

# T CELL REGULATION OF B CELL ACTIVATION

## T Cells Independently Regulate the Responses Mediated by Distinct B Cell Subpopulations

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It has been well established that T cells exert a number of important regulatory effects on the activation of B cells by antigen. T helper ( $T_H$ )<sup>1</sup> cells, for example, are required for the generation of antibody responses to the large class of T-dependent antigens (1-3). The complexity of such helper effects has been demonstrated by recent studies describing the existence of two distinct pathways of T-dependent B cell activation in these responses (4-6). These pathways differ in their requirements for major histocompatibility complex (MHC)-restricted cell interactions, in their requirements for covalent linkage of carrier and hapten, and in the identity of the B cell subpopulations which are activated (6). Thus, Lyb-5<sup>-</sup> B cells are activated by a pathway requiring both MHC restricted  $T_H$  cell-B cell interaction and carrier-hapten linkage, whereas the activation of Lyb-5<sup>+</sup> B cells is MHC restricted for T cell recognition of accessory cells only and requires neither restricted T-B interaction nor carrier-hapten linkage (6).

Because at least two pathways exist for the T-dependent activation of B cells, it is of importance to determine whether these distinct pathways can be independently modulated by regulatory influences such as those exerted by T suppressor ( $T_S$ ) cell populations. The present studies have been carried out in an effort to characterize the regulatory influences acting upon defined pathways of T cell dependent B cell activation. In these studies, it was first demonstrated that high concentrations of free carrier strongly inhibited the MHC-restricted in vitro responses of primed Lyb-5<sup>-</sup> B cells, whereas in contrast these same concentrations of free carrier failed to inhibit the in vitro activation of Lyb-5<sup>+</sup> B cells. The inhibition of Lyb-5<sup>-</sup> B cell responses by free carrier was shown to result from active suppression mediated by carrier-specific primed Lyt-1<sup>+</sup>2<sup>-</sup> T cells and to require the additional participation of unprimed Lyt-1<sup>-</sup>2<sup>+</sup> T cells. The activation of this suppression was antigen specific, whereas suppression, once activated, was antigen nonspecific in its effect.

### Materials and Methods

*Animals.* C57BL/10SgSn (B10), B10.A, and (C57BL/6 × C3H/HeJ)F<sub>1</sub> (B6C3F<sub>1</sub>) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. CBA/N, CBA/CaHN, and

<sup>1</sup> *Abbreviations used in this paper:* C, complement; FGG, fowl gamma globulin; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; Mlg, mouse immunoglobulin; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; SRBC, sheep erythrocytes; TNP, trinitrophenyl;  $T_H$ , T helper;  $T_S$ , T suppressor.

BALB/c mice were provided by the Small Animal Section, National Institutes of Health, Bethesda, MD.

*Antigens.* Keyhole limpet hemocyanin (KLH) (lot 730195, Calbiochem, San Diego, CA) and fowl gamma globulin (FGG) (N. L. Cappel Laboratories, Cochranville, PA) were conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, IL) as previously described (7). The degrees of substitution were 20 trinitrophenyl (TNP) residues/100,000 daltons KLH (TNP-KLH) and 9 TNP residues/100,000 daltons FGG (TNP-FGG).

*Antisera.* Monoclonal hybridoma anti-Lyt-1.2 antiserum (lot FPB-031) and anti-Lyt-2.2 antiserum (lot FPA 179) were purchased from New England Nuclear, Boston, MA, and used as cytotoxic reagents at final dilutions of 1:800 and 1:3200, respectively, on  $1 \times 10^7$  cells/ml. Monoclonal hybridoma anti-Thy-1.2 antiserum was a generous gift from Dr. P. Lake (Georgetown University, Washington, DC).

*Immunization.* Mice were immunized with 100  $\mu$ g of KLH, FGG, TNP-KLH, or TNP-FGG in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally 3-8 wk before use.

#### *Preparation of cells*

*T CELLS.* KLH or FGG-primed T cells were obtained as spleen cells nonadherent to anti-mouse immunoglobulin (anti-MIg) coated plastic dishes by the method of Mage et al. (8) modified as described previously (5).

*(B + ACCESSORY) CELLS.* (B + accessory) cells were prepared by depleting TNP-KLH-primed spleen cells of T cells by treatment with a T cell-specific cytotoxic rabbit anti-mouse brain serum (RAMB) + complement (C). The T cell specificity of this reagent has been previously characterized (7).

*CULTURE CONDITIONS.* Cultures were carried out as previously described (5). Cells were harvested and assayed for TNP-specific hemolytic plaque-forming cells (PFC).

*PFC Assay.* Sheep erythrocytes (SRBC) were conjugated with TNP (TNP-SRBC) and direct PFC (IgM) as well as total PFC (facilitated by rabbit anti-MIgG) were assayed on these conjugated target cells (5). IgG PFC were calculated as total PFC minus direct PFC. All points shown represent the arithmetic mean responses of triplicate or quadruplicate cultures.

## Results

*High Concentrations of Free Carrier Inhibit the T Cell-Dependent Activation of Lyb-5<sup>-</sup> B Cells.* T-dependent and predominantly IgG PFC responses are generated in vitro by primed B cells responding to low concentrations of carrier-hapten conjugate. These responses have been shown to require MHC-restricted T-B interaction as well as carrier-hapten linkage, and to be mediated by Lyb-5<sup>-</sup> B cells (5, 6). In contrast, predominantly IgM responses activated at higher concentrations of antigens are equally T dependent but require neither carrier-hapten linkage nor MHC-restricted T-B interaction, and are mediated by the activation of Lyb-5<sup>+</sup> B cells (5, 6). Consistent with these findings, it was observed in the present studies that KLH-primed T cells cooperated equally well with T-depleted (B + accessory) spleen cell populations from TNP-primed CBA/N (a mutant defective strain expressing only the Lyb-5<sup>-</sup> B cell subpopulation) or CBA/CaHN (a nondefective strain expressing both Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations) animals to generate predominantly IgG PFC in response to 0.001  $\mu$ g/ml TNP-KLH (Table I). In contrast, a predominantly IgM response to 10  $\mu$ g/ml TNP-KLH was generated only in normal CBA/CaHN B cells and not in defective CBA/N populations, demonstrating a requirement for Lyb-5<sup>+</sup> B cells in the pathway activated under these conditions (Table I).

To further analyze the regulatory role of antigen concentration in these pathways of B cell activation, graded concentrations of free carrier KLH were added to cultures of TNP-KLH-primed CBA/N or CBA/CaHN spleen cells responding to either high or low concentrations of TNP-KLH. The IgG responses of both CBA/N and CBA/

TABLE I  
*Distinct B Cell Subpopulations Are Activated by High or Low Concentrations of Antigen In Vitro*

(B + accessory) cells*	PFC/culture			
	0.001 $\mu\text{g/ml}$ TNP-KLH		10 $\mu\text{g/ml}$ TNP-KLH	
	IgG	IgM	IgG	IgM
CBA/CaHN (Lyb-5 <sup>+</sup> + Lyb-5 <sup>-</sup> )	1128 $\pm$ 96	249 $\pm$ 78	0	1032 $\pm$ 5
CBA/N (Lyb-5 <sup>-</sup> )	1027 $\pm$ 87	0	28 $\pm$ 28	48 $\pm$ 26

\*  $2 \times 10^6$  TNP-primed, RAMB + C treated spleen cells were co-cultured with  $1 \times 10^6$  KLH-primed B6C3F<sub>1</sub> T cells.

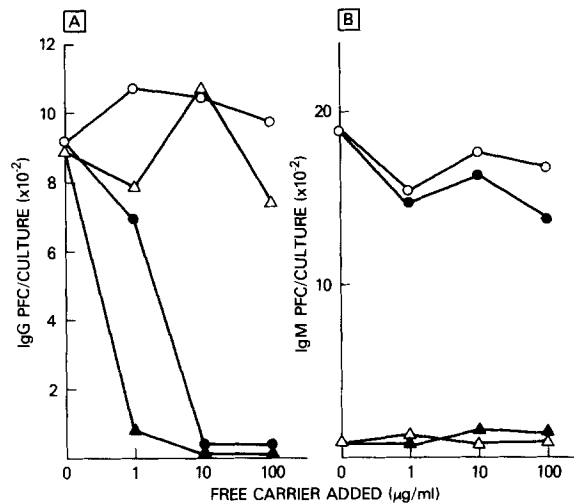


FIG. 1. Responses of CBA/N (Lyb-5<sup>-</sup>) B cells are suppressed by addition of free carrier.  $3 \times 10^6$  TNP-KLH-primed spleen cells of CBA/CaHN (○, ●) or CBA/N (△, ▲) origin were stimulated in vitro by 0.001  $\mu\text{g/ml}$  (A) or 20  $\mu\text{g/ml}$  (B) TNP-KLH. Graded dose of FGG (○, △) or KLH (●, ▲) were added to these cultures.

CaHN spleen cells to 0.001  $\mu\text{g/ml}$  TNP-KLH were consistently inhibited by concentrations of 10–100  $\mu\text{g/ml}$  KLH (Fig. 1 A). In contrast, the IgM response of CBA/CaHN spleen cells to 20  $\mu\text{g/ml}$  TNP-KLH (Fig. 1 B) or to 0.001  $\mu\text{g/ml}$  TNP-KLH (data not shown) was not significantly affected by the addition of free KLH (Fig. 1 B).

To test the antigen specificity of this observed inhibition, the effects of graded doses of KLH or FGG were examined on the IgG responses of primed spleen cells to either TNP-KLH or TNP-FGG. Responses to 0.001  $\mu\text{g/ml}$  TNP-KLH were generated by TNP-KH-primed B10.A spleen cells, but not by TNP-FGG-primed cells, confirming the specificity of priming in these populations (Fig. 2 A). These responses were inhibited by high concentrations of KLH but not by equivalent concentrations of FGG (Fig. 2 A). In contrast, responses of specifically primed spleen cells to TNP-FGG were inhibited by FGG but not by KLH (Fig. 2 B). These results thus demonstrate that free carrier induces in an antigen-specific fashion the inhibition of responses by Lyb-5<sup>-</sup> B cells to carrier-hapten conjugates.

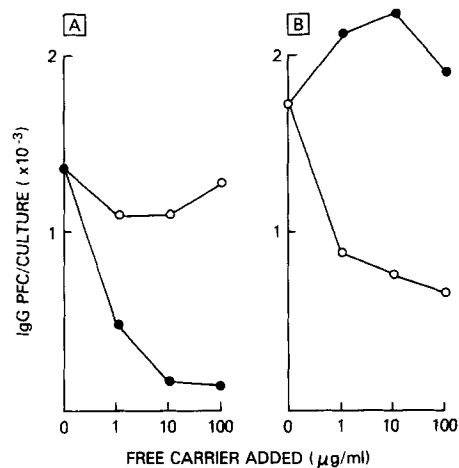


FIG. 2. Induction of suppression by free carrier is antigen specific.  $3 \times 10^6$  TNP-KLH-primed (A) or TNP-FGG-primed (B) B10.A spleen cells were stimulated in vitro by  $0.001 \mu\text{g/ml}$  TNP-KLH (A) or TNP-FGG (B) in the presence of graded dose of KLH (●) or FGG (○).

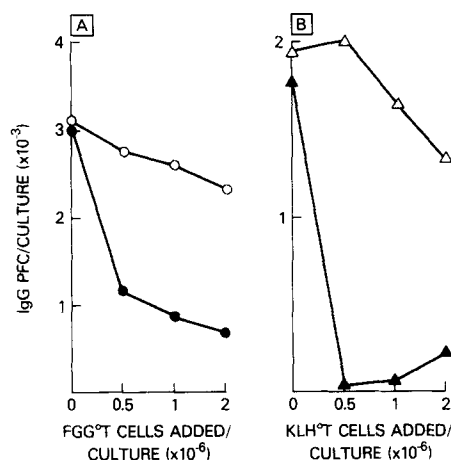


FIG. 3. Free carrier induces suppressor T cells in an antigen-specific manner.  $2 \times 10^6$  TNP-primed, RAMB + C-treated B10.A spleen cells were co-cultured with  $2 \times 10^6$  KLH-primed (A) or FGG-primed (B) B10.A T cells. (A) Cultures were stimulated by  $0.001 \mu\text{g/ml}$  TNP-KLH. Graded numbers of FGG-primed T cells were added to cultures with (●) or without (○)  $100 \mu\text{g/ml}$  FGG. (B) Cultures were stimulated by  $0.001 \mu\text{g/ml}$  TNP-FGG. Graded numbers of KLH-primed T cells were added to cultures with (▲) or without (△)  $100 \mu\text{g/ml}$  KLH.

*Response Inhibition by Free Carrier is Mediated by Antigen-specific Suppressor Cells.* Further studies were next carried out to examine the mechanism by which high concentrations of free protein carrier inhibit the IgG responses of Lyb-5<sup>-</sup> B cells. Experiments were first designed to distinguish between an active mechanism of response suppression and inhibition resulting from competition between free carrier and carrier-hapten conjugate. KLH-primed B10.A T cells cooperated with syngeneic TNP-primed (B + accessory) cells for responses to  $0.001 \mu\text{g/ml}$  TNP-KLH; and, in the absence of FGG-primed T cells, this response was not inhibited by the irrelevant carrier FGG (Fig. 3A). However, when graded numbers of FGG-primed B10.A T cells were added to

these cultures, high doses of FGG efficiently suppressed the response to TNP-KLH (Fig. 3A). KLH-primed T cells were similarly able to actively suppress the response to TNP-FGG in the presence of high doses of KLH (Fig. 3B). These results indicate that the inhibition of PFC responses by free carrier is in fact mediated by active suppression, and that this suppression is generated by carrier-primed T cells only in the presence of the specific carrier protein. Moreover, whereas the induction of this suppression in primed T cells is antigen specific, the effect of suppression, once generated, is antigen nonspecific.

*Suppression by Free Carrier Requires Interaction Between  $Lyt-1^{+}2^{-}$  and  $Lyt-1^{-}2^{+}$  T Cells.* To determine the cellular interactions involved in this antibody response suppression, the Lyt phenotypes of the T cell subpopulations required for suppression were analyzed. To further evaluate the requirement demonstrated above for the participation of  $Lyt-2^{+}$  cells in carrier-induced suppression, TNP-KLH primed B10.A spleen cells were treated with monoclonal anti-Lyt-2.2 + C and were then tested for their susceptibility to suppression by free carrier. Anti-Lyt-2.2 + C-treated spleen cells were competent to respond to TNP-KLH, demonstrating that T helper cells functioning in this response are  $Lyt-2^{-}$  (Fig. 4). In contrast, the ability of free KLH to suppress this response was completely eliminated by this pretreatment, indicating that  $Lyt-2^{+}$  cells are required for suppression (Fig. 4). Because the data presented above demonstrated that a population of specific carrier-primed T cells was required for suppression of antibody responses, the priming requirements of this  $Lyt-2^{+}$  population were examined. The susceptibility of TNP-KLH-primed B10 spleen cells to inhibition by high concentrations of KLH was first abrogated by treatment of KLH-primed T cells with anti-Lyt-2.2 + C, and the ability of unprimed B10 T cells to reconstitute this suppression was tested. It was found that the addition of unprimed T cells reconstituted the susceptibility to suppression, and that  $Lyt-1^{-}2^{+}$  cells were responsible for this reconstitution (Table II). These results suggested that both an unprimed  $Lyt-1^{-}2^{+}$  T cell population and an additional carrier-primed T cell population were necessary for suppression.

To further characterize the populations involved in carrier-induced suppression,

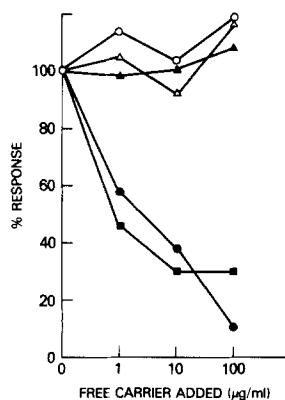


FIG. 4.  $Lyt-2^{+}$  cells are required for suppression induced by free carrier.  $3 \times 10^6$  TNP-KLH-primed B10.A spleen cells were stimulated by  $0.001 \mu\text{g/ml}$  TNP-KLH after treating with medium alone (○, ●), C alone (■), or anti-Lyt-2.2 + C (△, ▲) in the presence of  $100 \mu\text{g/ml}$  FGG (○, △) or  $100 \mu\text{g/ml}$  KLH (●, ■, ▲).

TABLE II  
*Lyt-1<sup>-</sup>2<sup>+</sup> Cells are Required for Suppression*

T cell treatment		IgG PFC/ culture	
KLH-primed T*	Unprimed T‡	TNP-KLH§	TNP-KLH§ + KLH
—¶	—	21	ND**
Untreated	—	2193 ± 152	273 ± 28
Anti-Lyt-2 + C	—	1694 ± 199	1248 ± 79
Anti-Lyt-2 + C	Untreated	1675 ± 229	105 ± 40
Anti-Lyt-2 + C	Anti-Lyt-1 + C	1910 ± 279	134 ± 134
Anti-Lyt-2 + C	Anti-Lyt-2 + C	1440 ± 305	1041 ± 62

\*  $1 \times 10^6$  KLH-primed B10 T cells were co-cultured with  $2 \times 10^6$  TNP-primed B10 (B + accessory) cells. T cells were untreated or treated with anti-Lyt-2 + C as indicated.

‡  $1 \times 10^6$  unprimed B10 T cells were added after treatment with either anti-Lyt-1 + C or anti-Lyt-2 + C as indicated.

§ 0.001  $\mu$ g/ml TNP-KLH.

|| 100  $\mu$ g/ml KLH.

¶ Not added.

\*\* Not done.

KLH-primed B10 T cells and FGG-primed B10 T cells were cocultured with TNP-primed (B + accessory) cells stimulated with 0.001  $\mu$ g/ml TNP-KLH with or without FGG. The helper T cells functioning under these conditions were Thy-1<sup>+</sup>, Lyt-1<sup>+</sup>2<sup>-</sup>, because treatment of KLH-primed T cells with either anti-Thy-1.2 + C or anti-Lyt-1.2 + C eliminated their helper activity, whereas anti-Lyt-2.2 + C did not (Table III, groups H, I, N). In the presence of untreated KLH-primed helper T cells, FGG-primed T cells induced suppression in the presence of FGG (Table III, group C), and pretreatment of FGG-primed T cells with anti-Lyt-2.2 + C did not affect this suppression (Table III, group D). Under these conditions, however, treatment of the FGG-primed T cells with either anti-Lyt-1.2 + C or anti-Thy-1.2 + C completely abrogated suppression, and a mixture of these treatment populations was also unable to induce suppression (Table III, groups E, F, G). These results demonstrated that carrier-primed Thy-1<sup>+</sup>, Lyt-1<sup>+</sup>2<sup>-</sup> T cells were required for the induction of suppression.

After treatment of the KLH-specific helper T cells with anti-Lyt-2.2 + C, untreated FGG-primed T cells still suppressed responses to TNP-KLH in the presence of FGG (Table III, group J). Treatment of the FGG-primed T cells with either anti-Lyt-1.2 + C or anti-Lyt-2.2 + C eliminated suppression under these conditions (Table III, groups K, L), but a mixture of the residual cells after treatment with anti-Lyt-1.2 + C or anti-Lyt-2.2 + C reconstituted the suppression (Table III, group M). These results indicate that interaction between Lyt-1<sup>+</sup>2<sup>-</sup> cells and Lyt-1<sup>-</sup>2<sup>+</sup> cells is required for the suppression of IgG responses in the presence of free carrier. This interaction further requires that the Lyt-1<sup>+</sup>2<sup>-</sup> cell population be specifically primed to the carrier inducing suppression, whereas the Lyt-1<sup>-</sup>2<sup>+</sup> cells express no such specificity.

### Discussion

Two distinct pathways have been described for the T<sub>H</sub> cell-dependent activation of different B cell subpopulations (4-6). The activation of Lyb-5<sup>-</sup> B cells requires the MHC-restricted interaction of T<sub>H</sub> cells with this B cell subset. In contrast, Lyb-5<sup>+</sup> B

TABLE III  
Interaction Between  $Lyt-1^+2^-$  and  $Lyt-1^-2^+$  T Cell Subpopulations Is Required for Suppression

Group	T cell treatment		IgG PFC/culture		Percent suppression ¶
	KLH-primed T*	FGG-primed T‡	TNP-KLH§	TNP-KLH§ + FGG	
A	—**	—	4 + 4	ND‡‡	ND
B	Untreated	—	2611 ± 182	2870 ± 211	—
C	Untreated	Untreated	2784 ± 134	1195 ± 230	57.1
D	Untreated	Anti-Lyt-2 + C	3859 ± 70	1003 ± 83	74.0
E	Untreated	Anti-Lyt-1 + C	3331 ± 163	3484 ± 249	—
F	Untreated	Anti-Thy-1 + C	3532 ± 282	4080 ± 117	—
G	Untreated	(Anti-Lyt-1 + C) + (Anti-Thy-1 + C)§§	2976 ± 436	3091 ± 194	—
H	Anti-Lyt-1 + C	—	120 ± 26	ND	ND
I	Anti-Lyt-2 + C	—	2510 ± 168	2846 ± 34	—
J	Anti-Lyt-2 + C	Untreated	2625 ± 87	734 ± 46	72.3
K	Anti-Lyt-2 + C	Anti-Lyt-2 + C	2332 ± 117	2203 ± 35	5.5
L	Anti-Lyt-2 + C	Anti-Lyt-1 + C	2188 ± 361	2452 ± 262	—
M	Anti-Lyt-2 + C	(Anti-Lyt-1 + C) + (Anti-Lyt-2 + C)	2083 ± 192	417 ± 51	80.0
N	Anti-Thy-1 + C	—	43 ± 21	ND	ND

\*  $2 \times 10^6$  T cells from KLH-primed B10 spleens were treated as indicated and co-cultured with  $2 \times 10^6$  TNP-primed, RAMB + C-treated B10 spleen cells.

‡  $1 \times 10^6$  T cells were added.

§ 0.001  $\mu$ g/ml TNP-KLH.

|| 100  $\mu$ g/ml FGG.

¶ Responses to TNP-KLH + FGG were compared to responses to TNP-KLH alone.

\*\* Not added.

‡‡ Not done.

§§  $1 \times 10^6$  anti-Lyt-1 + C-treated cells and  $1 \times 10^6$  anti-Thy-1 + C-treated cells were added.

|||  $1 \times 10^6$  anti-Lyt-1 + C-treated cells and  $1 \times 10^6$  anti-Lyt-2 + C-treated cells were added.

cells are activated through a pathway which does not require MHC-restricted  $T_H$  cell-B cell interaction (4-6). Since B cell responses can thus be mediated through alternative activation mechanisms, the possibility was considered that these alternative pathways might be independently regulated by  $T_S$  cell activity, a consideration previously suggested by other investigators (9). In fact, it was observed that responses of  $Lyb-5^-$  B cells to carrier-hapten conjugates were suppressed in the presence of high concentrations of free carrier, whereas responses of  $Lyb-5^+$  B cells were not inhibited. The activation of suppression was antigen specific, requiring the presence of carrier-primed T cells and a high concentration of the corresponding carrier. Once activated, however, the effect of this suppression was antigen nonspecific. The generation of carrier-induced suppression required the interaction of at least two T cell subpopulations, an antigen-specific (carrier-primed)  $Lyt-1^+2^-$  T cell and an  $Lyt-1^-2^+$  T cell population which exhibited no such specificity.

A number of alternative explanations may account for the selective effect of carrier-induced suppression on responses of the  $Lyb-5^-$  B cell subset. In several previously characterized systems, it has been concluded that  $T_S$  cells act upon the  $T_H$  cell populations involved in antibody response (10-16). If such a mechanism were involved

in the suppression described here, its selective effect could result from the participation of different  $T_H$  cell populations in the T dependent activation of  $Lyb-5^-$  and  $Lyb-5^+$  B cells. Alternatively, a single  $T_H$  cell functioning in responses of both B cell subsets might be selectively inhibited in its MHC-restricted activation of  $Lyb-5^-$  B cells. A second possibility is that carrier-induced suppression acts directly upon responding B cells and acts to specifically suppress the  $Lyb-5^-$  subpopulation. It is relevant in this regard that the same exposure to high concentrations of free carrier that induces suppression of  $Lyb-5^-$  B cell responses can also trigger  $T_H$  cell activity sufficient to activate responses by  $Lyb-5^+$  B cells (5, 6). Under these conditions, however, the suppression of  $Lyb-5^-$  responses requires the participation of  $Lyt-2^+$  T cells, whereas the activation of  $Lyb-5^+$  B cells exhibits no such requirement (Y. Asano, unpublished data), demonstrating that the signals mediating these two regulatory events are in fact different.

The activation of  $Lyb-5^-$  B cells in response to T-dependent antigens such as TNP-KLH requires the function of carrier-primed  $Lyt-1^+2^-$   $T_H$  cells. The suppression of these same antibody responses by high doses of carrier requires the participation of an antigen-primed  $Lyt-1^+2^-$  T cell and an unprimed  $Lyt-1^-2^+$  T cell. The  $Lyt$  phenotype of the first  $T_S$  population is thus identical to that of the  $T_H$  cells functioning in these responses. It is of interest, however, that the activation requirements demonstrated for  $T_H$  function and for  $T_S$  function in these responses are significantly different. Antigen-specific triggering of  $Lyt-1^+2^-$   $T_S$  activity occurs in response to free carrier and results in the subsequently antigen-nonspecific suppression of  $Lyb-5^-$  B cell responses. In contrast, the effector function of  $T_H$  cells for the activation of the same  $Lyb-5^-$  responses has been shown to be highly antigen specific, as reflected in the absolute requirement for carrier-hapten linkage in these responses (6). Efforts to determine whether these functions are mediated by the same or distinct T cell subpopulations are currently in progress through the use of additional cell surface phenotyping and with monoclonal  $T_H$  cell populations (6, 17).

The regulatory network described in this study resembles in several respects the system of antibody response suppression initially defined by Eardley and co-workers (14), as well as the  $T_S$  cell pathways subsequently characterized by a number of investigators (10-13, 15, 16). In each of these systems, a requirement has been demonstrated for interactions among T cell subsets in the mediation of suppression. In several systems, this suppressor network is initiated by the antigen-specific triggering of a primed  $Lyt-1^+2^-$  "afferent suppressor" or  $T_{S1}$  population (12-14, 16), consistent with the requirement for such a population demonstrated in the present report. In this work, an additional requirement was found for an  $Lyt-1^-2^+$  cell that did not require specific priming. This second T cell subpopulation is analogous to the "efferent suppressor" or  $T_{S2}$  also identified in a number of regulatory networks (10, 12-16). In contrast to a number of previous reports (10, 12-16), however, no requirement for an  $Lyt-1^+2^+$  cell was identified in the present work, a finding that may reflect only quantitative differences in the susceptibility of certain  $Lyt-1^+$  cells to lysis by antibody and C (18).

Recent findings have demonstrated the existence of two distinct pathways of T-dependent B cell activation. The present studies have revealed an additional dimension of this complexity in demonstrating that distinct B cell activation pathways can be independently regulated by  $T_S$  cell network influences. Thus, the MHC-restricted



$T_H$  cell activation of  $Lyb-5^-$  B cells is regulated by a  $T_S$  cell network, which is without apparent effect on the responses of  $Lyb-5^+$  B cells to the same antigen. Studies are currently in progress to further analyze the genetically restricted cell recognition events and effector mechanisms involved in this highly specific mode of immune response regulation.

### Summary

The present studies have been carried out to characterize the regulatory influences acting upon defined pathways of T cell-dependent B cell activation. In these studies, it was demonstrated that high concentrations of free carrier strongly inhibited the MHC-restricted in vitro T cell-dependent antibody responses of primed  $Lyb-5^-$  B cells to the corresponding carrier-hapten conjugate. In contrast, these same concentrations of free carrier failed to inhibit the T cell dependent responses of  $Lyb-5^+$  B cells to the same antigen. The inhibition of  $Lyb-5^-$  B cell responses by free carrier was shown to result from active suppression mediated by carrier-specific primed  $Lyt-1^+2^-$  T cells and to require the additional participation of unprimed  $Lyt-1^-2^+$  T cells. The activation of this suppression was antigen-specific, but suppression once activated was antigen nonspecific in its effect. These findings thus demonstrate that distinct pathways of B cell activation can be independently regulated by T suppressor network influences, and that these pathways therefore constitute potentially independent components of the immune response to a given antigenic stimulus.

The authors gratefully acknowledge Dr. A. Singer for his helpful discussions and critical reading of this manuscript. They wish to thank Dr. R. Germain, Dr. R. Schwartz, and Dr. A. Finnegan for their careful and critical readings and discussions of this manuscript, and Mr. J. Israel, Mr. F. Jones, and Ms. L. DeNenno for their expert animal care.

*Received for publication 14 December 1981.*

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