regulatory defects such as Ir gene control, allotype suppression, and X-linked immunodeficiency frequently result in the induction of epitope-specific suppression: the induced suppression is specific for antibody responses that tend to fail initially because of the defect (presumably because the initiation of such antibody production is delayed beyond the time when CTs mature to full function); e.g. Ir gene controlled responses, specific suppression for anti-hapten antibody responses is induced by immunizing with the hapten on an antigen that cannot stimulate IgG antibody production in the immunized animal; the suppression persists indefinitely and maintains its initial specificity, regardless of whether the inducing defect disappears.

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