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Epitope-specific regulation
III. Induction of allotype-restricted suppression for IgG antibody responses to individual epitopes on complex antigens*

Maternal antibody to the paternal Igh-1b (IgG_{2a}) allotype induces chronic suppression for Igh-1b (1b) production in (BALB/c × SJL)F_1 hybrid mice. These mice characteristically remain incapable of producing 1b antibody responses until about 3 months of age and then enter a remission phase during which they produce normal 1b antibody responses to antigens introduced initially at this time. Thus young allotype-suppressed mice do not produce 1b antibody responses to dinitrophenylated keyhole limpet hemocyanin (DNP-KLH); older mice however, stimulated initially with DNP-KLH after the onset of remission, produce normal 1b anti-DNP and 1b anti-KLH.
The suppression of 1b antibody responses in young allotype-suppressed mice prevents the expression, rather than the development, of 1b memory for priming antigen epitopes. Furthermore, it not only prevents the expression of such memory cells initially but results in the induction of a continued suppression that specifically prevents their expression after the onset of remission. Thus mice primed with DNP-KLH while allotype suppression is still active develop normal 1b memory for DNP and KLH but nonetheless fail to produce 1b anti-DNP and 1b anti-KLH responses, even when restimulated with DNP-KLH during remission. These mice also fail to produce 1b anti-DNP when stimulated with DNP on an unrelated carrier molecule, *i.e.*, with DNP-chicken gamma globulin (CGG).
This suppression is both epitope-specific and allotype-specific. That is, although 1b responses to DNP on CGG are suppressed, 1b responses to CGG epitopes on DNP-CGG proceed normally. Furthermore, there is no suppression of other isotype and allotype responses either to DNP or to the CGG epitopes. These data therefore define an Igh-restricted epitope-specific mechanism that can be induced to persistently suppress 1b antibody responses to epitopes introduced initially during active (1b) allotype suppression.

1 Introduction

Recently, an "epitope-specific" (E-sp) regulatory system** has been described that can be induced to suppress primary and subsequent IgG antibody responses to a given epitope (*e.g.*, the DNP hapten) presented on a variety of commonly used carrier proteins without interfering with the production of IgG antibodies to any of the other epitopes (*i.e.*, native structural determinants) on these proteins. Once induced to suppress antibody production to an epitope, this system continues to specifically suppress such antibody production regardless of the carrier molecule on which the epitope is subsequently presented [1–6].

Immunizing carrier-primed animals with DNP coupled to the priming carrier routinely induces the E-sp system to suppress IgG anti-DNP antibody production. This "carrier/hapten-carrier" immunization sequence generates normal IgG anti-DNP memory B cell populations, normal carrier-specific helper T cell activity and normal secondary IgG antibody responses to the carrier protein epitopes. Nevertheless, IgG anti-DNP responses are specifically suppressed to below primary levels in animals immunized with this sequence [1–6].

Studies presented here show that priming young allotype-suppressed (A-s) [7–12] mice with hapten-carrier conjugates induces an E-sp suppression identical to the suppression induced by carrier/hapten-carrier immunization except for the subset of anti-epitope responses suppressed, *i.e.* the suppression induced in A-s mice is restricted to 1b antibody responses and affects (1b) responses to all of the epitopes on the priming antigen. These differences demonstrate that the E-sp system can be selectively induced to suppress individual components of the primary and subsequent (anamnestic) IgG responses to epitopes on complex antigens according to the dictates of the

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Abbreviations: **A-s:** Allotype-suppressed **E-sp:** Epitope-specific **DNP:** 2,4-Dinitrophenyl hapten **KLH:** Keyhole limpet hemocyanin **CGG:** Chicken gamma globulin **RIA:** Radioimmunoassay(s)

** 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 which 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 2,4-dinitrophenyl (DNP) group 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.

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immunologic environment into which the epitopes are first introduced.

2 Materials and methods

The methods used in these studies have all been described in previous publications [*e.g.* 5, 13–15]. The affinity of anti-DNP antibodies in individual serum samples are measured by a recently developed radioimmunoassay (RIA) [13].

The experimental design was as follows: briefly, young A-s mice and age-matched controls were primed with 100 μg DNP-KLH (keyhole limpet hemocyanin) on alum during the acute phase of allotype suppression. About 6 weeks later, after the A-s mice have entered remission and are producing relatively normal serum Igh-1b levels, both groups were immunized again with 100 μg DNP-KLH or DNP-CGG (chicken gamma globulin) on alum. Anti-DNP and anti-carrier antibody levels in serum were monitored weekly, starting one week after the first immunization. Response levels 2 weeks after each antigenic stimulation are shown since these are representative of responses obtained throughout the period. Specific details for individual experiments are described in Table and Figure legends.

3 Results

3.1 Priming young A-s mice induces persistent suppression for priming antigen epitopes

$(BALB/c \times SJL)F_1$ hybrid mice normally produce about equal amounts of IgG_{2a} immunoglobulins carrying the maternally derived Igh-1a (1a) or the paternally derived Igh-1b (1b) allotype. When exposed perinatally to antibodies to the paternal 1b allotype, however, these mice become "chronically" suppressed for 1b production. Thus although their production of other serum allotypes and isotypes continues normally throughout life, they go through a characteristic series of changes vis à vis 1b allotype production as they age [7–12, 14].

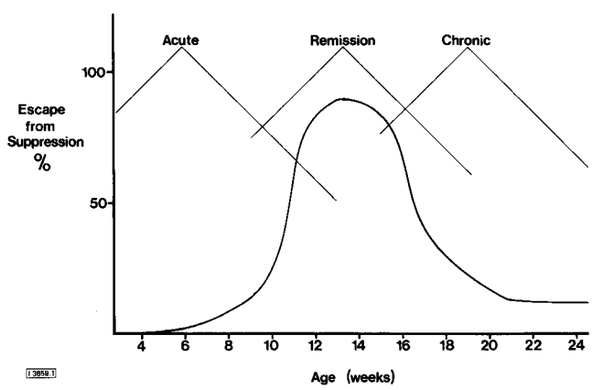

Figure 1. Time course for allotype suppression. $(BALB/c \times SJL)F_1$ mice exposed perinatally to maternal anti-Igh-1b were tested weekly for the presence of this allotype in serum. Those with > 10 μg/ml were scored as having escaped from suppression (*i.e.* in remission); however, for the experiments testing responses in remission animals, only those in full remission were used, *i.e.* > 500 μg/ml. This latter group constitutes about a third of the animals in remission. Data shown here represent a cumulative average of more than 50 litters tested during a 2–3-year period. Serum Igh-1b levels were measured by RIA [12, 14, 15].

The mice begin life with the maternal anti-1b antibody present and consequently remain in an "acute suppression" phase (from birth to about 10 weeks of age) during which they do not produce 1b allotype Ig. Next, they pass through a temporary "remission" phase (from about 10–20 weeks) during which they nearly always produce at least some 1b allotype and frequently show normal 1b allotype levels in serum. Finally, they enter the "chronic suppression" phase (from about 20 weeks until death) during which they usually terminate 1b production and only occasionally show serum 1b at low levels and for short periods of time (Fig. 1).

1b antibody responses to DNP-KLH in these mice vary according to whether they are primed during the acute or the remission phase. If they enter remission and produce detectable serum levels of 1b allotype within 1 to 2 weeks of being primed (either before or after priming) they tend to produce normal 1b anti-DNP and 1b anti-KLH responses (Table 1). If, in contrast, they are in the acute suppression phase when primed and do not shift into remission until a week or two has elapsed, their 1b anti-DNP and 1b anti-KLH responses are suppressed initially and remain suppressed throughout the remission period. Thus restimulation with antigen doses that would normally induce a 1b primary response during remission barely stimulates detectable 1b anti-DNP and 1b anti-KLH production in these animals (Table 2).

Whether primed during the acute or the remission phase, however, all mice immunized with DNP-KLH develop essentially normal 1b anti-DNP and 1b anti-KLH memory B cell populations (Table 3) and produce normal primary and secondary responses to DNP-KLH in all other isotypes and allotypes (see Tables 1 and 2). Thus the suppression of 1b antibody production in the mice primed during the acute suppression phase

Table 1. A-s mice produce normal Igh-1b antibody responses if primed during remission

Allotype suppression status when primed[b]	Antigen	IgG_{2a} antibody responses[a] Anti-DNP (μg/ml)		Anti-KLH (units)	
		Igh-1a	Igh-1b	Igh-1a	Igh-1b
Active	DNP-KLH	35	< 1	10	< 1
Remission	DNP-KLH	36	29	5	6
Control	DNP-KLH	33	35	5	5

a) Serum antibody levels were measured by RIA 2 weeks after priming. Levels shown remained essentially constant during the next 4–6 weeks despite marked increases in serum Igh-1b levels in actively suppressed animals that entered the remission phase during the test period. Arithmetic means of responses measured for 3–5 individuals in each group are shown. The majority of responses were closely grouped around the mean; however, responses in animals occasionally deviated by as much as 50%. Anti-KLH units are defined as the percentage of the anti-KLH antibody (of the indicated allotype) present in a standard secondary response antiserum to DNP-KLH.

b) For definition of allotype suppression status, see Sect. 3.1 or legend for Table 2. Mice were primed with 100 μg DNP-KLH on alum i.p.; experimental groups each contained 12 mice; control group contained 30 mice.

Table 2. Priming while Igh-1b allotype production is actively suppressed induces suppression for subsequent Igh-1b antibody responses to epitopes on the priming antigen

Allotype suppression status (when immunized)[a]				Igh-1 (IgG_{2a}) antibody responses (after second immunization)[b]					
First immunization[c]		Second immunization[c]		Anti-DNP (μg/ml)		Anti-KLH (units)		Anti-CGG (units)	
Status	Antigen	Status	Antigen	1a	1b	1a	1b	1a	1b
Active	DK	Remission	DK	130	< 5	60	< 5		
Active	DK	Remission	DC	260	10			14	14
Remission	DK	Remission	DC	316	195			13	18
Control	DK	Control	DK	127	110	25	40		

a) Antigens: DK=DNP-KLH; DC=DNP-CGG. Allotype-suppression status (see Sect. 3.1): Active (acute suppression phase), < 10 μg Igh-1b/ml serum 8–10 mice/group; remission (following acute suppression phase) and control (not exposed to maternal anti-Igh-1b), > 500 μg Igh-1b/ml serum.

b) Serum antibody levels tested by RIA 14 days after last indicated immunization. Anti-CGG and anti-KLH activity (units) expressed relative to an appropriate secondary response antiserum. Mean responses from 3–5 animals/group are shown (see legend for Table 1). Affinities of suppressed anti-DNP responses (when measurable) were roughly 50-fold lower than the control responses.

c) First immunization: 100 μg DNP-KLH on alum. Second immunization: 100 μg DNP-CGG on alum or 1 μg aqueous DNP-KLH. Similar results were obtained with 100 μg DNP-KLH on alum (data not shown). All antigens were injected i.p.

Table 3. Allotype suppression does not interfere with memory B cell development

DNP-KLH-primed B cells transferred[a] Allotype suppression status of donors when primed	No. of donors	Adoptive secondary IgG anti-DNP responses[b] Igh-1a	Igh-1b	Igh-4a	Igh-4b
Acute suppression phase	1	87	64	220	250
	1	99	81	320	270
Control (not suppressed)	3	90	80	250	280

a) (BALB/c × SJL)F_1 donors primed at 8 weeks of age; B cells remaining after 10^7 donor spleen cells were treated with monoclonal anti-Thy-1.2 and complement (to deplete T cells) mixed with syngeneic carrier-primed T cells (2.5×10^6 nylon-passed T cells from nonsuppressed KLH-primed mice and transferred to irradiated BALB/c mice (650 rd, 18 h previously). Recipients were immunized with 1 μg aqueous DNP-KLH at time of transfer.

b) Serum anti-DNP antibody levels (μg/ml) were measured by RIA. 2 weeks after transfer nylon-passed T cells transferred without B cells produced < 5 μg/ml anti-DNP antibody of each allotype.

continues throughout the remission phase despite the presence of ample carrier-specific help to support antibody responses to DNP-KLH and the presence of ample populations of memory B cells to give rise to 1b antibody responses if such responses were permitted.

3.2 Igh-1b antibody production is suppressed by the E-sp system

Further analysis of the suppression induced in A-s mice (BALB/c × SJL)F_1 indicate that it is mediated by a mechanism which is E-sp in addition to being allotype-specific. That is, this mechanism selectively prevents 1b antibody responses to epitopes on the antigen (DNP-KLH) used to induce suppression (during the acute allotype suppression phase) but does not interfere with 1b antibody responses to epitopes first presented during remission, even when these two types of epitopes are presented (during remission) on the same carrier molecule. The following studies, which describe the responses to DNP and to the epitopes which accompany it on two sequentially presented hapten-carrier conjugates (DNP-KLH and DNP-CGG) reveal this specificity quite clearly.

As the data in Table 2 show, mice primed with DNP-KLH during acute allotype suppression and then immunized with DNP-CGG during remission produce virtually no 1b antibody responses to the DNP hapten even though they produce normal primary-level 1b antibody responses to the other (CGG) epitopes on the DNP-CGG molecule. In principle, these mice should produce secondary-level 1b anti-DNP responses since they have comparable 1a and 1b anti-DNP memory B cell populations (see Table 3) and produce normal secondary-level 1a anti-DNP responses (see Table 2). Their inability to mount even a primary level 1b anti-DNP response to DNP-CGG indicates a substantially stronger suppression of this (anti-DNP) response that the contrast with the normal primary 1b anti-CGG response might suggest.

The suppression for 1b anti-DNP responses induced by immunization with DNP-KLH during acute allotype suppression therefore persists and prevents the expression of 1b anti-DNP memory B cells despite the presentation of the DNP hapten in a new antigenic context (on an unrelated carrier molecule) along with other determinants that successfully stimulate 1b antibody production. Therefore, except for being restricted to 1b antibody responses, this suppression is indistinguishable from the E-sp suppression of anti-DNP antibody production induced by the carrier/hapten-carrier immunization sequence [1–6].

The suppression of 1b anti-KLH responses induced in A-s mice (see Table 2) may at first appear to belie this conclusion since the E-sp suppression induced by KLH/DNP-KLH immunization characteristically suppresses anti-DNP, but not anti-KLH, antibody production [1–6]. However this striking

difference merely reflects the contrast between the mechanisms responsible for suppression induction in the two cases. That is, E-sp suppression induction for anti-DNP antibody responses in normal mice requires subsequent presentation of DNP on the priming carrier (carrier/hapten-carrier immunization) whereas similar suppression is induced in A-s mice just by priming with DNP-KLH. Thus, since the allotype-oriented mechanism inducing suppression in these mice does not depend on the distinction between initially and subsequently presented epitopes, suppression should be induced equally for 1b responses to all epitopes on DNP-KLH when presented as a priming antigen.

Data (not shown) from KLH/DNP-KLH immunizations in A-s mice support this conclusion. This immunization sequence, in which the KLH was presented during the acute suppression phase and the DNP-KLH was presented during remission, resulted in the suppression of all IgG isotype responses to DNP (due to carrier/hapten-carrier immunization) and the selective suppression of 1b anti-KLH responses (due to the operation of the mechanism that induces suppression for 1b antibody responses in young A-s mice). Thus, carrier/hapten-carrier immunization and allotype suppression can act in concert to induce an E-sp suppression that combines the specificities of the suppression induced by each of these mechanisms independently.

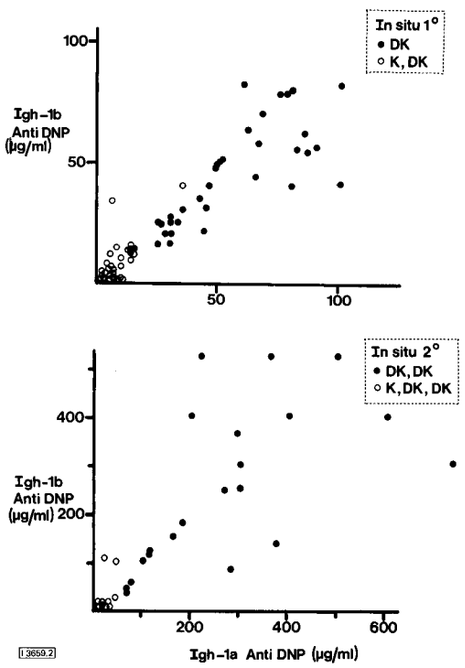

Figure 2. Individual differences in the Igh-1a and Igh-1b (IgG_{2a} allotype) anti-DNP responses in normal (not allotype-suppressed) (BALB/c × SJL)F_1 animals immunized either with DNP-KLH or the KLH/DNP-KLH sequence (which induces E-sp suppression). Axes indicate μg/ml anti-DNP antibody for the specified allotype in serum. Antibody levels were measured by RIA [10, 15] two weeks after the last antigenic stimulation. The "in situ 2°" label refers to animals that were restimulated with 100 μg DNP-KLH on alum 6 weeks after priming with the same amount of DNP-KLH (on alum). KLH-primed animals received 100 μg KLH on alum 6 weeks prior to the first DNP-KLH stimulation.

3.3 E-sp regulation in normal mice appears to be mediated by an allotype-restricted mechanism

Regulation of antibody production according to Ig heavy chain allotype is most clearly demonstrable when E-sp suppression is induced in A-s mice; however, antibody responses in control mice and in mice suppressed by exposure to the carrier/hapten-carrier sequence also show evidence of allotype-restricted regulation (particularly after two or more hapten-carrier stimulations). As data in Fig. 2 show, although allotype responses within each isotype tend to be concordantly regulated in allotype heterozygotes, occasional control animals produce substantially higher levels of one allotype than of the other.

Similarly, when E-sp suppression begins to wane following repeated antigenic stimulation and individual isotype responses selectively escape suppression [2], occasional allotype heterozygotes produce normal response levels of one allotype and remain completely suppressed for the other. Thus studies with normal (rather than A-s mice) foreshadowed the allotype-restricted regulation demonstrated here.

4 Discussion

We have shown that Igh-1b (1b) antibody responses to a specific set of epitopes are selectively suppressed in animals that otherwise produce normal IgG responses to these epitopes and furtheremore produce completely normal IgG responses (including 1b) to epitopes on other antigens. The joint activity of two independent suppression mechanisms (specific for allotype and epitope) cannot explain these findings. That is, an allotype suppression mechanism acting independently would suppress all Igh-1b responses; and an E-sp suppression mechanism acting independently would suppress both Igh-1a and Igh-1b (and other IgG) antibody responses to the target epitopes. Thus the extraordinary selectivity of the suppression demonstrated here (for 1b antibody responses to individual epitopes) defines a single, Igh-restricted, E-sp regulatory system capable of controlling the expression of memory B cells according to both the allotype and combining-site commitment such cells display.

The chronic allotype supression mechanism does not appear to be directly active in this regulatory system. Sequential immunization studies we conducted some time ago (circa 1968) indicate that "short-term" A-s mice (1a/1b progeny of C57BL/10 fathers and BALB/c mothers producing anti-1b antibodies) also develop an Igh-restricted E-sp suppression when immunized initially with sheep erythrocytes (SRBC) while the maternally induced allotype suppression is still in force. That is, the immunized (A-s) mice specifically fail to produce either primary or secondary 1b anti-SRBC responses, even when restimulated with SRBC after they have permanently recovered from allotype suppression and are fully capable of mounting normal 1b antibody responses to newly introduced antigens (L. A. Herzenberg and E. Rivera, unpublished observations).

These findings dissociate the specialized mechanisms responsible for chronic allotype suppression from the mechanisms that induce and mediate the Igh-restricted, E-sp suppression described here. Consequently, they define a causal connection between the early inability to produce a 1b allotype antibody response and the antigen-stimulated induction of specific sup-

pression for 1b antibody responses to (all) epitopes on the immunizing antigen.

At first glance, this would appear to distinguish the mechanism that induces this suppression from the carrier-specific mechanism that induces E-sp suppression in normal animals (for IgG anti-hapten responses after sequential immunization with a carrier protein and a hapten conjugated to the priming carrier). Nevertheless, the induction of suppression in both cases can be traced to a common condition: the failure to initiate a given set of anti-epitope responses before carrier priming is complete and the carrier-specific suppressor T cells (CT_s) that induce E-sp suppression have matured to full function.

Normally, the epitopes on a priming antigen (carrier) have the opportunity to induce stable IgG antibody production before the emergence of the CT_s population induced by the antigen. Carrier/hapten-carrier immunization, however, forces CT_s induction and maturation prior to the introduction of the "new" epitope (hapten). Allotype suppression similarly allows CT_s induced by a priming antigen to mature before 1b antibody responses to the epitopes on the antigen can be initiated. Thus, in each case, the initial advantage enjoyed by the epitopes on a priming antigen dissipates selectively and results in the selective induction of suppression for the compromised components of the antibody response, *i.e.* for IgG antibody responses to the "new" epitope in carrier/hapten-carrier-immunized animals and for 1b responses to the priming antigen epitopes in A-s mice.

These considerations, which indicate that anti-epitope responses must be rapidly initiated when an antigen is first introduced in order to protect such responses against subsequent suppression, outline perhaps the most novel property of the E-sp system: its bistable regulatory capability that permits it to be induced either to suppress individual anti-epitope responses or to support such responses and actively prevent the induction of suppression for them. We discuss this unique regulatory capability more completely in the second publication in this series [2] which presents evidence directly demonstrating the bistable properties of the individual (Igh-restricted, E-sp) regulatory elements that together comprise the "E-sp system".

The mechanism that underlies the Igh restriction in the system, however, is more appropriately considered here. This restriction, clearly visible in the specific suppression of 1b anti-epitope responses (in antigen-primed A-s mice) is also apparent in the selective suppression of individual isotype anti-hapten responses that frequently occur in carrier/hapten-carrier-immunized animals (either when suppression is weak initially or wanes after repeated hapten-carrier stimulation [2]). Thus E-sp regulatory cells must be capable of recognizing Igh constant-region structures (determinants).

The cells that mediate the allotype-restricted regulation described here have to recognize allotypic determinants; however, the cells that mediate the isotype-specific regulation in carrier/hapten-carrier mice could recognize either allotypic or isotypic determinants, since nearly all allotypic determinants are unique to individual isotypes and consequently identify their respective isotypes unambiguously [16]. In fact, isotypic and allotypic determinants are functionally equivalent in allotype homozygotes. Therefore, if the allotype responses for a given isotype response in allotype heterozygotes tend to be concordantly regulated in carrier/hapten-carrier animals, allotype-restricted regulation could account for the observations in these animals as well.

This hypothesis is favored both by the occasionally disproportionate allotype representation in anti-epitope responses in normal hapten-carrier-primed allotype heterozygotes and by the sporadic nonconcordant "escapes" from E-sp suppression evident in carrier/hapten-carrier-primed heterozygotes. These rare disparities could also be explained by random variation in the number of memory B cell clones of each allotype represented in the memory populations in individual animals (*i.e.* by "clonal dominance"); however, although Occam's razor has occasionally been known to cut too close, we lean towards the idea that the E-sp system recognizes allotypic determinants under all circumstances.

Thus, on the basis of the evidence presented here and in the preceding two publications in this series [1, 2], we suggest that Igh constant region (isotype/allotype) representation in antibody responses is controlled by allotype-restricted E-sp elements that tend to operate concordantly for a given isotype, except when stimulatory conditions (such as those used here) induce the system to uniquely suppress production of antibodies carrying a particular allotype.

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