

EPITOPE-SPECIFIC REGULATION  
II. A Bistable, Igh-restricted Regulatory  
Mechanism Central to Immunologic Memory\*

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Bistable systems, by definition, have two alternative steady states with mutually exclusive functions. When confronted initially with a stimulus favoring one state or the other, these systems move rapidly to the favored state. Stabilization mechanisms then maintain the initially induced state, so that a substantially stronger signal is required to move to the other steady state than would have been required to establish that state initially. Thus, bistable systems tend to remain as initially induced, but nonetheless remain capable of shifting to the alternate state if stimulatory conditions so dictate.

The electronic binary (“flip-flop”) circuit is frequently cited as the typical example of a bistable system; however, systems with similar behavior are well known in biology (e.g., enzyme induction in bacteria and hormonal regulation in higher organisms). In addition, as we show here, the epitope-specific regulatory system that selectively controls antibody production to individual epitopes on antigenic (carrier) molecules (1–4)<sup>1,2</sup> operates as a typical bistable mechanism.

This system, we will show, is composed of Igh-restricted, epitope-specific elements that can be induced to either support or suppress the production of antibody molecules with distinctive Ig heavy chain constant region (isotype) and combining-site structures. Because these elements are independently inducible, different types of immunizations with an epitope such as DNP (dinitrophenyl hapten on a carrier molecule) can induce either suppression for all IgG anti-DNP responses, support for all such responses or selective suppression for certain isotype or allotype responses, and concomitant support for others.

The selective isotype regulation demonstrated here in IgG anti-DNP responses

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<sup>1</sup> We have previously called this regulatory mechanism hapten-specific, using the term hapten in its more general sense (synonymous with epitope) to indicate a relatively small structure that induces antibody production when presented on a larger (carrier) molecule. This term, however, is also commonly used to distinguish artificially added structures, such as the dinitrophenyl phenyl group (DNP) from the native epitopes on a carrier molecule (antigen). Therefore, to avoid ambiguity, we have now substituted the term epitope-specific for the previous nomenclature.

<sup>2</sup> Herzenberg, L. A., T. Tokuhisa, D. R. Parks, and L. A. Herzenberg. Epitope-specific regulation. III. Induction of allotype-restricted suppression for IgG antibody responses to individual epitopes on complex antigens. Manuscript submitted for publication.

obtained following various sequential immunizations with carrier proteins and DNP-carrier conjugates shows that the Igh restriction of epitope-specific regulation demonstrated for IgG<sub>2a</sub> allotypes in allotype-suppressed mice<sup>2</sup> extends to the regulation of all isotype responses. In addition, the characteristic pattern of isotype responses obtained when suppression is weak defines a hierarchy of suppressibility among the isotypes that (as a practical matter) provides a reliable index for analyzing the strength of the suppression induced or maintained during various immunization sequences and consequently for analyzing the stability of epitope-specific regulation.

Thus, by examining the isotype responses in individual animals immunized with different carrier and hapten-carrier sequences, we show that the individual elements in the epitope-specific system are typically bistable in that they tend to maintain their initially induced regulatory state despite antigenic stimulations that, *de novo*, would induce them to the alternate state. That is, when induced to support an antibody response, these elements largely prevent the subsequent induction of suppression for that response; and, when induced to suppress a response, they tend to continue to do so despite subsequent immunizations that normally induce substantial support for antibody production.

In discussing these findings, we point out (*a*) that most of the properties of epitope-specific regulation have been described in idiotype, allotype, or carrier-specific regulatory systems; (*b*) that the novel bistable properties of the system are recognizable in well-known characteristics of primary and anamnestic (memory) responses; (*c*) that these properties are also recognizable in mechanisms that maintain partial or complete "nonresponsiveness" to individual epitopes; and (*d*) that cell-mediated immune responses are apparently mediated by a similar epitope-specific system.

### Materials and Methods

The methods used for studies presented here are described in the preceding paper (3).

### Results

The isotypes represented in anti-DNP antibody responses differ characteristically in their sensitivity to the suppression mediated by the epitope-specific system (Table I). IgM responses are the most refractory to suppression. These responses are indistinguishable in control (hapten-carrier-primed) and suppressed (carrier/hapten-carrier-immunized) mice and remain so after subsequent stimulation with the hapten on the same or an unrelated carrier. IgG anti-hapten responses, in contrast, are suppressed in essentially all carrier/hapten-carrier-immunized animals and, for some isotypes, tend to remain suppressed despite repeated hapten stimulations.

IgG<sub>1</sub> anti-hapten responses differ qualitatively from responses in the other three IgG isotopes vis a vis initial sensitivity to suppression induction and maintenance of suppression once induced. Data from >2,000 animals examined in the course of studies exploring the mechanisms that induce and mediate epitope-specific suppression (2) indicate that IgG<sub>1</sub> responses in individual animals (*a*) overlap with control responses more frequently after initial suppression induction under optimum conditions; (*b*) tend to be suppressed in fewer animals under suboptimum conditions for suppression induction; and (*c*) escape from suppression more frequently than the other IgG isotypes after a given number of restimulations with the hapten.

For example, as data in Fig. 1 show, IgG<sub>1</sub> responses escape from suppression in

TABLE I  
*Epitope-specific System Selectively Regulates Isotype Representation in  
 Antibody Responses*

Immunizations‡	Relative anti-DNP levels in serum*				
	IgM	IgG <sub>1</sub>	IgG <sub>3</sub>	IgG <sub>2b</sub>	IgG <sub>2a</sub>
—, DNP-KLH	1	1	1	1	1
KLH DNP-KLH	2	0.5	0.3	0.4	0.2
—, DNP-KLH DNP-KLH	3	10	8	13	7
KLH DPN-KLH DNP-KLH	4	8	2	1	0.3

\* Mean responses (normalized to primary response to DNP-KLH) measured by RIA 2 wk after last immunization (3). See Fig. 1 or Table II for representative (absolute) IgG<sub>1</sub> and IgG<sub>2a</sub> responses in individual animals. IgG<sub>1</sub> responses in KLH/DNP-KLH/DNP-KLH animals were broadly distributed (see Fig. 2); other responses were more tightly grouped around the mean response shown.

‡ 100  $\mu$ g each antigen on alum at 4-wk intervals; 10 or more (BALB/c  $\times$  SJL)F<sub>1</sub> mice per group.

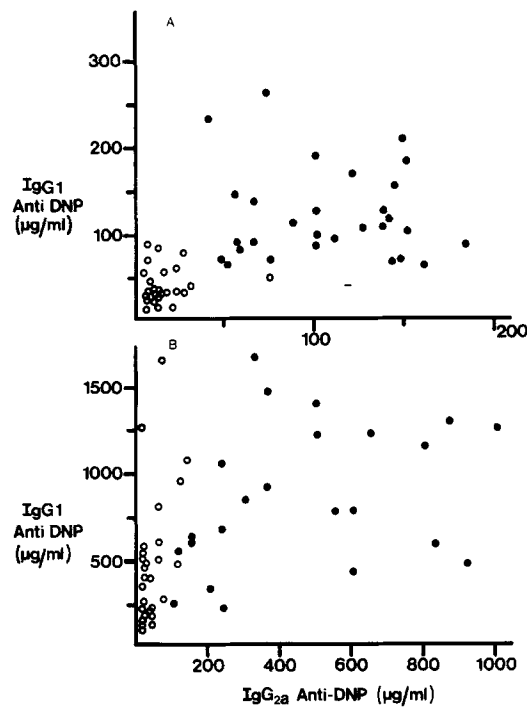


FIG. 1. The epitope-specific system selectively regulates isotype (subclass) representation in antibody responses. Each point in the figure represents the anti-DNP responses obtained in an individual (BALB/c  $\times$  SJL)F<sub>1</sub> animal. Animals were immunized with 100  $\mu$ g alum-precipitated KLH (K) or DNP-KLH (DK) at 6-wk intervals. Anti-DNP responses (serum antibody levels) were measured by RIA (3) 2 wk after the last indicated immunization. A, *in situ* 1° DNP-KLH (●) and KLH, DNP-KLH (○); B, *in situ* 2° DNP-KLH, DNP-KLH (●) and KLH, DNP-KLH, DNP-KLH (○).

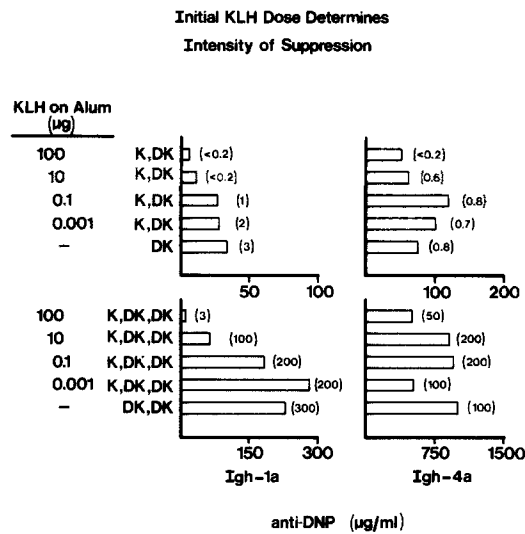


FIG. 2. Initial KLH dose determines the intensity of the anti-DNP suppression induced by the KLH/DNP-KLH immunization sequence. Animals (BALB/c) were stimulated first with KLH, as indicated, and then twice with DNP-KLH (100 µg on alum) 4 wk and 6 wk later. Responses shown were measured 2 wk after each of the last two stimulations. Data in parentheses after each response bar shows the average affinity ( $K_a M^{-1} \times 10^6$ ) for the response (3). Anti-KLH responses (not shown) were optimum at the 100 µg KLH dose but were substantial even at the 10 µg dose (30–100% of the primary and subsequent responses, depending on the group tested). Responses to aqueous KLH were lower initially but comparable to the KLH plus alum-stimulated responses after two or more stimulations. K, KLH; DK, DNP-KLH.

about one-half the keyhole limpet hemocyanin (KLH)<sup>3</sup>/DNP-KLH-immunized animals reimmunized with DNP-KLH 6 wk later, whereas IgG<sub>2a</sub> anti-DNP responses remain suppressed in essentially all of these animals. IgG<sub>2b</sub> and IgG<sub>3</sub> responses behave similarly to IgG<sub>2a</sub>. That is, responses in these latter three isotypes tend to escape concordantly when suppressed animals have been immunized three or more times with the hapten, even though each of these isotypes escaped from suppression independently in 10–20% of the (roughly 200) animals in which all IgG isotype responses were examined (data not shown).

This characteristic isotype hierarchy prevails in animals in which the induction of epitope suppression is either genetically impaired (2, 3) or experimentally minimized by immunizing initially with low doses of the carrier protein (Fig. 2). In fact, whenever suppression is weak initially or begins to wane after repeated antigenic stimulation, IgG<sub>1</sub> antibody responses are always the first to appear (2). Thus, the distinction between IgG<sub>1</sub> and the other more suppressible IgG isotypes provides a reliable index for evaluating experimental conditions that interfere with suppression induction.

*Epitope-specific Regulation Is Igh Restricted.* We show here that the epitope-specific system selectively regulates the production of the various isotypes represented in a given anti-epitope response. Furthermore, we show that the epitope-specific system can be induced to suppress Igh-1b (IgG<sub>2a</sub> allotype) responses to individual epitopes in an allotype heterozygote without interfering with production of the (allelically

<sup>3</sup> Abbreviations used in this paper: DNP, dinitrophenyl hapten; TNP, trinitrophenyl hapten; PC, phosphoryl-choline hapten; CCG, chicken gamma globulin; KLH, keyhole limpet hemocyanin; TGAL ((T,G)-A-L), poly-L-(tyrosine, glutamic acid)-poly-DL-alanine-poly-L-lysine; RIA, solid-phase radioimmuno assay.

determined) Igh-1a responses to the same epitopes.<sup>2</sup> These findings demonstrate that epitope-specific regulation is mediated by independent elements whose regulatory potential is restricted to controlling the production of antibodies with the same or closely related combining-site structures and a single heavy chain constant region structure (allotype/isotype).

This Igh constant region restriction appears to be based on the recognition of allotypic rather than isotypic structures. That is, because isotypic structures are shared between allotypically different heavy chains, isotype-restricted regulation cannot explain the selective regulation of Igh-1b allotype antibodies. Allotype-restricted regulation, in contrast, can clearly account for selective isotype regulation because nearly all Igh allotypic structures are unique to (and thus can identify) the heavy chain isotype on which they are found. Thus, it is likely that the selective regulation of both isotype and allotype representation in individual anti-epitope responses derives from a requirement for recognition of polymorphic (allotypic) regions of Igh heavy chain constant regions.

*Initiation of Antibody Production Specifically Impairs Subsequent Suppression-Induction.* Studies conducted in the early 1970's (5) demonstrated that carrier-primed animals immunized with DNP on the priming carrier produce relatively normal secondary anti-DNP responses if the animals have previously been immunized with the hapten on an unrelated carrier. These data, obtained before the "suppression era," were commonly interpreted as indicating that antihapten memory B cells cannot be induced *de novo* by carrier/hapten-carrier immunization but, once present, can be expressed normally in response to immunization with the hapten-carrier conjugate presented in the sequence.

We now show, however, that anti-hapten anti-DNP memory B cells develop normally but are not expressed after carrier/hapten-carrier immunization (3). Furthermore, we show directly that anti-DNP responses fail in carrier/hapten-carrier-immunized animals because this immunization sequence induces the epitope-specific system to suppress the expression of these memory cells. Thus, viewed from a contemporary perspective, the restoration of responsiveness demonstrated in the early 1970's and reproduced here (see below) would appear to be the result of mechanisms that interfere with the induction of epitope-specific suppression once a primary antihapten response has been initiated.

Data presented in Table II confirm this conclusion by showing that an ongoing primary response attenuates rather than prevents the subsequent induction of suppression by the carrier/hapten-carrier sequence. In essence, the anti-hapten responses obtained under these conditions are typical of responses obtained whenever the suppression-induction stimulus is weakened, i.e., IgG<sub>1</sub> tends to be produced normally, whereas IgG<sub>2a</sub>, IgG<sub>2b</sub>, and IgG<sub>3</sub> responses tend to be suppressed.

For example, primary IgG<sub>1</sub> anti-DNP responses in DNP-KLH-primed animals generally rise to normal secondary response levels when these animals are immunized subsequently with the CGG/DNP-CGG sequence. IgG<sub>2a</sub> anti-DNP responses, in contrast, rise to secondary levels in some animals but become suppressed in others. Sometimes this suppression is incomplete; however, in many animals, the ongoing primary IgG<sub>2a</sub> anti-DNP response essentially terminates after completion of the CGG/DNP-CGG immunization sequence and IgG<sub>2a</sub> anti-DNP levels in serum fall to the minimal, low-affinity anti-DNP response levels characteristic of epitope-specific sup-

TABLE II  
*Prior Hapten-Priming Impairs Epitope-specific Suppression Induction*

Immunizations*	IgG anti-DNP responses in individual animals*	
	IgG <sub>2a</sub>	IgG <sub>1</sub>
	$\mu\text{g/ml (Ka)}$	
DNP-CGG, —, DNP-KLH	68 (70)	45 (40)
	66 (100)	60 (200)
	168 (50)	150 (50)
DNP-KLH, —, DNP-CGG	36 (70)	180 (70)
	106 (>400)	150 (300)
	184 (150)	550 (300)
DNP-CGG, KLH, DNP-KLH	12 (1)	210 (100)
	16 (4)	360 (300)
	33 (5)	600 (300)
DNP-KLH, CGG, DNP-CGG	6 (<0.3)	30 (200)
	37 (40)	70 (40)
	60 (200)	200 (150)
KLH, DNP-CGG, DNP-KLH	10 (<0.3)	25 (1)
	17 (0.5)	40 (5)
	124 (100)	190 (20)
CGG, DNP-KLH, DNP-CGG	5 (1)	430 (80)
	22 (2)	90 (20)
	24 (0.5)	150 (30)

\* Each line in the table shows the response from an individual animal measured by RIA (4) 2 wk after the last (third) antigenic stimulation. Low level (suppressed) responses are substantial overestimates of the actual amount of antibody being produced at time of measurement and should be corrected downward by roughly 5  $\mu\text{g}$  to account for the contribution of primary response antibody that has not decayed in the 2 wk between challenge and test.  $(Ka) = Ka M^{-1} \times 10^6$  (4).

‡ 100  $\mu\text{g}$  of indicated antigen on alum injected intraperitoneally into BALB/c mice at 0, 6, and 8 wk; primary responses to DNP were normal in all mice.

pression (Table II). IgG<sub>2b</sub> and IgG<sub>3</sub> responses also tend to be completely suppressed in these latter animals, although the concordance is not absolute (data not shown).

Epitope-specific suppression can therefore be induced, and subsequent memory B cell expression can be prevented, even after a primary response has been initiated. Nevertheless, the initiation of a primary response clearly impairs the subsequent induction of suppression, particularly for certain isotypes. Thus, our findings define an overall regulatory system capable of selectively regulating IgG isotype responses to individual epitopes and of maintaining (or shifting) the responses produced according to the conditions under which the epitope is introduced.

Similar isotype selectivity was noted in the original studies (cited above) demonstrating that ongoing primary responses attenuate the effects of carrier/hapten-carrier immunization. This failure to restore certain isotype responses was (reasonably) discounted at the time as perhaps the result of selective "adjuvant effects" on memory B cell priming (5); however, reconsidered now, the carefully reported data from these studies essentially provides the outlines of the bistable, Igh-restricted epitope-specific regulatory system described here.

*Epitope-specific Regulation Is Bistable.* DNP-CGG/KLH/DNP-KLH immunization results in the production of substantially better anti-DNP responses than KLH/DNP-KLH/DNP-CGG immunization, even though the responding animals have been immunized with the same amounts of each antigen at similar intervals (compare Table II and Fig. 2). Thus, antigenic stimulations that induce strong suppression in naive animals are no longer capable of inducing such suppression once an antihapten response has been established; and, similarly, antigenic stimulations that induce antibody production in naive animals are relatively ineffective in doing so once suppression has been established.

Comparison of the anti-DNP responses produced after DNP-CGG/KLH/DNP-KLH immunization with responses produced in KLH/DNP-KLH-suppressed animals when the KLH dose is reduced 10-fold (from 100  $\mu\text{g}$  to 10  $\mu\text{g}$ ) demonstrates that the initiation of a primary antihapten response is functionally equivalent to reducing the strength of the carrier-specific mechanism that induces the epitope-specific system to suppress antibody production (Table II and Fig. 2). In both cases, the suppression obtained is restricted to the "more suppressible" isotypes and is demonstrable in essentially the same proportion of immunized animals.

Weakening the effector mechanism that maintains epitope-specific suppression has a similar effect. That is, KLH/DNP-KLH-immunized animals stimulated repeatedly with either DNP-KLH or DNP-CGG eventually produce normal secondary-level IgG antihapten responses. However, while recovering from suppression, these animals pass through a stage during which they produce selectively suppressed responses comparable to those produced by animals in which suppression-induction is initially impaired either by prior hapten-priming or by suboptimum carrier stimulation (e.g., compare Tables I and II and Fig. 2). Thus, the order of antigenic stimulations determines the responses produced at any given point in a stimulation sequence, but the rules governing which responses are suppressed are the same, whether animals are in transition from suppression to full responsiveness or vice versa.

The epitope-specific elements that individually control the production of antibodies with common Ig heavy chain and antibody combining-site structures, therefore, have the following properties: (a) they can be independently induced to provide either support or suppression for antibody production; (b) they tend to maintain their initially induced state despite subsequent antigenic stimulation(s) that would induce the alternate state in unprimed animals; and (c) they remain capable of shifting to the alternate state when confronted with sufficiently strong stimulation favoring establishment of that state. Thus, these elements are typically bistable, and, acting as a system, constitute a unique adjunct to immunologic memory that permits the conditions surrounding the first exposure to an epitope to strongly influence the composition of initial and subsequent antibody responses to that epitope.

### Discussion

Surprisingly, although the epitope-specific regulatory system introduces a fundamentally new mechanism central to the control of heterogeneous antibody responses, almost every aspect of this mechanism appears to have been studied previously. The T cell control of B cell expression according to Ig combining-site commitment, for example, has been extensively examined in idio-type-suppression systems (e.g., 6-9). Similarly, selective control according to Ig heavy chain constant region structure

recalls evidence from “chronic” allotype suppression studies in which suppressor T cells specifically prevent production of antibodies carrying one of the two parental IgG<sub>2a</sub> heavy chain allotypes in an allotype heterozygote (10, 11). Thus, the effector mechanism mediating epitope-specific suppression is novel mainly in its ability to combine the properties of Ig-oriented mechanisms that have previously been studied independently.

Similarly, although we (and many of our colleagues) were initially surprised to find that immunization with (what we now call) the carrier/hapten-carrier sequence resulted in suppressed rather than augmented antihapten responses, a cursory literature search revealed that this phenomenon was well known some time ago. In fact, the first (and often cited) papers demonstrating that spleen cells from carrier-primed animals provide carrier-specific help for antihapten memory B cells in adoptive cotransfer assays also noted clearly that the carrier-primed donors used for these experiments produce very little antihapten antibody when stimulated *in situ* with the homologous hapten-carrier conjugate (12, 13).

During the intervening years, this unexplained response failure came to be attributed to interference with antihapten memory B cell development. Our studies refute this hypothesis directly by showing that these mice have normal antihapten memory B cell populations (1, 3). Furthermore, we show that the expression of these memory B cells is specifically suppressed, that carrier/hapten-carrier immunization induces this suppression (which is mediated by the epitope-specific system), and, finally, that carrier-specific suppressor T cells (CTs) present in carrier-primed mice induce such epitope-specific suppression when confronted with the hapten on the priming carrier.

Carrier priming, of course, has long been known to generate CTs capable of suppressing adoptive and *in vitro* antihapten responses to haptens presented on the priming carrier (e.g., 14–16). The presence of these cells, however, did not appear relevant to the specific failure of the *in situ* antihapten response in carrier/hapten-carrier-immunized animals, largely because of confusion introduced by assuming that the CTs specificity for the carrier protein meant that they controlled antibody production by depleting carrier-specific help. CTs activity *in situ* thus seemed minimum except in tolerized animals (e.g., 17) and, in any event, could not explain a specific inability to produce antibody to the “new” epitope (hapten) on the carrier.

Our recent studies (conducted with Dr. Masuru Taniguchi in his laboratory at Chiba University, Chiba, Japan), however, demonstrate clearly that CTs suppress antibody production by inducing epitope-specific suppression rather than by reducing the supply of carrier specific help (3, 18). This conclusion, which is entirely consistent with data from previous CTs studies, is based on a more extensive analysis showing that the specificity of the suppression obtained in CTs recipients is identical to the suppression obtained in carrier/hapten-carrier-immunized animals. CTs, therefore, emerge in a new regulatory role (as inducers of specific suppression for *in situ* responses to epitopes presented on the carrier protein under certain conditions), which explains the previously puzzling presence of these cells (19) in spleens from animals recently primed with a carrier protein and producing a normal anti-carrier antibody response.

The bistable mechanism that permits the epitope-specific system to establish either support or suppression for individual antibody responses constitutes perhaps the most surprising capability of this system given the data available from currently studied immunoregulatory mechanisms. However, this apparently unprecedented capability



becomes quite familiar when considered within the context of common experience garnered in the production of antibody "reagents" for use in immunogenetic, phylogenetic, or structural studies. Serologists have long known that antibody responses in individual animals tend to become fixed early in the response with respect to the epitopes detected and the isotypes produced. Such response characteristics, commonly attributed to (B cell) "clonal dominance," flow naturally from the operation of a bistable regulatory system that maintains initially defined response states for individual determinants on complex antigens.

That is, the initial presentation of an antigen (i.e., a carrier molecule and its native epitopes) must generate something of a horserace between the operation of mechanisms that stabilize epitope-specific elements to support antibody production (and prevent suppression induction) and the maturation of carrier-specific CTs that, once functional, induce the remaining "unstabilized" epitope-specific elements to suppress antibody production. Furthermore, the outcome of this response-determining race must be largely during the first few days after antigenic stimulation because KLH-specific CTs, for example, mature within a week of priming (3, 19).

Thus, responses to epitopes on complex antigens can be expected to vary from individual to individual, particularly when such epitopes have an inherently low probability of inducing antibody production rapidly. Although certain epitopes might mimic DNP and universally induce stable antibody production, most will fall prey to the suppression-induction mechanism (independently) in at least some of the immunized animals. Therefore, individual animals will tend to produce antibodies to more or less random subsets of the epitopes on an immunizing antigen, and the initial specificity pattern of the response will tend to be maintained when animals are repeatedly re-immunized. In other words, the consequences expected from the operation of a bistable regulatory system such as we have described predict the response patterns commonly observed in serologic studies.

Studies on epitope-specific regulation in allotype-suppressed mice,<sup>2</sup> however, directly demonstrate the importance of this bistable mechanism in determining the course of subsequent responses to the epitopes on a priming antigen. We have shown that young Igh-1b (1b) allotype-suppressed mice primed with DNP-KLH cannot produce 1b responses to epitopes on the priming antigen. Thus, when these mice enter a remission period during which they can produce normal 1b antibody responses to newly introduced epitopes, they remain specifically unable to produce 1b anti-DNP and 1b anti-KLH antibodies. Furthermore, they fail to produce 1b responses to the DNP hapten presented during remission on chicken gamma globulin (CGG), even though they produce normal 1b antibody responses to the CGG determinants on the stimulating (DNP-CGG) antigen.<sup>2</sup>

Other isotype and allotype responses to DNP-KLH epitopes in the allotype-suppressed mice are initiated normally after DNP-KLH stimulation and proceed normally thereafter. Thus, the inability to produce 1b antibody responses to the epitopes presented on DNP-KLH during the period when allotype suppression is active results in the induction of a stable and specific suppression for subsequent 1b antibody responses to these epitopes, and this epitope-specific suppression persists when the allotype suppression mechanism ceases its activity. In other words, the epitope-specific system maintains the characteristics of the overall antibody response

defined by conditions in the regulatory environment when the animal first encounters a given epitope.

These findings independently demonstrate the bistable regulatory potential of the epitope-specific system shown (in this publication) by the contrast between the minimum suppression obtained for anti-DNP responses in DNP-CGG/KLH/DNP-KLH-stimulated animals and the strong suppression obtained after KLH/DNP-KLH/DNP-CGG stimulation. In addition, they independently demonstrate the Igh-restricted regulation provided by the individual elements that comprise this system, demonstrable in carrier/hapten-carrier-immunized animals mainly by their ability to selectively regulate isotype rather than allotype.

Taken as a whole, therefore, the evidence we have presented indicates that the epitope-specific system plays a central role in defining the magnitude, specificity, and isotype/allotype composition of primary and anamnestic (memory) responses. This system is clearly subordinate to mechanisms that influence memory B cell development; however, its ability to maintain itself in its initially induced state and to selectively control the expression of memory B cells makes it a key element in determining how an animal responds to previously encountered epitopes.

In essence, it provides an effector mechanism through which initial immunization conditions can establish the production of functionally relevant isotype responses and prevent production of functionally deleterious responses without sacrificing the potential for producing a different type of response at a later date. Furthermore, it offers a versatile alternative or adjunct to deletional-type tolerance mechanisms in that it can maintain partial or complete tolerance to epitopes that have succeeded in generating substantial memory B cell activity.

Analogous mechanisms apparently regulate cellular immune responses. For example, recent studies (20) demonstrate that the induction of allergic encephalomyelitis (AE) by an encephalitogenic peptide-carrier conjugate can be inhibited (suppressed) by prior immunization with the carrier protein, i.e., by carrier/hapten-carrier immunization. Similarly, the mechanisms regulating delayed-type hypersensitivity (21) show a specificity for epitopes not unlike the mechanisms described here. Thus, although the epitope-specific system as such has only recently been recognized, the consequences of epitope-specific regulation have apparently been known in various guises for many years.

The cell interactions responsible for this intricately balanced regulatory system, in contrast, are not readily extractable (at least as a unit) from any of the currently known mechanisms controlling antibody production or cellular immunity. Such interactions, however, constitute the heart of the regulatory "circuit" model we proposed some time ago (22, 23). The bistable "core" circuit in this model is Igh restricted and epitope specific; it regulates the expression of memory B cells, and it is induced to suppress or support antibody production by various "environment-sensing" (carrier-specific, allotype-specific) auxiliary circuits. This theoretical exercise, therefore, suggests a plausible (although not necessarily correct) cellular basis for epitope-specific regulation.

The induction of epitope-specific suppression by either carrier-specific or allotype-specific mechanisms is predictable (with hindsight) from the principles that guided construction of this model. Similarly, recent studies (24) demonstrating contrasuppressive regulation fit well within its framework. That is, regulatory cells, such as the

carrier-specific suppressor T cell and its contra-suppressor T cell counterpart, would be expected to vie with one another (and with other types of regulatory cells) for the dominating position vis a vis the stabilization of epitope-specific elements to support or suppress responses.

In essence then, the evidence we present casts the epitope-specific system as an integrative central mechanism responsible for shaping humoral and cellular responses according to the dictates of the regulatory environment when an antigen is first introduced. The complexity inherent in such a system is staggering; but the confusion it generates for us (as observers) is clearly balanced by the stunning simplicity of a mechanism evolved to funnel broadly diverse regulatory influences through a single set of gates that ultimately say "yea" or "nay" to the production of the various antibodies and cellular responses possible after antigenic stimulation.

### Summary

Antibody responses to commonly used antigens are regulated by an epitope-specific system composed of Igh-restricted elements responsible for controlling the isotype and allotype responses mounted to each of the epitopes on the antigen. Because these elements can be independently induced to either suppress or support antibody production, this system as a whole provides an effector mechanism capable of selectively controlling the amount, affinity, isotype representation, and epitope-specificity of an antibody response.

Sequential immunizations with a carrier molecule and a hapten conjugated to that carrier (carrier/hapten-carrier immunization) induce suppression for IgG responses to the hapten. IgG<sub>2a</sub>, IgG<sub>2b</sub>, and IgG<sub>3</sub> responses are easily suppressed, whereas IgG<sub>1</sub> responses tend to be more resistant. Once induced, suppression tends to be maintained; however, repeated stimulation with the hapten (on any carrier) eventually induces antibody production, first for IgG<sub>1</sub> and later for the more suppressible isotypes (IgG<sub>2a</sub>, IgG<sub>2b</sub>, IgG<sub>3</sub>).

Antibody production, once initiated, also tends to be maintained. Ongoing IgG antihapten responses in animals primed with a hapten-carrier conjugate can be suppressed by subsequent carrier/hapten-carrier immunization (using a different carrier molecule); however, the suppression induced under these circumstances is substantially weaker, i.e., it mainly affects the more suppressible isotypes and is only strong enough to detect clearly in about one-half the immunized animals. Thus, the initiation of antibody production impairs the subsequent induction of suppression, and the initial induction of suppression tends to prevent subsequent initiation of antibody production.

This reciprocal relationship defines a bistable regulatory mechanism, i.e., one that tends to maintain its initially induced state but is capable of shifting to the alternate state when stimulatory conditions so dictate. The operation of such a mechanism permits conditions surrounding the first immunization with an epitope (hapten) to strongly influence but not absolutely determine which and how many of the anti-epitope memory B cells generated by that immunization will subsequently be expressed. Thus, epitope-specific regulation, although subordinate to mechanisms that control memory B cell development (as opposed to expression), plays a key role in determining the magnitude, affinity, and isotype representation of anamnestic (memory) responses produced in response to previously encountered epitopes.

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