A REGULATORY ROLE FOR THE MEMORY B CELL AS SUPPRESSOR-INDUCER OF FEEDBACK CONTROL

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Homeostasis is a hallmark of the immune system, ensuring an elastic response to infection from an otherwise quiescent lymphocyte population and development of memory that persists for decades (1–3). It is not unexpected that multiple feedback mechanisms have evolved to restrict the duration and intensity of humoral responses in vivo and assert homeostasis. Regulation by antibody (4, 5) or antiidiotypic antibody (6, 7) is well documented, and surface marker studies have enabled a fine dissection of the cellular elements of feedback control (8–10). It is now agreed that the target for suppression is the T helper (T_H)[1] cell that may contribute to its own demise by initiating a feedback loop mediated by a suppressor-inducer cell (11, 12). Further complexity has been introduced to the system with the definition of contrasuppression—requiring a contrasuppressor-inducer cell—to regulate the suppressor cell (13–15). The existence of two regulatory circuits provides for symmetry and balance between opposing forces of help/contrasuppression and suppression/suppressor-induction.

Most model systems, designed to analyze feedback control of antibody production, are concerned with primary responses to sheep erythrocytes (SRBC) in vitro (12, 13), whereas systems for the induction of suppressors in vivo require either tolerogenic doses of antigen (16) or hapten conjugated to nonimmunogenic carriers; (17, 18). In this report we describe a potent and antigen-specific suppressor system for the regulation of secondary responses in vivo—initiated by the double adoptive transfer of memory cells—and provide evidence of a central role for the antigen-responsive B cell, as a suppressor-inducer of feedback control.

Materials and Methods

Mice. CBA/Ca mice were bred under specific pathogen-free conditions at this Institute and used at 2–3 mo of age.

Antigens. Pooled normal immunoglobulin (HGG) was donated by Blood Products Laboratory, Elstree, Herts, United Kingdom, and keyhole limpet hemocyanin (KLH) purchased from Calbiochem, La Jolla, CA. Dinitrophenyl (DNP) -HGG and DNP-KLH were used at substitution ratios of 8–12 and 263, respectively.

Immunizations. Mice were immunized by intraperitoneal injection of 100–200 μg antigen

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[1] *Abbreviations used in this paper:* C′, guinea pig complement; B hapten, hapten-specific B cells; DNP, dinitrophenyl; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HGG, human Ig; IPFC; indirect plaque-forming cells; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; SRBC, sheep erythrocytes; T_H, T helper (cells); T_S, T suppressor (cells).

absorbed onto alum and mixed with 2×10^9 *Bordetella pertusis* organisms, and used 3–6 mo later.

Antisera. Anti-Thy-1.2 serum was obtained by hyperimmunization of AKR (Thy-1.1) mice with CBA (Thy-1.2) thymocytes; cytotoxic titer 1:200. Mouse monoclonal antibody to Thy-1.2 (clone NIMF2-97-13) was grown as an ascitic tumor in (AKR × BALB/c)F_1 mice. Rat monoclonal anti-Thy-1 (clone NIMR-1) (19) was maintained as an ascitic tumour in Lou rats. Rat monoclonal hybridoma to Lyt-2 (clone 53-6.7), isolated by Ledbetter and Herzenberg (20), was maintained in tissue culture.

Cytotoxic Depletion of Cells. Spleen cells (4×10^8) were depleted of T cells by incubating with 4 ml anti-Thy-1.2 serum (1:4 dilution in minimal essential medium [MEM]) or monoclonal antibody (1:200) for 30 min at 3°C, washed, and incubated for a further 30 min at 37°C with 3 ml guinea pig complement (C′) (1:3 dilution, agarose absorbed).

Selection of Lymphocyte Subpopulations on the Fluorescence-activated Cell Sorter (FACS). FACS-II (B-D FACS Systems, Becton, Dickinson & Co., Mountain View, CA) was operated on a visible line (488 nm) with a sorting rate of 5×10^3 cell/s. Cells were collected at 0–3°C into 10-ml centrifuge tubes containing 1 ml 50% FCS in MEM.

B CELL SELECTION. $3–5 \times 10^8$ lymphocytes were incubated in 1:30 normal rabbit serum for 30 min on ice, to block F_c receptors, washed, and incubated in 4 ml of 1:30 dilution of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse Ig for a further 30 min, then diluted to 3×10^6/ml with MEM (10% fetal calf serum [FCS]) for cell sorting.

THY-1^+ CELL SELECTION. Spleen cells were incubated in normal rat serum (1:30 dilution) for 30 min, washed, then incubated for a further 30 min in FITC-conjugated monoclonal rat anti-Thy-1 (clone NIM-R1) (19).

LYT-2^+ CELL SELECTION. Spleen cells were incubated in undiluted tissue culture supernatant of monoclonal rat anti-Lyt-2 (clone 53-6.7), washed, then incubated in FITC-rabbit anti-rat Ig, preabsorbed with mouse Ig coupled to Sepharose 4B.

Cell Transfer. Recipient mice were irradiated with 850 rad from a ^{60}Co source, and injected 3 h later with syngeneic spleen cells combined with 10 μg of aqueous antigen.

Indirect Plaque-forming Cell (IPFC) Assay. Splenic IPFC were assayed 6 d after cell transfer by a slide modification of the Jerne hemolytic plaque assay, in which DNP-Fab anti-SRBC was used to coat erythrocytes or, alternatively, HGG was coupled by the chromic chloride method (21). IPFC were developed with a polyspecific rabbit anti-mouse Ig in the presence of goat anti-mouse-μ serum to inhibit direct PFC. Values for IPFC are expressed as the arithmetic mean ± SEM for three of more spleens assayed individually at two or more dilutions.

Results

Transient Loss of Memory on Double Adoptive Transfer. If memory cells for a thymus-dependent antigen are adoptively transferred, from one irradiated recipient to another, the response is lost after a critical time in the primary host. For instance, in a double adoptive transfer of DNP-HGG-primed spleen cells, a cyclic change occurs in the anti-hapten response with an apparent loss of memory after 6–7 d in the initial recipient (Fig. 1).

To ascertain whether this transient loss of memory was a defect in either B or T cell populations, T_H function was assayed in a similar transfer system: HGG-primed cells were placed in the primary host for various times and assayed for help by a further transfer with hapten-carrier primed B cells, the read out population. Here again, there was a cyclic change in helper function (Fig. 2), closely mirrored by the anti-DNP responses of the preceding experiment.

Generation and Specificity of Suppressor Cells. There were several likely and trivial reasons for the loss of helper activity on adoptive transfer: a majority of T_H might have entered the recirculatory pool and be absent from the spleen at the time of assay, or changes in the seeding efficiency of T_H in an irradiated environment; but

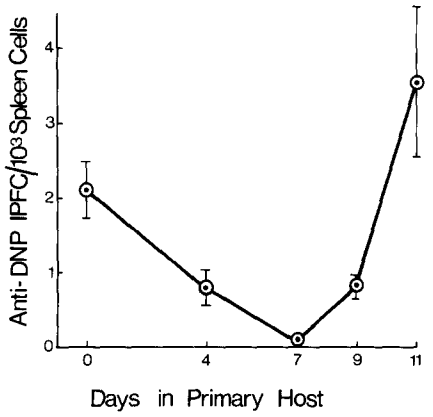

FIG. 1. Loss of an anti-hapten memory response after adoptive transfer. Lethally irradiated CBA mice (primary hosts) were reconstituted with 3×10^7 DNP-HGG-primed spleen cells from syngeneic donors and 10 μg aqueous antigen. At various times, as indicated, recipient spleen cells (2×10^7) were further transferred to irradiated recipients (secondary hosts). Anti-DNP IPFC expressed as the arithmetic mean ± SEM for four spleens assayed at two or more dilutions 6 d after cell transfer.

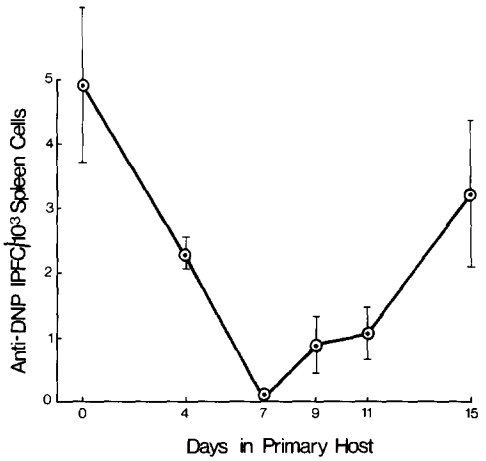

FIG. 2. Cyclic change in helper function of carrier-primed cells after adoptive transfer. Irradiated primary hosts were reconstituted with 3×10^7 HGG-primed spleen cells and 10 μg aqueous HGG. At various times thereafter, helper function of recipient spleen cells was assayed by adoptive transfer (10^7) with DNP-HGG-primed cells (10^7) to secondary irradiated hosts.

more significantly, T_S might have been recruited, particularly since the decline in T_H activity was coincident with a peak in the plaque response of the primary host.

Suppressors were therefore assayed for, by their ability to inhibit a secondary anti-hapten response, by co-transfer with spleen cells primed to DNP-HGG or DNP-KLH, an irrelevant carrier. It was apparent that the spleens of irradiated recipients, 6 d after reconstitution with HGG-primed cells, were a potent source of suppressors, able to entirely ablate a secondary anti-DNP response in a carrier-specific manner (Table I, lines 1 and 3); the response to hapten on an unrelated carrier was unaffected by the suppressors (lines 4 and 6).

Suppression of Both Anti-Hapten and Anti-Carrier Responses. Since responder and

TABLE I

Generation of Antigen-specific Suppressor Cells by Secondary Adoptive Transfer

Hapten-primed spleen cells (10^7)	HGG-primed spleen cells (2×10^7)	HGG-primed cells from primary host (2×10^7)*	Antigen given at transfer (10 μg)	Anti-DNP IPFC‡ 10^6 spleen cells
1. DNP-HGG	−	−	DNP-HGG	6,184 ± 123
2. DNP-HGG	+	−	DNP-HGG	11,836 ± 3,000
3. DNP-HGG	−	+	DNP-HGG	472 ± 38
4. DNP-KLH	−	−	DNP-KLH + HGG	17,968 ± 981
5. DNP-KLH	+	−	DNP-KLH + HGG	19,368 ± 3,562
6. DNP-KLH	−	+	DNP-KLH + HGG	13,916 ± 2,779

* Spleen cells from irradiated mice reconstituted with 3×10^7 cells from HGG-primed donors and 10 μg HGG on day −6.

‡ IPFC values are the arithmetic means ± SEM for three to four spleens assayed at two or more dilutions, 6 d after cell transfer.

TABLE II

Suppression of the Response to Both Haptenic and Carrier Determinants

DNP-HGG-primed spleen cells (10^7)	HGG-primed cells from primary hosts* (2×10^7)	DNP-HGG-primed spleen cells from primary hosts‡ (2×10^7)	IPFC/10^6 spleen cells	
			Anti-DNP	Anti-HGG
1. +	−	−	7,153	4,690
2. +	+	−	200	1,290
3. +	−	+	1,507	1,662

* Spleen cells from lethally irradiated mice reconstituted with HGG-primed cells and 10 μg HGG on day −6.

‡ Spleen cells from lethally irradiated mice reconstituted with DNP-HGG-primed cells and 10 μg aqueous DNP-HGG on day −6.

suppressor population shared a common carrier HGG, antigenic competition between hapten and carrier determinants, or feedback by anti-carrier antibody, rather than "active" suppression, were also candidate mechanisms. However, there was no significant plaque response to either hapten or carrier in the co-transfer assay for suppressors (Table II).

Antigen Sequestration and Competition for Biological Space. It is known that changes occur in the recirculatory behavior of lymphocytes after encounter with antigen, and a large number of carrier-primed cells were likely to have been activated in the primary host, both by antigen and the additional stimuli of an irradiated environment. rior activation might then confer a selective advantage over hapten-carrier primed cells on adoptive transfer, either by competition for available antigen or competition for biological space, but appear as "active" suppression. In this case, the balance might be redressed by the adoptive transfer of hapten-carrier-primed cells and the putative suppressors at different times. An equivalent suppression was obtained when the two populations were transferred together, or on consecutive days, arguing against such artifacts of competition (Table III).

Negative Selection of Suppressors. Our initial attempts to demonstrate the T cell origin of suppression were unsuccessful since treatment with anti-Thy-1 antibody and C′, either a monoclonal antibody or a high titer alloantiserum used routinely to remove

TABLE III

Suppression Is Not Due to Antigen Sequestration or Altered Seeding of Cells in Recipient Spleens

Time of cell transfer*			Anti-DNP IPFC 10^6 spleen cells
DNP-HGG-primed spleen cells (10^7)	HGG-primed spleen cells (2×10^7)	HGG-specific suppressor cells (2×10^7)‡	
1. Day 0	Day 0	—	3,828 ± 759
2. Day 0	—	Day 0	58 ± 49
3. Day 0	Day +1	—	5,260 ± 98
4. Day 0	—	Day +1	203 ± 60

* Irradiated secondary hosts were given DNP-HGG-primed cells and 10 μg antigen and, at the same time or 24 h later, carrier-primed or HGG-specific suppressor cells.

‡ Spleen cells from irradiated recipients (primary hosts) reconstituted with 3 × 10^7 cells from HGG-primed donors and 10 μg HGG on day −6.

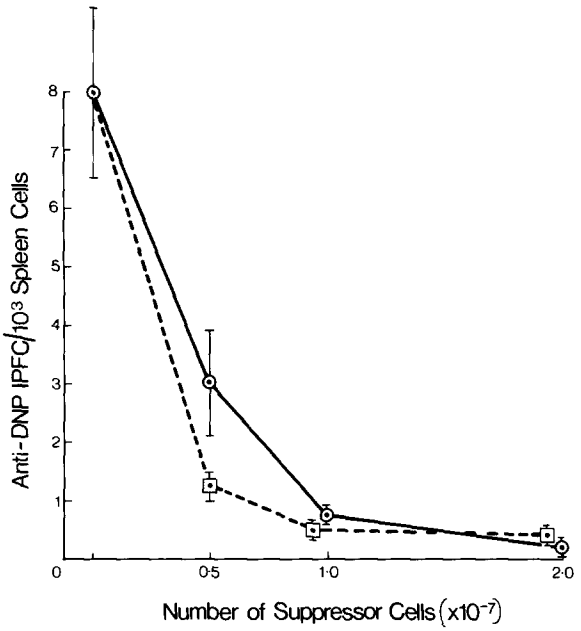

FIG. 3. Failure to abrogate suppression with anti-Thy-1 C′ treatment. HGG-specific suppressor cells were untreated (⊡) or treated with anti-Thy-1-2 + C′ (⊙) before variable numbers were transferred with a constant number (10^7) of DNP-HGG-primed cells to irradiated secondary hosts.

T_H, failed to abrogate their effect: inhibition of anti-hapten responses was obtained, with variable numbers of suppressors and a constant number of hapten-carrier primed cells, before or after treatment with anti-Thy-1 antibody and C′ (Fig. 3).

Negative selection is seldom satisfactory for defining the surface phenotype of effector cells—as witnessed by the minimal but significant expression of Lyt-1 by killer/suppressor cells (22). The finding that suppressors were refractory to antibody and C′ may have been due to minimal expression of Thy-1, or to the involvement of another cell type, such as the B cell or macrophage, although the specificity of

suppression would discount the latter. Positive selection was necessary, therefore, to provide an unequivocal answer.

Positive Selection of Suppressor T Cells. Suppressor cells were stained directly with a fluorescein-conjugated rat monoclonal antibody to Thy-1 and fluorescent cells (26% of the splenic lymphocyte population) positively selected for in the cell sorter. Alternatively, a Thy-1^- fraction was obtained by treatment with a monoclonal antibody and C′. The selected populations were assayed for suppressor function by adoptive transfer with hapten-carrier-primed spleen cells at a 2:1 ratio of effector to responder. Potent suppression was obtained with Thy-1^+ cells, thereby confirming the presence of effector T cells, but inhibition was also obtained with the putative Thy-1^- population (Table IV).

Positive Selection of Lyt-2^+ Cells. In a further experiment, the Lyt-2 phenotype of suppressor T cells was established by positive selection after indirect staining with a rat monoclonal antibody, clone 53-6.7 (20). There was an equivalent supression, on a per cell basis, with either total spleen or FACS-purified Lyt-2^+ cells (Table V).

Suppressor Induction by Carrier-specific B Cells. Although positive selection had confirmed that the suppressor was a Lyt-2^+ T cell (Tables III and IV), the resistance to anti-Thy-1 antibody and C′ treatment required some explanation (Fig. 3). The possibility still remained that suppresion could be achieved by both Lyt-2^+ T cells

TABLE IV

Positive Selection of Suppressor T Cells

DNP-HGG-primed spleen cells (5×10^6)	HGG-specific suppressor cells (10^7)*	Anti-DNP IPFC/spleen
1. +	—	22,500 ± 4,667
2. +	Total spleen	2,821 ± 264
3. +	Thy-1^+ cells‡	1,042 ± 507
4. +	Thy-1^- cells§	1,375 ± 408

* Spleen cells from irradiated mice reconstituted with HGG-primed cells and 10 μg HGG on day −6.

‡ Spleen cells were stained with FITC-conjugated monoclonal rat anti-Thy-1 (26% Thy-1^+ cells) and fluorescent cells collected with the FACS (>95% Thy-1^+ cells).

§ Prepared by treatment with anti-Thy-1.2 and C′.

TABLE V

Positive Selection of Lyt-2^+ Suppressor Cells

DNP-HGG-primed spleen cells (5×10^6)	HGG-specific suppressor cells (10^7)*	Anti-DNP IPFC/10^6 spleen cells
1. +	—	3,720 ± 356
2. +	Total spleen	1,308 ± 259
3. +	Lyt-2^+ cells‡	1,175 ± 413

* Spleen cells from irradiated recipients reconstituted with HGG-primed cells on day −6.

‡ Spleen cells were stained with rat monoclonal anti-Lyt-2, followed by FITC-conjugated rabbit anti-rat Ig, and fluorescent cells were collected with the FACS (>98% Lyt-2^+).

TABLE VI

Carrier-specific B Cells, Positively Selected from a Suppressive Cell Population, Induce Suppression of an Anti-hapten Response

DNP-HGG-primed spleen cells (5×10^6)	HGG-specific suppressor cells (10^7)*	Anti-DNP IPFC/spleen
1. +	—	9,806 ± 3,838
2. +	Spleen cells	2,888 ± 1,438
3. +	Ig^+ cells‡	2,269 ± 428

* Spleen cells from irradiated mice reconstituted with HGG-primed cells on day −6.

‡ Spleen cells were stained with FITC-conjugated rabbit anti-mouse Ig (60% Ig^+) and fluorescent cells positively selected with the FACS (>98% Ig^+).

and non-T cells, and the same criterion of positive selection was required to exclude a role for the B cells.

A suppressive population, generated by parking carrier-primed cells in the primary host for 6 d, was stained with a fluorescent anti-Ig reagent and fluorescent B cells selected for in the FACS. On further transfer with hapten-carrier-primed spleen cells, suppression was obtained with either total spleen cells, or purified carrier-specific B cells (Table VI). Since both helper activity (Fig. 2) and anti-carrier plaque responses (Table III) were minimal under the conditions of double adoptive transfer, it was reasonable to consider that B cells were exerting their effect in an indirect manner, rather than by secreted antibody.

Abrogation of B_{DNP} Suppressor-Induction by X Irradiation. In the above experiment (Table VI), the suppressive B cells, primed to carrier, and responder spleen cells, primed to hapten-carrier, shared the same carrier molecule and therefore had common specificites for their respective T cells. If the inhibitory action of $B_{carrier}$ on anti-DNP responses was not a direct effect but a consequence of suppressor-induction in the hapten-primed population, a reciprocal mechanism would be expected from "suppressive $B_{DNP\text{-}HGG}$," generated by a similar double adoptive transfer protocol, namely, the induction of suppressor T cells (T_S) in a carrier-primed but otherwise naive population. Consequently, $B_{DNP\text{-}HGG}$ would be trapped in an autoregulatory loop—unable to receive help from autochthonous or heterologous T_H—unless T_S and their precursors were removed. This prediction was tested and confirmed by the following experiment.

T_H and T_S exhibit different susceptibilities to X irradiation: T_H are radioresistant in situ, but T_S are exquisitely sensitive (23). This difference was exploited to demonstrate suppressor-induction by $B_{DNP\text{-}HGG}$ of a carrier-primed population and its abrogation by irradiation. Lethally irradiated recipients were reconstituted with hapten-carrier-primed cells; 6 d later, recipient spleens were depleted of T cells and the residual $B_{DNP\text{-}HGG}$ transferred to normal irradiated recipients, either alone or with carrier-primed cells (a source of T_H and T_S); alternatively, $B_{DNP\text{-}HGG}$ were transferred to carrier-primed, irradiated recipients (a source of T_H but not T_S). No response could be elicited from the hapten-carrier-primed population on adoptive transfer from the primary to the secondary host, either alone (Table VII, lines 1, 3) or supplemented with carrier-primed spleen cells (line 2). In striking contrast, transfer of total spleen, or $B_{DNP\text{-}HGG}$, to carrier-primed, X-irradiated recipients produced a substantial anti-

TABLE VII

Abrogation of B_{hapten} Suppressor-Induction By Adoptive Transfer to Carrier-primed, Irradiated Recipients

	Donor cells		Irradiated recipient	Anti-DNP IPFC/10^6 spleen cells
	DNP-HGG-specific* spleen cells from primary hosts (10^7)	HGG-primed spleen cells (10^7)		
1.	+	−	Normal	<20
2.	+	+	Normal	<20
3.	Anti-Thy-1 + C′	+	Normal	<20
4.	+	−	HGG primed‡	2,178 ± 272
5.	Anti-Thy-1 + C′	−	HGG primed‡	2,788 ± 367

* DNP-HGG-primed spleen cells (2×10^7) were adoptively transferred, with 10 μg aqueous DNP-HGG, to primary irradiated hosts. 6 d later, recipient spleen cells were or were not treated with anti-Thy-1 + C′ before transfer to normal, or carrier-primed, irradiated mice.

‡Carrier-primed, irradiated recipients were primed with HGG (as described in Materials and Methods) 4 mo previously.

DNP response (lines 4, 5). These findings did not favor a direct and suppressive effect of B cells, mediated by antibody or other secreted product, but rather a suppressor-inducer mechanism. Accordingly, the irradiation procedure would have eliminated suppressors and their precursors from the carrier-primed recipients, permitting cooperation between radioresistant T_H and $B_{DNP\text{-}HGG}$, but in the normal recipients, receiving viable, carrier-specific cells, suppressor-induction by $B_{DNP\text{-}HGG}$ would be dominant over $B_{DNP\text{-}HGG}$-T_H cellular cooperation.

Discussion

The double adoptive transfer system, described herein, provides a suitable in vivo model for feedback control. The distinguishing features of the system are the generation of suppressors by secondary challenge, their appropriate appearance at the peak of the plaque response, and most significantly, the central role demanded of the antigen-responsive B cell as an inducer of feedback suppression.

A suppressive effect, mediated by B cells, raises the specter of feedback by antiidiotypic or anti-carrier antibody, and it is indeed impossible to exclude a role for antibody in this system, even though the data favor an indirect mechanism of suppressor-induction. The suppressive elements, both B and T cells, are carrier specific; therefore, inhibition of an anti-hapten response is unlikely to require idiotype-antiidiotype interaction. And a somewhat less cogent argument may be mustered against carrier antibody: the suppressive effect of B cells occurs in the absence of their companion T cells, while an anti-carrier antibody response is a thymus-dependent process. It would be necessary, therefore, to argue that help for a carrier antibody response is recruited from the indicator population (hapten-carrier primed) at the expense of an anti-hapten response, and this would also constitute a suppresser-inducer mechanism.

There are several precedents in the literature for B cell involvement in feedback control, other than by classical antibody. Zubler et al. (24, 25) made some pertinent observations on feedback regulation of primary antibody responses to SRBC, in vitro,

using a protocol for the induction of antigen-specific suppressors first described by Eardley and Gershon (26). They made the unexpected observation that the suppressor cell, generated by preculture with antigen, was a B cell, and that suppression was mediated by antibody. Anti-hapten responses were unaffected by precultured and suppressive B-cells and therefore, by inference, neither were carrier-specific T_H. The converse is true here: suppression is carrier specific but hapten nonspecific.

The induction of suppressor mechanism(s) by the B cell and its progeny are well documented in a variety of systems (27–31). For instance, Calkins et al. (29–32) found that in co-cultures of spleen cells, primed or unprimed to SRBC, the anti-SRBC plaque response was less than that of either population cultured alone. This was attributed to a novel cell-cell interaction whereby a subset of primed B cells, with the surface characteristics of plasma cells, activated the precursors of T_S in a population not previously exposed to antigen. Similar studies in vivo have shown that adoptive transfer of immune B cells (33) or B cell blasts (34) activate specific feedback suppressor mechanisms in naïve recipients. Examples so far cited in the literature of suppressor-induction by B cells have required the presence of unprimed T cells as a source of suppressors.

In the present system, suppressor-induction occurs between primed cell populations in a potent and reciprocal manner: $B_{\text{hapten-carrier}}$ elicits suppressors in a carrier-primed population and B_{carrier} elicits suppressors in a hapten-carrier-primed population, and both provide a carrier-specific, but hapten nonspecific feedback mechanism. The potency of suppression may reflect the mode of induction of T suppressors and B suppressor-inducers. The adoptive transfer of memory cells is an immune response *in extremis*, and the combined effects of antigen stimulation and hemopoeitic stress may synchronize a significant proportion of the population in one functional mode to account for the potency of suppression. Nevertheless, the induction of a secondary response is a prerequisite for the generation of T suppressors and B suppressor-inducers—transiently and appropriately at the peak of the plaque response. This temporal feature of help vs. suppression must argue for physiological significance to the in situ conditions of homeostasis.

Several independent feedback mechanisms may act, in vivo, in a "fail-safe" and structured manner to conserve memory and prevent clonal exhaustion, their recall being dictated by the strength of immunogenic challenge. The cellular elements of T cell-T cell interaction have been defined, both for T suppressor-induction (12) and T contrasuppressor-induction (13, 15) and their role in feedback control demonstrated. If the normal route of induction for T suppressors and contrasuppressors is via the T cell, then what is the need for a further induction system mediated by the B cell? Given the hemopoietic stress of double adoptive transfer required to demonstrate its existence, the B suppressor-inducer may provide an override mechanism to prevent clonal exhaustion under conditions of excessive immune challenge or acute infection. Alternatively, the B cell may serve as a fail-safe for the T suppressor-inducer circuit in conditions that otherwise bypass suppression, such as anomolous route of antigen presentation or polyclonal activation. In this regard, we have no information as to whether both B and T inducers are recruited by the double adoptive transfer.

Finally, the participation of antigen-responsive B cells in feedback control may provided a means to establish clonal dominance over competitors, not necessarily responding to common determinants, since the suppressor mechanism is carrier

specific but hapten nonspecific. For instance, in the response to a complex antigen of n serological determinants, the promiscuous induction of B cells to hapten 1 would initiate suppression against the residual $(n - 1)$ determinants and T_S would then enforce a restricted response, be it for idiotype, allotype, class, or affinity. Whatever the role, or occasion, for the recruitment of B inducers, the existence of such a mechanism does make provision for autoregulation of the antibody response.

It is possible that there are many hitherto unrecognized permutations to suppression, suppressor-induction, and contrasuppression fashioned by the contrasting demands of homeostasis and infection, which constitute a complex network of feedback controls that are both precise and stringent. It is tempting, therefore, to speculate whether the B inducer mechanism, defined herein, is self-limiting or in turn requires some regulation.

Summary

A regulatory role is proposed for the antigen-responsive B cell, as suppressor-inducer of feedback control during the secondary response in vivo. In a double adoptive transfer of memory cells primed to a thymus-dependent antigen from one irradiated host to another, antigen-specific suppressors are generated after a critical time in the primary recipient, able to entirely ablate a secondary anti-hapten response. Positive cell selection in the fluorescence-activated cell sorter confirmed that suppression was mediated by an Lyt-2^+ T cell; however, positively selected B cells were also inhibitory and able to induce suppressors in a carrier-specific manner: B_{hapten} induced suppressors in a carrier-primed population, and $B_{carrier}$ induced suppressors in a hapten-carrier population. At the peak of the antibody response in the primary host, memory B cells and their progeny were unable to differentiate further to plasma cells due to their intrinsic suppressor-inducer activity, but this autoregulatory circuit could be severed by adoptive transfer to carrier-primed, X-irradiated recipients.

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