EPITOPE-SPECIFIC REGULATION

I. Carrier-specific Induction of Suppression for IgG Anti-Hapten Antibody Responses*

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In this series of publications (1)[1] we defined the properties of an epitope-specific regulatory system (2–4) that operates centrally to control the amount, affinity, and isotype/allotype composition of antibody responses to individual epitopes on complex antigens. This system, which has gone unrecognized as such despite more than 10 yr of intensive study of the cells and cell interactions controlling antibody production, provides a versatile Igh-restricted effector mechanism that selectively shapes primary and subsequent antibody responses to a given epitope according to the dictates of the regulatory environment when the epitope is first introduced.

For example, we show that priming young allotype-suppressed mice induces the epitope-specific system to selectively suppress allotype-marked (Igh-1b) antibody responses to all epitopes on the priming antigen.[1] This suppression then persists so that the animals fail to produce Igh-1b responses to the priming-antigen epitopes when reimmunized after the onset of the characteristic midlife remission from allotype suppression (during which *de novo* immunizations induce normal Igh-1b antibody responses).

Immunizing carrier-primed mice with a "new" epitope coupled to the priming carrier also induces the epitope-specific system to suppress antibody production; however, under these conditions, antibody responses to the newly introduced epitope are selectively suppressed, and anti-carrier responses proceed normally (2–4).[2] Studies presented here trace the induction of this suppression to the *in situ* activity of carrier-specific suppressor T cells (CTs)[3] and show further that the epitope-specific system

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[2] We 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.

[3] *Abbreviations used in this paper:* CTs, carrier-specific suppressor T cell; DNP (or D), dinitrophenyl hapten; TNP, trinitrophenyl hapten; PC, phosphoryl-choline hapten; CGG (or C), chicken gamma globulin; KLH (or K), keyhole limpet hemocyanin; TGAL ((T,G)-A--L), poly-L-(tyrosine, glutamic acid)-poly-DL-alanine-poly-L-lysine; RIA, solid-phase radioimmune assay.

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constitutes the major, if not only, effector mechanism through which CTs control antibody production.

That is, we define the characteristics of the epitope-specific suppression in these "carrier/hapten-carrier" immunized animals, show that it is induced by a carrier-specific mechanism, and then show that "classical" CTs generated according to protocols originated by Tada and colleagues (4, 5) duplicate this function in adoptive recipients. Thus, we demonstrate directly that CTs regulate antibody production by inducing typical epitope-specific suppression (rather than by depleting carrier-specific help, as previously believed) and thereby identify an *in situ* regulatory role for these cells.

In addition, we present (summarized) data from an extensive series of *in situ* immunization experiments indicating that the carrier-specific and epitope-specific mechanisms described here represent general regulatory processes. For example, these studies show that epitope-specific suppression can be induced with diverse antigens administered under widely different immunization conditions in a variety of mouse strains.

We discuss the relationship of these suppressive mechanisms to the overall regulation of antibody responses in the second publication in this series (1), which shows that the individually specific, IgH-restricted elements that comprise the epitope-specific system can be induced either to support or suppress antibody production and then tend to maintain themselves as initially induced during subsequent immunizations. This novel bistable regulatory capability provides the key to understanding how carrier-primed animals can produce normal primary and secondary IgG anti-carrier antibody responses, even though they have CTs that induce suppression for antibody responses to new determinants presented on the carrier.

Materials and Methods

Mice. Unless otherwise indicated, BALB/c or (BALB/c × SJL/JHz)F_1 mice bred in our colony were used between 2 and 5 mo of age.

Antigens. Keyhole limpet hemocyanin (KLH) (Pacific Bio-Marine Laboratories Inc., Venice, CA), chicken gamma globulin (CGG) (United States Biochemical Corp., Cleveland, Ohio), and bovine serum albumin (BSA) were used; DNP11-KLH, DNP8-CGG, DNP5-BSA, and DNP-41-BSA were prepared as previously described (7–8).

Antibodies. Analyses of antibody levels in serum were performed using monoclonal antibodies to Igh-1a [Igh(1a)8.3], Igh-1b [Igh(1b)5.7], Igh-4a [Igh(4a)10.9], and Igh-4b [Igh(4b)12.8], and with affinity-purified rabbit anti-IgM and IgG3 reagents (7–9).

Serum Antibody Levels and Affinity. The solid-phase radioimmune assays (RIA) have been described in detail (7, 8). The amount of antibody in a test sample is determined by comparison with the antibody bound from a "standard" secondary response antiserum. Antibody levels are expressed as units (percentage of standard) per ml test serum or μg antibody per ml, depending on whether μg per ml values have been determined for the standard serum, e.g., the anti-DNP standard serum used here has 100 μg/ml of Igh-1b anti-DNP.

Mean antibody responses shown represent the arithmetic average of the individual responses of four animals (generally) per experimental group. Data are presented for responses measured at 2 wk after the last indicated antigenic stimulation because these responses are representative of the serum antibody levels and affinities that persist for at least an additional 4 wk in virtually all animals tested.

Average affinities shown for anti-DNP responses were determined from the ratio of antibody bound to DNP5-BSA and DNP41-BSA (at room temperature). We have shown previously that this ratio is proportional to log Ka (affinity) (7). In the studies presented here, affinity values were determined from the equation $Ka = 1.18 \times (10^6)\ (e^{3.75}r)$, where r is the amount of a

particular isotype or allotype bound to DNP5-BSA) per the amount bound to DNP41-BSA at a given dilution (see ref. 7). This equation is defined from the binding ratios obtained with purified anti-DNP hybridomas or myeloma antibodies of known affinity.

Immunizations. Unless otherwise declared, animals were immunized with 100 μg of the indicated carrier protein or hapten-carrier conjugate on alum, regardless of whether the immunization represented the first (primary) stimulation or a subsequent stimulation with the same or a different antigen.

Cell Transfer Studies. The general methods used here for preparing and injecting cell suspensions have been described previously (7). Details for individual experiments are presented with each figure and table.

Results and Discussion

In the analysis that follows, we measure the amount, affinity, and isotype composition of anti-hapten and anti-carrier antibody responses occurring in individual animals exposed to sequential immunizations with carrier proteins and hapten-carrier conjugates.

Carrier/Hapten-Carrier Immunization Induces Suppression for IgG Anti-Hapten Antibody Production. Data in Table I show that priming with either of two commonly used carriers markedly and persistently impairs IgG antibody production to a new epitope (the DNP hapten) subsequently presented on the priming carrier. For example, anti-DNP responses in animals immunized first with KLH and then twice with DNP-KLH (100 μg each antigen on alum) are roughly 10-fold lower in magnitude and 100-fold lower in affinity than the normal secondary anti-DNP responses obtained in

TABLE I

Anti-hapten Antibody Production Is Specifically Suppressed in Carrier/Hapten-Carrier-immunized Mice

Immunizations			In situ IgG_{2a} antibody responses*					
			Anti-DNP				Anti-carrier	
Carrier	Primary DNP	Secondary DNP	Primary		Secondary		Anti-KLH units	Anti-CGG units
			μg/ml	Ka‡	μg/ml	Ka‡		
—	DNP-KLH		35	(5)	—	—	15	—
—	DNP-KLH	DNP-KLH	—	—	120	(300)	130	—
—	DNP-KLH	DNP-CGG	—	—	60	(100)	—	9
—	DNP-CGG		13	(1)	—	—	—	11
—	DNP-CGG	DNP-CGG	—	—	85	(400)	—	100
KLH			—	—	—	—	20	—
KLH	DNP-CGG		20	(2)	—	—	—	21
KLH	DNP-KLH		5	(<0.3)	—	—	170	—
KLH	DNP-KLH	DNP-KLH	—	—	9	(0.5)	370	—
KLH	DNP-KLH	DNP-CGG	—	—	6	(<0.3)	—	8
CGG			—	—	—	—	—	12
CGG	DNP-KLH		15	(0.6)	—	—	ND§	—
CGG	DNP-CGG		5	(<0.3)	—	—	—	70
CGG	DNP-CGG	DNP-CGG	—	—	7	(2)	—	210

* BALB/c × SJL mice were injected intraperitoneally with 100 μg of the indicated antigen on alum at ~6-wk intervals.

* Serum antibody levels were measured by RIA 2 wk after last immunization. Anti-carrier antibody expressed as percentage of antibody in a "standard" secondary response serum pool.

‡ Ka $M^{-1} \times 10^6$ measured by RIA (8).

§ Not done.

control animals just immunized twice with DNP-KLH. Similarly, IgG anti-DNP antibody responses remain at or below primary level in CGG/DNP-CGG/DNP-CGG-immunized animals. The IgG_{2a} responses shown are representative of IgG_{2b} and IgG_3 responses obtained. IgG_1 responses tend to be somewhat less suppressible and to escape from suppression somewhat more frequently (1).

Immunizing carrier-primed animals with DNP on an unrelated carrier molecule, in contrast, does not interfere with antibody production to DNP. KLH-primed animals stimulated with DNP-CGG similarly produced normal primary and secondary anti-hapten responses, etc. (see Table I). Therefore, the impaired anti-DNP responses in animals immunized sequentially with a carrier protein and a hapten (DNP) conjugated to the same carrier protein are specifically the result of immunization with this carrier/hapten-carrier sequence.

The landmark papers (10, 11) demonstrating the presence of carrier-specific helper T cells in carrier-primed mice also noted that the donors used for these adoptive transfer experiments (surprisingly) failed to respond to haptens presented subsequently on the priming carriers. These *in situ* response failures were later ascribed either to impaired anti-hapten memory B cell development in carrier/hapten-carrier immunized animals or to insufficient carrier-specific helper T cell activity capable of supporting a primary response to the new hapten presented on the priming carrier. Evidence presented below, however, rules out both of these explanations and shows directly that anti-hapten responses fail because they are specifically suppressed after carrier/hapten-carrier immunization.

Anti-Hapten Memory B Cells and Carrier-specific Helper T Cells Develop Normally in Carrier/Hapten-Carrier Immunized Mice. Adoptive transfer studies with a protocol that favors detection of relatively minor differences in memory development (7) show directly that hapten-carrier conjugates stimulate normal anti-hapten memory B cell development in carrier/hapten-carrier immunized mice. Splenic B cell populations (T-depleted) from KLH-primed mice immunized with DNP-KLH 3 wk before transfer give rise to adoptive anti-DNP memory responses equivalent in magnitude, affinity, and isotype representation to control responses produced by memory B cell popula-

TABLE II

Epitope-specific Suppression Does Not Interfere with Anti-Hapten Memory B Cell Development

DNP-primed donors (BALB/c × SJL)				Anti-DNP adoptive secondary response*	
−9 wk	−3 wk	Status	Cells transferred‡	IgG_{2a}	IgG_1
				μg/ml	
KLH	DNP-KLH	Suppressed	Spleen (T + B)	32	250
—	DNP-KLH	Control	Spleen (T + B)	120	125
KLH	DNP-KLH	Suppressed	T-depleted spleen (B)	104	300
—	DNP-KLH	Control	T-depleted spleen (B)	90	125

100 μg each antigen intraperitoneally on alum.

* Serum anti-DNP levels measured by RIA 7 d after transfer.

‡ 10^7 spleen cells (BALB/c × SJL) or remaining (T-depleted) cells from 10^7 spleen cells after treatment with monoclonal anti-Thy-1.2 plus complement. T-depleted populations were supplemented with 10^7 KLH-primed T cells (as a source of carrier-specific help). Recipients (600 rad irradiated BALB/c) were injected intravenously with the indicated cell populations plus 1 μg aqueous DNP-KLH.

tions from mice immunized only with DNP-KLH (see Table II). Similarly, IgD+ and IgD− memory B cell activity (7) is equivalent in these B cell populations (data not shown). Thus, carrier/hapten-carrier immunization impairs anti-hapten memory B cell expression rather than development.

This impairment is not because of interference with carrier-specific help. Carrier/hapten-carrier-immunized animals have ample carrier-specific help to support either primary or secondary antibody responses to most of the determinants on the immunizing conjugate, even though they produce only minimum anti-DNP responses when stimulated with such conjugates, i.e., primary and secondary anti-CGG and anti-KLH responses proceed normally in all cases (Table I). Thus, anti-hapten antibody production fails in these animals despite the presence and apparently normal potential of the two types of lymphocytes currently known to be required for such antibody production (anti-hapten memory B and carrier-specific helper T). In other words, this response failure is caused by the induction of active suppression rather than by a deficit of the requisite cells.

An Epitope-specific Effector Mechanism Mediates the Suppression Induced in Carrier/Hapten-Carrier-immunized Mice. The specificity of the suppression-effector mechanism is demonstrated most clearly by the failure of the potential secondary anti-hapten response in carrier/hapten-carrier-immunized animals stimulated subsequently with the hapten on a second (unrelated) carrier molecule. For example (as indicated above), KLH/DNP-KLH-immunized mice stimulated with DNP-CGG produce normal anti-CGG primary responses but fail to produce more than a minimum anti-DNP response, and CGG/DNP-CGG-immunized mice show a similar specific inability to produce anti-DNP antibody when stimulated with DNP-KLH (Table I). Thus, regardless of which carrier is used in the carrier/hapten-carrier immunization sequence and which is used subsequently to test the specificity of the suppression, the result is the same: the anti-DNP response is specifically suppressed while the antibody responses to carrier epitopes proceed normally.

These findings rule out nonspecific suppressive mechanisms such as interference with antigen handling, processing, or presentation. In addition, they exclude mechanisms that interfere with carrier-specific help because these kinds of mechanisms should either affect responses to all epitopes on the hapten-carrier conjugate equally, or they should have a more drastic suppressive effect on the primary responses to the carrier epitopes than on the potential secondary responses (to DNP). Therefore, the mechanism mediating suppression in carrier/hapten-carrier mice is "epitope-specific" in the sense that it selectively suppresses antibody production to one of the epitopes (DNP) on a complex antigen.

Carrier-specific Suppressor T Cells Induce Epitope-specific Suppression. The specificity of the mechanism responsible for inducing (as opposed to mediating) suppression in carrier/hapten-carrier-immunized mice parallels the specificity of the well-known carrier-specific suppressor T cells (CTs) that are generated by KLH priming (4, 5) and suppress adoptive or in vitro responses to DNP on KLH but not on unrelated carriers. That is, DNP-KLH immunization induces suppression (for anti-DNP responses) in KLH-primed animals, whereas DNP-CGG immunization does not; and DNP-CGG induces suppression in CGG-primed mice, whereas DNP-KLH does not. Furthermore, the overall properties reported for the suppression obtained with KLH-

specific CTs foreshadow the properties of epitope-specific suppression (e.g., selective interference with high-affinity anti-hapten antibody production).

Studies conducted in collaboration with Dr. Masaru Taniguchi (in his laboratory at Chiba University, Chiba, Japan) confirm the surprising implications of the above findings. These studies show directly that CTs and CTs factors generated and tested according to protocols developed by Tada and collaborators (4, 5) regulate antibody responses by inducing epitope-specific suppression. That is, KLH-specific CTs transferred to nonirradiated recipients challenged immediately after transfer with DNP-KLH induce a specific and persistent suppression for IgG antibody responses to DNP. Thus, these recipients produce normal anti-KLH responses but show typical suppressed responses to DNP injected subsequently on KLH or on an unrelated carrier (Table III). Soluble KLH-specific suppressor factors induce a similar suppression (12).

In a related series of adoptive co-transfer studies (2) (conducted in our own laboratory), we have shown that epitope-specific suppression is difficult to induce or demonstrate in irradiated recipients. This is consistent with evidence presented by Eardley and Gershon (13) showing that the same carrier-primed T cells that provide help for adoptive responses in irradiated recipients will suppress such responses if co-transferred with T cells from an unprimed donor. These peculiar properties of response regulation in "reconstituted" animals probably explain how KLH-primed animals can be used as an excellent source of KLH-specific help for adoptive anti-DNP responses to DNP-KLH and yet be induced to suppress *in situ* anti-DNP responses by immunization with this same hapten-carrier conjugate.

Analysis of the characteristics of the *in situ* mechanism responsible for inducing epitope-specific suppression provide further evidence indicating that CTs act by inducing epitope-specific suppression. For example, studies summarized in Table IV show (*a*) that carrier-immunization protocols used to generate CTs populations also generate the *in situ* carrier-specific mechanisms that induce epitope-specific suppression; (*b*) that the "kinetics" of CTs appearance match the kinetics of the *in situ* suppression-induction mechanism (2, 14); and (*c*) that the genetic elements that govern CTs activity (2, 7) also govern *in situ* suppression induction.

TABLE III

Carrier-specific Suppressor T Cells Induce Epitope-specific Suppression

KLH 1° cells transferred	Antigen (weeks)			IgG_{2a} antibody response (RIA)		
	0	4	8	Anti-DNP	Anti-KLH	Anti-CGG
None	DNP-KLH	DNP-KLH		120	9	NT*
None	DNP-KLH	DNP-KLH	DNP-CGG	100	NT	35
Spleen	DNP-KLH	DNP-KLH		20	8	NT
Spleen	DNP-KLH	DNP-KLH	DNP-CGG	10	NT	20
Spleen (T-depleted)	DNP-KLH	DNP-KLH		140	11	NT
	DNP-KLH	DNP-KLH	DNP-CGG	125	NT	39

Donors primed with 100 μg aqueous KLH at −4 and −2 wk. 50×10^6 spleen cells transferred intravenously to BALB/c (nonirradiated) recipients; Recipients challenged with 100 μg of each antigen at indicated times; Antibody response measured 2 wk after last antigenic stimulation; Anti-DNP response is μg/ml; Anti-KLH is percent secondary response standard serum.

* Not tested.

Specificity data from previous CTs studies have generally been interpreted as indicating that CTs regulate antibody responses by controlling the supply of carrier-specific help; however, viewed in retrospect, this evidence is insufficient to distinguish a carrier-specific suppression-effector mechanism from a carrier-specific suppression-induction mechanism coupled with an epitope-specific effector mechanism. Thus, by extending the earlier protocols to include examination of anti-carrier as well as antihapten antibody responses and by testing CTs-suppressed recipients for their subsequent ability to respond to DNP on an unrelated carrier, we obtained evidence that is entirely consistent with the earlier findings but leads to a strikingly different conclusion concerning the role CTs play in regulating responses.

In sum, we now show definitively that CTs activity in adoptive assays is functionally identical to the activity of the carrier-specific mechanism that induces epitope-specific suppression in KLH/DNP-KLH-immunized animals. Consequently, we conclude that CTs induced when an animal first encounters a carrier protein serve subsequently (*in situ*, in vitro, or in appropriate adoptive recipients) to induce suppression for new epitopes subsequently encountered on that carrier.

Epitope-specific Suppression Is a General Regulatory Process. Tables IV and V summarize data from our laboratory and elsewhere, demonstrating that the epitope-specific system regulates antibody production to a variety of antigens and can be induced by widely different immunization conditions. In essence, these studies (2) show that varying the hapten, carrier, age, or strain of the animals immunized, the intervals between carrier and hapten-carrier injection, or the dose or form of the injected carrier protein has very little effect on the induction of suppression by the carrier/

TABLE IV

Epitope-specific Suppression Is a General Regulatory Process: Immunization Conditions That Permit Suppression Induction by the Carrier/Hapten-Carrier Sequence

	Parameter examined*	Result
Carriers	KLH, CGG, OVA, TGAL	All effective except when genetically restricted‡§
Epitopes	DNP, TNP	All effective; suppression also inducible for responses to KLH epitopes§
Persistence	KLH/DNP-KLH, then DNP-KLH or DNP-CGG up to 1 yr later	Anti-DNP suppression equally strong
Carrier dose	KLH: 1, 10, or 100 μg on alum	Strong suppression induced with 100 μg; weak suppression with 10 μg; no suppression with 1 μg
Adjuvants	Alum + *B. pertussis*, alum alone, CFA, aqueous antigen	*B. pertussis* with the carrier prevents suppression-induction; other adjuvants do not interefere; aqueous antigen somewhat better suppression induction
Timing	1–13 wk between KLH and DNP-KLH	Suppression equally strong with all intervals; <1 wk and >13 wk not tested
Age	KLH immunization at 8 wk to >6 mo	Suppression equally strong at all ages

* For reference to data cited, please see text.

‡ Preliminary evidence indicates that suppression induced with certain carriers is relatively easily overcome by subsequent immunization with DNP-CGG or DNP-KLH. Primary immunization with DNP coupled to these carriers, e.g., sheep erythrocytes (in our hands), tends to induce predominantly IgM and IgG_3 anti-DNP responses rather than the full range of isotype responses induced by DNP conjugates of the carriers listed in this table.

§ See Table V.

TABLE V

Epitope-specific Suppression Is a General Regulatory Process: Genetic and Regulatory Conditions That Permit Suppression Induction

Parameters tested*		Result
Mouse strains	BALB/c, BAB/14, SJL, SJA, SJL, × BALB/c, C3H, C3H.SW, A/J, C57BL/10, C57BL/6	Suppression inducible in all strains
IR gene control	TGAL/TNP-TGAL in C3H (H-2k) and C3H.SW (H-2b) congenic mice	Suppression stronger in C3H (nonresponders); suppression in C3H.SW comparable to suppression induced by KLH/DNP-KLH
Carrier function genes (not in H-2)	KLH in A/J and C57BL/10	Specific impairment of suppression induction by KLH/DNP-KLH sequence
Idiotypes	MOPC-460, MOPC-315	Suppression induced for both by KLH/DNP-KLH (Igh^a-460 exempt)
Cell transfers	KLH-primed T cells containing CTs and CTh activity; DNP-KLH to recipients	Suppression-induction favored in nonirradiated recipients; help favored in irradiated recipients
Chronic allotype suppression	DNP-KLH priming in young mice "acutely" suppressed for Igh-1b allotype	Epitope-specific suppression induced for Igh-1b anti-DNP and Igh-1b anti-KLH responses; suppression active during midlife remission from allotype suppression

* See text for references.

hapten-carrier sequence. Only two protocol modifications tested impaired suppression induction: injecting lower carrier-protein doses or injecting *Bordetella pertussis* (but not complete Freund's adjuvant) together with the carrier protein.

Interestingly, the carrier/hapten-carrier sequence induces strong epitope-specific suppression for anti-DNP responses in animals carrying an immune response gene (Ir-1a) (15), previously believed to prevent responsiveness to the carrier molecule; i.e., TGAL/TNP-TGAL immunization induces persistent suppression for IgG responses to trinitrophenyl (TNP) on KLH or CGG in C3H (H-2k, Ir-1a) animals (4). Non-major histocompatibility complex gene(s) that specifically interfere with CTs induction by carrier immunization (16), in contrast, markedly impair suppression induction by the carrier/hapten-carrier sequence, e.g., by KLH/DNP-KLH in A/J mice (Table V).

Table V also refers to studies presented in the third publication in this series,[1] demonstrating that the immunization of young allotype-suppressed mice with DNP-KLH induces an allotype-restricted, epitope-specific suppression for Igh-1b allotype antibody responses to all determinants on the DNP-KLH molecule. This suppression persists throughout the characteristic midlife remission from allotype suppression during which normal Igh-1b antibody responses are produced to newly introduced antigens. Thus, direct immunization with hapten-carrier conjugates induces epitope-specific suppression in an immunologically compromised animal, and the overall specificity of the suppression induced reflects the conditions in the regulatory environment that led to the induction of suppression.

Taken together, these various studies demonstrate that epitope-specific suppression is a broadly occurring and robust regulatory process clearly discernible within the

confines of antibody responses commonly taken as representative of the "normal" functioning of the immune system.

Is Epitope-specific Regulation Consistent with the Known Properties of Antibody Responses? The findings presented here, clearcut in themselves, nevertheless appear paradoxical when considered within the context of normal secondary (anamnestic) antibody responses. That is, if restimulation with an antigen leads to augmented antibody production, shouldn't responses to a new hapten on the antigen also be augmented rather than suppressed; or, conversely, given the antigen-induced presence of CTs capable of inducing epitope-specific suppression for antibody responses to new determinants presented on a priming antigen, shouldn't CTs also induce suppression for responses to the "old" determinants as well?

The answer to this key question lies in perhaps the most novel property of the epitope-specific system: its ability to provide either stable support or stable suppression for individual anti-epitope responses, depending on how it is first induced. That is, if the system is induced to support antibody production for a given epitope before CTs mature, then that anti-epitope response will be "protected" by the time CTs become active. Therefore, the mature CTs population present (after about a week) in carrier-primed animals will induce suppression for responses to new determinants introduced on the priming carrier but will not hamper established antibody responses to the epitopes present on the carrier molecule itself. We discuss this point more fully in the accompanying publication (1).

Summary

The epitope-specific regulatory system selectively controls IgG antibody production to the individual (haptenic) determinants on a complex antigen. This system can be specifically induced to suppress primary and secondary IgG antibody responses to dinitrophenyl hapten (DNP) without interfering with antibody responses to epitopes on the carrier molecule on which the DNP is presented. Furthermore, once induced, it will specifically suppress responses to DNP presented on unrelated carrier molecules. Results summarized here obtained using widely different immunization conditions, and a variety of haptens and carrier molecules indicate that this regulatory system controls antibody production in most T-dependent antibody responses.

Carrier-specific suppressor T cells (CTs) that arise shortly after priming with a carrier molecule such as keyhole limpet hemocyaninin (KLH) induce the epitope-specific system to suppress *in situ* and adoptive antibody responses to epitopes (e.g., DNP) presented subsequently on the priming carrier. These well-known regulatory T cells are commonly believed to regulate antibody production by interfering with carrier-specific help; however, by repeating the original CTs transfer experiments with additional controls that define the specificity of the mechanism mediating suppression in CTs recipients, we show that KLH-specific CTs regulate responses by inducing typical isotope-specific suppression for anti-DNP responses when the recipients are immunized with DNP-KLH. Thus, whether KLH-primed animals are immunized directly with DNP-KLH (KLH/DNP-KLH immunization sequence) or whether T cells from these animals are challenged with DNP-KLH in (nonirradiated) recipients, anti-DNP responses are persistently suppressed while anti-carrier responses proceed normally.

The aqueous KLH-priming protocols usually used to generate CTs are marginally

more effective in priming for *in situ* suppression-induction than the alum KLH-priming protocols commonly used to generate KLH-specific helper T cells and used here in KLH/DNP-KLH immunizations. Thus, studies presented show that priming with an antigenic (carrier) molecule simultaneously prepares the animal for the production of typical secondary (anamnestic) antibody responses to epitopes on the priming antigen and for the induction of epitope-specific suppression for antibody production to determinants presented subsequently on the same antigenic molecule. We discuss the mechanism(s) responsible for this duality and its significance for antibody responses in an accompanying publication that describes the bistable regulatory capabilities of the epitope-specific system.

As indicated in the text, part of these studies were conducted in Dr. Masaru Taniguichi's laboratory, Chiba University, Chiba, Japan. We were fortunate to be able to collaborate with Dr. Taniguichi in the series of experiments reported here. Most of the work reported here, however, was conducted in Dr. Leonard A. Herzenberg's laboratory at Stanford. We are also fortunate to have had the benefit of Dr. Herzenberg's support, advice, and criticism throughout this project. We also thank Dr. Kyoko Hayakawa for helpful scientific contributions to these studies, Mr. F. T. Gadus for excellent technical assistance, Ms. Jean Anderson and Ms. Debra Parks for editorial help, and Mr. Wayne Moore for computer support that greatly aided the preparation of this manuscript.

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