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Epitope-Specific Regulation

IV. *In Vitro* Studies with Suppressor T Cells Induced by Carrier/Hapten-Carrier Immunization

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Sequential immunization with a carrier molecule and a new epitope (hapten) conjugated to the carrier (carrier/hapten-carrier immunization) induces specific suppression for IgG antibody production to the new epitope (hapten) on the carrier. Once induced, this "epitope-specific" suppression persists and specifically suppresses subsequent *in vivo* IgG antibody responses to the hapten presented on the same or on an unrelated carrier molecule. *In vitro* studies presented here characterize the surface markers and specificity of suppressor T cells generated in carrier/hapten-carrier-immunized animals. Thus we show (1) that spleen cells from these donors suppress *in vitro* IgG anti-hapten antibody production by cocultured hapten-primed spleen cells; (2) that some but not all of the suppressor cells carry surface Lyt-2; (3) that at least some of the suppressor cells have receptors for the inducing hapten (DNP); and (4) that, unlike the suppression obtained *in vivo*, the *in vitro* suppression extends to IgG responses to unrelated carrier protein epitopes presented in association with the inducing hapten.

INTRODUCTION

Immunizing carrier-primed animals with a hapten coupled to the priming carrier has two functionally opposite effects: it stimulates the development of anti-hapten memory B cells and it induces a persistent "epitope-specific" suppression that prevents the immediate and subsequent expression of these memory cells even when the hapten (epitope) is later introduced on an unrelated carrier molecule (1). Carrier-specific T cells induce this suppression, apparently by "presenting" the inducing epitope via a hapten-carrier bridge (2). The suppression itself, however, is mediated by a unique effector mechanism that selectively controls antibody production to individual epitopes regardless of the carrier molecule on which the epitopes are presented (2).

This epitope-specific effector mechanism is Igh restricted in the sense that it selectively controls the expression of memory cells committed to producing IgG anti-hapten antibodies with different Ig heavy chain isotypes or allotypes (3, 4). Furthermore, certain isotypes tend to be suppressed more easily than others (3). In essence, whenever suppression is weak or wanning, IgG_1 responses tend to be produced while IgG_{2a}, IgG_{2b}, and IgG_3 responses tend to be suppressed. Thus the detection of epitope-specific suppression often requires measurement of individual isotype responses (such as in studies presented here).

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We have induced epitope-specific suppression for IgG responses to a variety of haptens presented on a variety of carrier molecules (2). Most of our studies, however, have been conducted with two commonly used hapten-carrier conjugates (and carrier molecules): DNP-CGG and DNP-KLH (dinitrophenyl hapten coupled to chicken γ-globulin and to keyhole limpet hemocyanin, respectively).[1] As we have shown (2), IgG anti-DNP responses are dramatically suppressed in CGG/DNP-CGG- (or KLH/DNP-KLH-) immunized animals and tend to remain so when these animals are subsequently immunized either with DNP-CGG or with DNP-KLH. Anti-CGG and anti-KLH responses, in contrast, proceed without interference and reach the primary or secondary IgG response level appropriate to the number of stimulations with the carrier protein.

In studies presented here, we use CGG/DNP-CGG immunization to induce suppressor T cells that actively suppress *in vitro* IgG antibody responses to DNP-KLH by cocultured spleen cells from DNP-KLH-primed donors. The characteristics of these suppressor T cells (surface markers, receptor specificity, functional activity) indicate that the regulatory T-cell populations in carrier/hapten-carrier-immunized mice are even more complex than previously supposed.

MATERIALS AND METHODS

Animals. BALB/c CrSlc mice were purchased from Shizuoka Experimental Animal Laboratory, Company, Ltd., Hamamatsu, Japan. (BALB/c × SJL)F_1 mice were raised in the animal facility at Stanford University.

Antigens. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem, Behring Corporation, American Hoechst Corporation, San Diego, California. Ovalbumin (OVA) and bovine serum albumin (BSA) were obtained from Sigma Chemical Company, St. Louis, Missouri. Chicken γ-globulin (CGG) was purchased from the United States Biochemical Corporation, Cleveland, Ohio. Dinitrophenylated KLH (DNP_{580}-KLH), OVA (DNP_{11}-OVA), BSA (DNP_{28}-BSA), and CGG (DNP_7-CGG) were prepared as previously described (5). *Bordetella pertussis* vaccine (BPV) was obtained from the Chiba Serum Institute, Chiba, Japan.

Antisera. Rabbit anti-mouse immunoglobulin (anti-MIg), anti-mouse IgG_{2a}, and anti-mouse IgG_1 were produced in our laboratory. Monoclonal anti-Igh-1a (8.3) was produced by Dr. V. T. Oi, Stanford University (6). Monoclonal rat anti-Thy-1.2 (H-12), anti-Lyt-2 (53-6.72), and fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat Ig were kindly provided by Dr. J. A. Ledbetter, Stanford University (7).

Immunizations. Responder mice were primed with 100 μg of DNP-KLH and 10^9 of BPV intraperitoneally (ip). The epitope-specific suppressed mice were primed with 100 μg of alum-precipitated CGG ip. These mice were boosted with 100 μg of alum-precipitated DNP-CGG ip 4 weeks after the initial priming of CGG (CGG/DNP-CGG priming).

Immunofluorescence staining and fluorescence-activated cell sorting for cells with anti-Thy-1 and with anti-Lyt-2. Spleen cells were treated with monoclonal rat anti-

[1] Abbreviations used: BPV, *Bordetella pertussis* vaccine; BSA, bovine serum albumin; CGG, chicken γ-globulin; CTs, carrier-specific suppressor T cells; DNP, dinitrophenyl; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; ip, intraperitoneally; KLH, keyhole limpet hemocyanin; MIg, mouse immunoglobulin; OVA, ovalbumin; PBS, phosphate-buffered saline; RIA, solid-phase radioimmunoassay; Ts, suppressor T cells.

Thy-1.2 or anti-Lyt-2 and stained with FITC-mouse anti-rat Ig as described by Ledbetter *et al.* (7). This does not stain surface Ig^+ B cells in spleen. With a fluorescence-activated cell sorter (FACS-IV), Thy-1^+ and Thy-1^- or Lyt-2^+ and Lyt-2^- cells were sorted on the basis of the amount of FITC-labeled anti-rat Ig bound (8).

Enrichment of hapten-binding T cells. To eliminate surface Ig^+ B cells in spleen cells, 1×10^7 cells in 3 ml of RPMI 1640 (GIBCO Laboratories, Grand Island Biological Company, Grand Island, New York) enriched with 1% fetal calf serum (FCS, GIBCO) and 0.1% NaN_3 were applied to a petri dish (Falcon 3002, Falcon Labware, Div. of Becton–Dickinson and Company, Oxford, California) precoated with 300 μg of rabbit anti-MIg in 3 ml of phosphate-buffered saline (PBS), pH 7.2, as described previously (9). Cells not bound to anti-MIg-coated dishes (90% are Thy-1^+ T cells and Ig^+ cells are less than 5%) were further applied to petri dishes precoated with 300 μg of DNP-BSA or BSA in 3 ml of PBS to enrich antigen-binding T cells. A final recovery of antigen-binding T cells was about 1% of original spleen cells.

In vitro culture system. A modified Mishell–Dutton coculture system using 96-well microtiter plates (Falcon 3042) was used to detect the activity of suppressor cells derived from CGG/DNP-CGG-primed mice. Various numbers of responding spleen cells from DNP-KLH-primed mice were cocultured with spleen cells from the CGG/DNP-CGG-primed mice in 0.1 μg/ml of antigen and 2×10^{-5} *M* 2-mercaptoethanol at 37°C in 5% CO_2 in air for 8 days.

Measurement of the antigen-specific antibody responses. A solid-phase radioimmunoassay (RIA) was used to measure IgG_1 and IgG_{2a} antibody responses as described elsewhere (10).

RESULTS

CGG/DNP-CGG-Immunized Mice Have Epitope-Specific Suppressor Cells

DNP-KLH-primed spleen cells respond to *in vitro* stimulation with DNP-KLH by producing relatively high levels of IgG_{2a} and IgG_1 anti-DNP antibodies detectable in radioimmunoassay of 8-day-culture supernatants (see Table 1). This response is largely suppressed by the addition of spleen cells from mice immunized sequentially with CGG and DNP-CGG (CGG/DNP-CGG) but is unaffected by spleen cells from mice immunized only with DNP-CGG. The possibility of nonspecific suppression can be easily ruled out just by increasing the cell numbers in the coculture systems. Thus this coculture assay reveals the presence of a suppressor cell population specifically generated by carrier/hapten-carrier immunization.

The *in vitro* suppression mediated by this cell population is clearly epitope specific since it impairs anti-DNP antibody responses by cells responding to DNP on a different carrier molecule (KLH) than that used to induce the suppressor population (CGG). Furthermore, it is similar to the *in vivo* epitope-specific suppression induced by carrier/hapten-carrier immunization in that IgG_{2a} antibody responses are more easily suppressed than IgG_1 responses when suppression is suboptimal (3), e.g., when smaller numbers of CGG/DNP-CGG-immunized spleen cells are added in the coculture assay (see Table 1).

Comparison of the anti-carrier antibody responses in suppressed animals and suppressed cultures, however, reveals a key difference between the two systems. The *in vivo* suppression is specific for antibody responses to the DNP hapten and does not detectably affect antibody production to other epitopes on the carrier molecules on

TABLE 1

Carrier/Hapten-Carrier Immunization Induces Suppressor Cells That Prevent *in Vitro* Secondary Antibody Responses by Cocultured DNP-KLH-Primed Spleen Cells

Spleen cell donor immunization ($\times 10^6$/ml)[a]			IgG anti-DNP response (ng/ml ± SD)[b]	
DNP-KLH	CGG/DNP-CGG	DNP-CGG	IgG_{2a}	IgG_1
3	—	—	106 ± 12	1175 ± 150
4	—	—	80 ± 16	900 ± 100
5	—	—	92 ± 18	1375 ± 75
3	1	—	27 ± 4	1175 ± 125
3	2	—	8 ± 2	475 ± 110
3	—	1	55 ± 14	750 ± 75
3	—	2	63 ± 11	1000 ± 75

[a] Spleen cells from BALB/c mice were used in this experiment. Donors were always immunized with 100 μg of the indicated antigen. DNP-KLH immunization: 6 weeks prior to culture; DNP-CGG immunization: 4 weeks prior to culture; CGG/DNP-CGG immunizations: 4-week intervals prior to culture. The indicated number of spleen cells was cultured for 8 days with 0.1 μg/ml of DNP-KLH.

[b] Anti-DNP antibodies in the culture supernatants were measured by RIA. Standard deviations were calculated from six wells per group.

which the DNP hapten is presented (1). The *in vitro* suppression, in contrast, extends to all epitopes that accompany DNP on the carrier molecule on which it is presented. Thus, in addition to suppressing anti-DNP antibody production, cells from CGG/DNP-CGG-immunized animals suppress anti-KLH responses in cultures stimulated with DNP-KLH but not in case of stimulation with KLH (see Table 2).

TABLE 2

Suppression of *In Vitro* Anti-Carrier Antibody Responses Occurs Only for Epitopes That Accompany DNP on the Carrier Molecule

Spleen cell donor immunization ($\times 10^6$/ml)[a]			IgG_{2a} antibody response[a]	
DNP-KLH	CGG/DNP-CGG	*In vitro* antigen[b]	anti-DNP (ng/ml)	anti-KLH[c] (units)
3	—	DNP-KLH	71 ± 22	60 ± 20
4	—	DNP-KLH	76 ± 10	35 ± 5
3	1	DNP-KLH	12 ± 4	9 ± 3
3	—	KLH	—	77 ± 24
4	—	KLH	—	97 ± 10
3	1	KLH	—	56 ± 5
3	—	DNP-OVA + KLH	—	86 ± 10
4	—	DNP-OVA + KLH	—	97 ± 8
3	1	DNP-OVA + KLH	—	79 ± 15

[a] See legend to Table 1.

[b] Indicated cells were cultured with 0.1 μg/ml of antigens.

[c] Anti-KLH responses were expressed as percentages × 100 responses in a "standard" secondary anti-KLH antiserum pool.

This suppression of anti-carrier antibody production is not due to nonspecific "bystander" effects, since it is ineffective in suppressing antibody production to epitopes on protein antigens that do not carry DNP, for example, DNP-OVA plus KLH stimulation (see Table 2). Furthermore, it is not simply due to "relocation" of suppressor cells in a more responsive environment, since spleen cells transferred from carrier/hapten-carrier-immunized donors (KLH/KLH/DNP-KLH-immunized, in this case) to irradiated recipients show the typical in situ specificity pattern for suppression. As Table 3 shows, anti-KLH responses proceed normally at secondary response levels when such recipients are stimulated with 1 μg aqueous DNP-KLH, and, similarly, anti-CGG responses proceed normally at very low primary levels in recipients stimulated with 10 μg aqueous DNP-CGG.

Thus CGG/DNP-CGG immunization induces at least two kinds of DNP-specific suppressor activities: one, operative *in vivo* (and also possibly *in vitro*), specifically suppresses IgG antibody production to DNP; and a second, operative primarily *in vitro,* suppresses antibody production to protein epitopes that accompany DNP on a carrier molecule.

Some of the T Cells Active in Vitro in Epitope-Specific Suppression Are Lyt-2⁻

FACS separation of CGG/DNP-CGG donor spleen cells shows that nearly all of the suppressive activity is recovered in the Thy-1^+ cell population (see Table 4). This suppression is not attributable to anti-Thy-1.2 antibodies carried into the culture by the stained and sorted population, since a similarly stained population derived from DNP-CGG (nonsuppressed) donors did not affect the *in vitro* antibody production (data not shown). Furthermore, B-cell depletion experiments (on anti-MIg-coated petri dishes) show that suppressive activity remains with the nonadherent population enriched for splenic T cells (see Table 6) and cytotoxic depletion studies demonstrate

TABLE 3

Epitope-Specific Suppression in Adoptive Recipients

Expt	Spleen cell donor immunization[a]	Recipient immunizations	Antigen dose (μg)	Igh-1a response in recipients[b] Anti-DNP (μg/ml)	Anti-KLH (units)[c]	Anti-CGG (units)[c]
I	KLH, KLH, DNP-KLH	DNP-KLH	1	10	230	—
	—, —, DNP-KLH	DNP-KLH	1	400	240	—
II	KLH, KLH, DNP-KLH	DNP-CGG	10	5	—	1.0
	—, —, DNP-KLH	DNP-CGG	10	114	—	1.0

[a] All donors were (BALB/c × SJL)F_1 mice; all recipients were BALB/c (650 R irradiated). In Experiment I, donors were exposed to 100 μg aqueous KLH twice (1 and 2 weeks) followed by 100 μg DNP-KLH on alum 2 weeks after; 2×10^7 spleen cells transferred. In Experiment II, donors were exposed to 100 μg of each antigen on alum at 6-week intervals; 1×10^7 spleen cells transferred.

[b] All recipients were injected with spleen cells and antigen (1 μg aqueous DNP-KLH or 10 μg aqueous DNP-CGG) and boosted 6 weeks later with the same antigen (100 μg on alum). Responses were measured 14 days after last antigen stimulation and calculated from three animals per group.

[c] Anti-KLH and anti-CGG responses were expressed as percentages of antibody in a "standard" secondary serum pool.

TABLE 4

Suppressor Cells from CGG/DNP-CGG-Immunized Animals Are THY-1-Bearing (T) Cells

DNP-KLH-immunized spleen[a] ($\times 10^6$/ml)	CGG/DNP-CGG-immunized spleen[a]		IgG anti-DNP response (ng/ml)[a]	
	$\times 10^6$/ml	Treatment[b]	IgG_{2a}	IgG_1
3	—		43 ± 5	2580 ± 750
4	—		36 ± 3	1300 ± 630
5	—		27 ± 5	1900 ± 500
3	2	Unseparated	2 ± 1	130 ± 30
3	2	Thy-1^+	2 ± 1	380 ± 50
3	2	Thy-1^-	20 ± 5	1830 ± 380

[a] See legend to Table 1.

[b] Spleen cells from BALB/c mice were treated with monoclonal rat anti-Thy-1.2 followed by FITC-conjugated mouse anti-rat Ig. Thy-1-positive cells were separated with a FACS-IV.

that the suppressor cells in an CGG/DNP-CGG-immunized spleen are killed by anti-Thy-1 (data not shown). Thus (not surprisingly), the suppression detected in the coculture assay is due to the activity of Thy-1^+ (suppressor T) cells generated in CGG/DNP-CGG-primed animals.

Data from FACS separation studies with anti-Lyt-2, in contrast, contradict the current paradigm. In spite of recent publications about Lyt-2^- suppressor T cells (11–13) suppressive activity is usually recovered among Lyt-2^+ cells (14). However, the cells responsible for *in vitro* epitope-specific suppression essentially partition equally (in terms of suppressive activity) between the Lyt-2^+ and the Lyt-2^- sorted cell populations (see Table 5). Thus we obtained strong suppression with cells that did not carry detectable levels of Lyt-2.

These sorting experiments (repeated three times) presented no special problems: cells were stained with a monoclonal anti-Lyt-2 antibody; the FACS staining profile

TABLE 5

Hapten-Specific Suppressor T Cells Belong to LYT-2^+ and LYT-2^- T-Cell Subsets

DNP-KLH-immunized spleen[a] ($\times 10^6$/ml)	CGG/DNP-CGG-immunized spleen[a]		Anti-DNP response (ng/ml)[a]	
	$\times 10^6$/ml	Treatment[b]	IgG_{2a}	IgG_1
3	—		94 ± 25	375 ± 160
4	—		70 ± 20	415 ± 115
3	1	—	11 ± 5	100 ± 35
3	1	Unseparated	13 ± 4	40 ± 10
3	0.75	Lyt-2^+	25 ± 6	190 ± 90
3	1	Lyt-2^-	33 ± 8	140 ± 25

[a] See legend to Table 1.

[b] Spleen cells were treated with monoclonal rat anti-Lyt-2 followed by FITC-conjugated mouse anti-rat Ig. Lyt-2-positive and -negative cells were sorted by a FACS-IV.

(logarithmic) showed the typical narrow distribution for Lyt-2^+ cells and the clearcut distinction between Lyt-2^+ and Lyt-2^- cells; the frequency of Lyt-2^+ cells ranged between 10 and 15% of the initial spleen cell suspension; and the suppression obtained with the sorted cells followed the typical isotype distribution pattern for this type of suppression. Therefore we see no technical basis for discounting these data and conclude that some of the suppressor T cells responsible for the *in vitro* epitope-specific suppression described here lack Lyt-2 determinants.

Some T Cells Active in Vitro in Epitope-Specific Suppression Bind Specifically to the Inducing Hapten (DNP)

Absorption ("panning") experiments with DNP-BSA-coated petri dishes demonstrate that at least some suppressor T cells from CGG/DNP-CGG-immunized animals have specific receptors for DNP (see Table 6). In these experiments, we incubated B-depleted spleen cells from immunized donors for 1 hr in petri dishes coated with either DNP-BSA or BSA and then harvested both the "floating" (unbound) cells and the cells that remained bound to the dishes after several washes. Under these conditions, cells responsible for the suppressive activity in the original spleen cell suspension remained in the "floating" population (either because they lacked receptors for DNP or because the conditions used were not adequate to exhaustively deplete DNP-binding cells from the residual "floating" population). Nevertheless, a population of cells that specifically attach to the DNP-BSA-coated dishes (but not to BSA-coated control dishes) was recovered and these cells strongly suppressed anti-DNP antibody production in the coculture assay.

The DNP-bound suppressor cells also appear to suppress anti-KLH responses (mounted by cocultured DNP-KLH-primed cells responding to DNP-KLH); however, we are hesitant about drawing conclusions on this point, since the overall anti-KLH responses in several of these experiments were quite low (although the anti-DNP responses were comparable). Thus while we have shown that CGG/DNP-CGG im-

TABLE 6

Specific Enrichment of DNP-Binding Suppressor T Cells

DNP-KLH-immunized spleen[a] ($\times 10^6$/ml)	CGG/DNP-CGG-immunized spleen[a]		IgG_1 anti-DNP[a] (ng/ml)
	$\times 10^6$/ml	Treatment[b]	
3	—		238 ± 63
4	—		450 ± 125
5	—		625 ± 175
3	1	—	88 ± 13
3	2	—	100 ± 38
3	1	T enriched	25 ± 13
3	2	T enriched	13 ± 3
3	0.4	Bound to DNP-BSA	50 ± 25
3	0.4	Bound to BSA	288 ± 75

[a] See legend to Table 1.

[b] Spleen cells from BALB/c mice were first applied to anti-MIg-coated dishes to deplete B cells. Aliquots of the resulting T-enriched (nonbound) cell population were applied to DNP-BSA- or BSA-coated dishes. Nonbound cells were then removed and the bound cells were recovered for testing.

munization stimulates DNP-binding suppressor T cells that suppress anti-DNP antibody production, we do not at present know whether these cells participate in the *in vitro* suppression of antibody responses to carrier protein epitopes presented in association with DNP.

DISCUSSION

The studies presented here were initially aimed at defining the characteristics of the cells that specifically suppress anti-hapten antibody responses in carrier/hapten-carrier-immunized animals. To this end, we developed an *in vitro* coculture assay in which anti-hapten antibody production is suppressed by spleen cells from carrier/hapten-carrier-immunized mice but is not affected by spleen cells from hapten-carrier-primed animals. Then, using this assay, we showed that the *in vitro* suppression is mediated by a population of Thy-1^+ cells that is heterogeneous with respect to surface Lyt-2 expression and (most likely) to the presence of receptors that allow binding to hapten-coated petri dishes.

We also showed, however, that the scope of the *in vitro* suppression extends well beyond the scope of the suppression demonstrable *in vivo* in carrier/hapten-carrier-immunized animals. That is, the *in vivo* suppression is specifically limited to IgG anti-hapten responses and does not detectably influence anti-carrier antibody production (1) while the *in vitro* suppression interferes with antibody production both to the inducing hapten and to the carrier protein epitopes that accompany it on hapten-carrier conjugates (see Table 2). Thus, the most distinctive characteristic of the *in vivo* suppression, its specificity for antibody responses to the inducing hapten, disappears when cells from suppressed donors are assayed for suppressive activity *in vitro.*

The mechanisms underlying this specificity difference resolve to two alternatives: either the broader specificity of the *in vitro* suppression reflects the activity of a single suppressor population whose specificity degenerates *in vitro* or it reflects the *in vitro* expression of an additional suppressor population whose activity is largely cryptic *in vivo.* Our data do not distinguish between these alternatives; however, the "second suppressor" mechanism can be reconciled more easily with the known properties of regulatory T cells (15–17). The results shown in Table 5 also support the "second suppressor" mechanism, since there are at least two distinct suppressor T cells (Lyt-2^+ and Lyt-2^-) in carrier/hapten-carrier-immunized animals. Thus we suggest the following explanation for our findings:

CGG/DNP-CGG immunization induces two DNP-specific systems of suppressor cells. One system, defined principally by our *in vivo* studies, suppresses anti-DNP antibody production regardless of the carrier on which the DNP is presented and does not affect anti-carrier antibody production. The effector mechanism in this epitope-specific system is functionally analogous to an idiotype-specific suppressor effector mechanism, since it controls the production of antibodies with particular combining-site structures. Thus we tentatively see the suppression of anti-DNP responses in the CGG/DNP-CGG-immunized donors used here as a concerted idiotype suppression extending to most of the DNP-specific combining site structures normally represented in the anti-DNP antibodies produced in primary and secondary responses.

The DNP-binding cells that we isolated in our "panning" experiments could well include some of these idiotype-specific suppressors, whose DNP-specific receptors

define the idiotypes (combining sites) that are suppressed *in vivo* and perhaps *in vitro* as well. The highly specific suppressive activity of these cells, however, is difficult to discern *in vitro* because the donor spleen cell suspension contains DNP-specific cells (in the "second" suppressor system) whose activity results in the suppression of antibody responses to essentially all epitopes on the hapten-carrier conjugate used to stimulate *in vitro* antibody production (see Table 2).

The suppressor cells in this second system appear to be functionally analogous to carrier-specific suppressor T cells (CTs) operating in their recently redefined role as inducers of epitope-specific suppression (2). That is, KLH-specific CTs generate suppression for IgG antibody productions to epitopes (such as DNP) presented on KLH molecules under appropriate conditions, e.g., in KLH/DNP-KLH-immunized animals (2). Similarly, the DNP-specific suppressors described here apparently use DNP as a "carrier determinant" and generate suppression for IgG antibody production to carrier protein epitopes on DNP-bearing molecules (see Table 2). Thus the DNP-specific "CTs" probably act by directly or indirectly stimulating the "idiotype-specific" effector suppressor cells discussed above via "reverse" hapten-carrier bridges in which the protein epitopes on the DNP-bearing molecule constitute the "presented" haptens to which antibody responses are regulated.

Casting DNP as a "carrier determinant" returns to an old question. In principle, regulatory T cells that recognize and use DNP as a carrier determinant should be commonplace since the DNP hapten bound to a carrier molecule is formally equivalent to any other epitope on the molecule. In practice, however, this broadly used hapten tends to stimulate antibody production more frequently than the average epitope on a protein antigen; and, perhaps for the same reasons, it only rarely serves as a carrier determinant that "presents" other hapten-carrier conjugate epitopes to responding cells in the immune system. By and large, cells that use hapten as a carrier determinant have principally been recovered from animals immunized with hapten coupled to self antigens, e.g., by "skin painting" donors or immunizing them with hapten on mouse lymphocytes or immunoglobulin (18–20).

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