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Carrier-priming leads to hapten-specific suppression

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Studies over the past two decades have established[1,2] that 'helper' T lymphocytes primed to a 'carrier' protein increase B-lymphocyte antibody responses to 'haptenic' determinants on the carrier protein. T cells from donors primed with one commonly used carrier protein, keyhole limpet haemocyanin (KLH), have been shown by many workers to augment B-cell IgG anti-dinitrophenyl (DNP) responses in adoptive recipients stimulated with DNP-KLH. Therefore, we were surprised recently to find that the KLH-primed mice we normally use as donors for carrier-specific helper T cells in adoptive transfers[3] show reduced rather than augmented IgG *in situ* anti-DNP antibody production when stimulated with DNP-KLH. Here we show that hapten-specific regulation of antibody production is responsible for this response failure. That is, that KLH-priming before DNP-KLH exposure reduces the production of IgG anti-DNP antibody without interfering with the anti-KLH response or with the development of anti-DNP memory B cells, and that IgG anti-DNP production remains low after further stimulation with DNP on KLH or an unrelated carrier. Thus initial stimulation with a carrier-protein creates an oddly compromised animal in which IgG antibody production to a 'new' haptenic determinant on the carrier is kept minimal despite the presence of normal anti-hapten memory and carrier-specific help capable of supporting other responses to the hapten–carrier conjugate.

Figure 1 shows that very little IgG anti-DNP antibody is produced in KLH-primed mice stimulated with 100 μg DNP-KLH on alum and stimulated again 6 weeks later with 1 μg of aqueous DNP-KLH. The average affinity of this minimal anti-DNP response is 10–100-fold lower than the anti-DNP affinities obtained in mice not previously exposed to KLH (Table 1). The Igh-1a (IgG2a) anti-DNP responses from

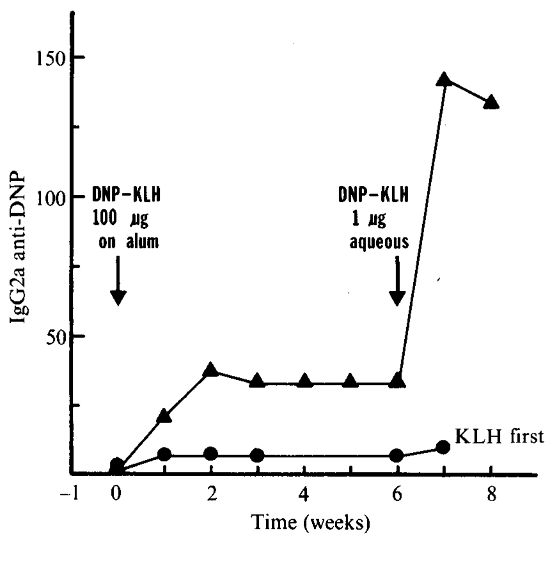

Fig. 1 Carrier-priming prevents hapten–carrier stimulation of anti-hapten antibody responses. (BALB/c × SJL)F_1 hybrids were used for these experiments. BALB/c and BAB/14 mice showed similar results. Data for Igh-1a (IgG2a) allotype anti-DNP responses are shown here. Protocol and assay details are as described in Table 1 legend. ●, Primed with 100 μg KLH at −6 weeks; ▲, no prior antigenic exposure.

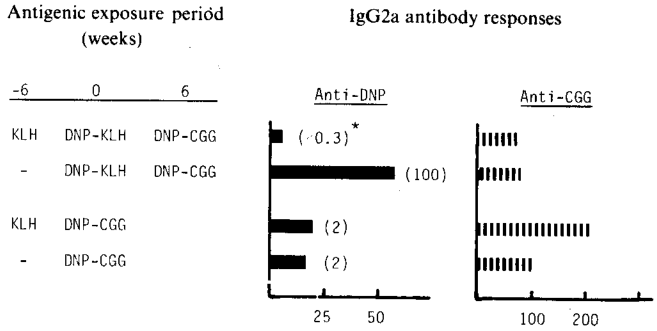

Fig. 2 Homologous hapten–carrier conjugate induces hapten-specific suppression in carrier-primed mice. Each indicated antigenic stimulation was given as 100 μg of antigen on alum, i.p. Responses were measured 1 week after last indicated stimulation; RIA units shown are relative to 'standard' antiserum pools for each antigen. Mean K_a (in parentheses, $\times 10^6$) was determined from RIA binding. Assay details are as described in Table 1 legend.

Igh^a/Igh^b (BALB/c × SJL)F_1 hybrids shown are similar to Igh-1b responses in the same mice. Production of IgG_1 anti-DNP is also reduced in carrier-primed mice but often increases with repeated DNP-KLH stimulation. IgM responses, in contrast, are not reduced (data not shown). These findings are representative of results obtained using a variety of protocols in which DNP-KLH was injected 1–6 weeks after KLH and at different doses either on alum or in aqueous form (see Table 1).

Table 1 also shows that although the anti-DNP response is severely reduced in the KLH-primed mice exposed repeatedly to DNP-KLH, the anti-KLH response in these mice proceeds normally (that is, increases with each subsequent exposure to KLH determinants). As both these responses require help from carrier-primed T cells, the specific and continued failure of the anti-DNP response cannot be ascribed simply to the lack of such help. Nor can this failure be ascribed to deficiencies in anti-DNP memory B-cell development. Adoptive transfer data in Table 2 show that splenic memory B cells generated by DNP-KLH in KLH-primed mice (groups I and III) are indistinguishable from memory populations generated by our usual protocol for priming anti-DNP memory B cells (group II)[3]. Both the magnitude and the affinity of B-cell memory in each of these three groups are essentially the same. Thus functional memory B cells are clearly present but generally remain silent in mice primed first with KLH (on alum) and then exposed repeatedly to DNP-KLH.

In situ and memory responses from mice primed with aqueous DNP-KLH (group III, Tables 1 and 2) show that inadequate priming reduces all responses to the hapten–carrier conjugate rather than specifically reducing anti-DNP antibody production. These results therefore indicate that alum priming with KLH (free or hapten-conjugated) is required to induce adequate carrier-specific help for optimal *in situ* antibody production and memory B-cell development. Furthermore, since anti-DNP memory development occurs normally in KLH (on alum)-primed mice exposed to DNP-KLH, these results confirm that adequate carrier-specific help is present and operative with respect to DNP-KLH in these mice, even though the anti-DNP antibody production is minimal.

Finally, data in Fig. 2 show that exposure of KLH-primed mice to DNP-KLH renders these mice incapable of responding to DNP conjugated to an unrelated carrier protein but does not interfere with response to the carrier protein itself, that is chicken γ-globulin (CGG). Figure 2 also shows that mice not exposed to DNP-KLH respond normally to

Table 1 Carrier-priming reduces the amount and affinity of anti-hapten responses stimulated by the hapten–carrier conjugate but does not interfere with anti-carrier response

Antigenic stimulation			*In situ* IgG2a antibody responses			
Group	Protocol	Time (weeks)	Anti-KLH units	Anti-DNP units	Anti-DNP Mean K_a ($\times 10^6$)
I	Hapten-carrier first				
	DNP-KLH (100 μg on alum) →	0			
		2	15	35	(5)
		6	15	30	(8)
	DNP-KLH (1 μg aqueous) →	6			
		7	130	140	(100)
		8	120	120	(500)
II	Carrier first				
	KLH (100 μg on alum) →	−6			
		−4	20	<1	(<0.3)
	DNP-KLH (100 μg on alum) →	0			
		2	170	5	(<0.3)
		6	ND	5	(<0.3)
	DNP-KLH (1 μg aqueous) →	6			
		7	370	9	(0.5)
III	Hapten-carrier first				
	DNP-KLH (100 μg aqueous) →	0			
		2	4	3	(<0.3)
IV	Carrier first				
	KLH (100 μg on alum) →	−6			
	DNP-KLH (100 μg aqueous) →	0			
	2	2	140	5	(<0.3)

(BALB/c × SJL)F_1 hybrid mice (Igh^a/Igh^b), approximately 10 weeks old at the beginning of the stimulation protocols, were used. All antigens were injected intraperitoneally (i.p.). Mice were bled from the tail at indicated times and serum assayed for anti-KLH and anti-DNP activities by solid phase radioimmunoassay (RIA). Data for the Igh-1a (IgG2a) allotype response are shown here; Igh-1b allotype responses were similar. IgG1 responses were somewhat higher (see text). Units of antibody are equal to the percentage of allotype anti-DNP antibody in a standard serum pool of BALB/c × SJL adoptive secondary response sera[12]; 1 units $\simeq 1\ \mu g\ ml^{-1}$ of anti-DNP antibody. Mean $K_a(M^{-1})$ values are estimated from the ratio of RIA binding obtained on DNP_{12} bovine serum albumin (BSA) and DNP_{42}BSA. Studies with monoclonal anti-DNP antibodies have shown that this ratio is proportional to log K_a determined by fluorescence quenching with ε-DNP-lysine[3]. ND, not determined.

Table 2 Hapten–carrier stimulation of anti-hapten memory B cells is normal in carrier-primed mice

Memory B-cell donors form of antigenic stimulation			Adoptive IgG anti-DNP response	
Group	KLH	DNP-KLH	Units	Mean K_a ($\times 10^6$)
I	*	Alum	73	10
II	Alum	Alum	73	8
III	*	Aqueous	18	0.7
IV	Alum	Aqueous	50	8

Donors (BALB/c × SJL) from groups listed in Table 1 were killed 3 weeks after the first indicated stimulation with DNP-KLH. Splenic B cells were prepared by cytotoxic depletion of T cells using monoclonal anti-Thy-1.2 (30-H12, J. Ledbetter). Cells obtained from 10^7 spleen cells were co-transferred with 2×10^6 nylon-passed syngeneic T cells from KLH-primed donors (100 μg on alum plus 10^9 *Bordatella pertussis* at least 6 weeks before transfer). Recipients (650 rad irradiated BALB/c) were stimulated with 1 μg aqueous DNP-KLH (intravenous) at time of transfer and bled 1 week later for assay. Assay details are as described in Table 1 legend. Igh-1b (IgG2a) response data are shown here. Igh-1a (IgG2a) and IgG1 response data were similar. Responses in mice receiving nylon T (no donor B) were three units of very low affinity antibody. The proportion of IgD^+ memory (also an index of memory population maturity[3] was similar in groups I, II and IV and higher in group III, indicating less maturation (to IgD^-) in mice primed with aqueous antigen. Anti-KLH memory was similar in groups I, II and IV and lower in group III.

*No KLH injection.

DNP-CGG. Therefore, we conclude first that the induction of non-responsiveness to DNP requires exposure to hapten on the carrier used initially for priming, and second that the effector mechanism responsible for preventing production of the anti-DNP antibody is hapten-specific and does not depend on specific interaction with the carrier protein.

Since the effector mechanism prevents production of antibodies with particular variable region structures (that is the majority of those capable of combining with DNP) and since it 'actively' suppresses anti-DNP responses by co-transferred DNP-primed populations in adoptive transfer recipients (data not shown), we tentatively regard the non-responsiveness for DNP demonstrated here as due to the induction of a broad idiotype suppression[4,5] occasioned by exposure of carrier-primed animals to DNP conjugated to the carrier used for priming.

The characteristics of *in situ* antibody responses in a number of other immunization systems indicate that the inability of carrier-primed mice to produce IgG antibody to 'new' haptenic determinants on the carrier is a general regulatory phenomenon which applies even to haptenic determinants presented with the carrier when these initially fail to stimulate an antibody response. For example, we have observed that repeated stimulation of individual mice producing antibodies to IgG2a allotypic determinants elicits production of very little antibody to determinants not detected in the early antibody populations. "Original antigenic sin" studies[6] with viral antigens also suggest that initial reactivity defines the scope of subsequent antibody production. Finally, although we (and some of our colleagues) were surprised that carrier-primed mice respond poorly *in situ* to a 'new' hapten on a hapten–carrier conjugate, the basic elements of this finding have already been described[1,2,7].

In fact, extensive evidence (albeit otherwise interpreted) has been presented demonstrating that suppression for anti-hapten responses develops when carrier-priming precedes exposure to hapten–carrier conjugates. 'Carrier-specific' suppression, for example[8], is usually defined by the ability of carrier-primed mice to suppress the response to a hapten conjugated to the priming carrier but not to the same hapten on a non-related carrier. Our demonstration that hapten–carrier conjugates induce specific anti-hapten suppression in similarly primed mice suggests (but does not prove) that in some cases this so-called carrier-specific suppression may really be hapten-specific. A similar argument applies to studies of feedback suppression[9].

Evidence[1,2,7] demonstrating that carrier-priming reduces subsequent responses to hapten–carrier conjugates has been largely ignored because hapten and carrier immunization protocols were developed that yielded the expected *in situ* secondary anti-hapten responses in carrier-primed animals (for example, ref. 10). Once these studies showed that carrier-primed T cells help hapten-primed B cells *in situ*, the matter of the anomalous response failures faded into the background and investigations shifted to exploring the mechanism of T–B interactions in adoptive transfer systems that avoid problems associated with *in situ* responses.

The conditions required to demonstrate carrier-specific help *in situ*, however, are of interest: before challenge with carrier and hapten–carrier conjugate, animals must be primed with the hapten (on an unrelated carrier)[10]. This suggests that the induction of hapten-specific suppression fails when cell populations that normally arise during hapten priming (that is, anti-hapten memory B cells and their idiotype-specific helper T cells) are present before the carrier/hapten–carrier exposure sequence. Which, if either, population prevents suppression induction is unclear; however, the participation of idiotype-specific help would be consistent with the idea that antibody responses are controlled by idiotype-specific "core regulatory circuits"[11] configured such that they tend to stabilize in either a suppression or a help mode (depending on whether more suppressor or more helper T cells are induced by the initial priming conditions).

In any event, the dramatic reduction of the high affinity IgG anti-DNP response that we have shown to occur in KLH-primed mice subsequently exposed to DNP-KLH now brings hapten-specific regulatory processes into the arena of commonly studied heterogeneous antibody responses. Furthermore, it suggests an even closer functional interrelationship between carrier-specific and haptern (idiotype?)-specific regulation than previously supposed, as it indicates that carrier-specific priming contributes to the induction of hapten-specific suppression. This offers some entirely new perspectives on the mechanisms involved in response regulation and possibly on mechanisms of tolerance induction and prevention of autoantibody production.

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