

ROLE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN  
T CELL ACTIVATION OF B CELL SUBPOPULATIONS  
Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B Cell Subpopulations Differ in Their  
Requirement for Major Histocompatibility Complex-restricted  
T Cell Recognition\*

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In general, the interaction between T helper (T<sub>H</sub>)<sup>1</sup> cells and antigen-presenting accessory cells is genetically restricted by products of the major histocompatibility complex (MHC; 1-3). In contrast, the cellular interactions between T<sub>H</sub> cells and B cells in the generation of primary and secondary IgM and IgG responses both in vivo and in vitro have variously been reported as restricted by the MHC (4-8) or not restricted by the MHC (9-13). Because genetic restrictions imposed on T cell interactions by products of the MHC are best understood as a requirement for the recognition by T cells of the MHC determinants expressed by non-T cells, this controversy reflects uncertainty about whether T<sub>H</sub> cells must necessarily recognize the MHC determinants expressed by B cells in order to trigger them to secrete antibody. However, underlying this controversy is the possibility that both T<sub>H</sub> and B cell populations are functionally heterogeneous. Indeed, it has been suggested that two distinct subpopulations of T<sub>H</sub> cells exist, one of which is genetically restricted in its interactions with B cells and one which is not (14). Similarly, it is conceivable that there might exist two distinct subpopulations of B cells that have identical antibody repertoires but differ in their activation requirements such that one B cell subpopulation requires a genetically restricted interaction with T<sub>H</sub> cells, whereas the other B cell subpopulation does not, perhaps because it is responsive to soluble activating factors secreted by T<sub>H</sub> cells.

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<sup>1</sup> *Abbreviations used in this paper:* C, complement; Con A, concanavalin A; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain; SAC, spleen adherent cells; SRBC, sheep erythrocytes; SN, supernates; T<sub>H</sub>, T helper; TNP, trinitrophenyl.

B cells can be separated into two subpopulations of approximately equal size based on their expression of the differentiation antigen, Lyb-5, which is a determinant encoded by a single locus with two allelic forms, Lyb-5.1 and Lyb-5.2 (15). B cells that express the Lyb-5 determinant are a late-appearing subpopulation in normal mice and are virtually absent in mutant CBA/N mice (15, 16). Thus, Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cell subpopulations either represent two distinct developmental stages of the same B cell differentiation pathway or represent two distinct B cell lineages.

The experiments reported here have assessed the activation by T<sub>H</sub> cells of Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cell subpopulations in T<sub>H</sub> cell-dependent primary IgM responses. These experiments were performed both in vitro and in vivo and were designed to clearly distinguish T<sub>H</sub> cell recognition of MHC determinants expressed on accessory cells from T<sub>H</sub> cell recognition of MHC determinants expressed on B cells. The results of these experiments demonstrate that Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations do differ in their genetic requirements for activation by T<sub>H</sub> cells. Specifically, these experiments demonstrate that T<sub>H</sub> cell recognition of the MHC determinants expressed on B cells is not required for activation of the Lyb-5<sup>+</sup> B cell subpopulation, but is required for activation of the Lyb-5<sup>-</sup> B cell subpopulation.

### Materials and Methods

*Animals.* C57BL/10Sn (abbreviated B10), B10.A, CBA/J, and C3H.SW (abbreviated C.SW) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. CBA/CaHN, CBA/N, and F<sub>1</sub> mice derived from matings between DBA/2 and CBA/N mice were obtained from the Small Animal Section, National Institutes of Health, Bethesda, Md., or from Flow Laboratories, Rockville, Md. Because the failure of Lyb-5<sup>+</sup> B cells to develop in CBA/N mice is X-linked, F<sub>1</sub> male offspring from crosses between CBA/N ♀ and DBA/2 ♂, (CBA/N × DBA/2)F<sub>1</sub> ♂ (abbreviated CBD2F<sub>1</sub>), are hemizygous for the *xid* gene and are deficient in Lyb-5<sup>+</sup> B cells, whereas F<sub>1</sub> male offspring from crosses between DBA/2 ♀ and CBA/N ♂, (DBA/2 × CBA/N)F<sub>1</sub> ♂ (abbreviated D2CBF<sub>1</sub>), do not possess the *xid* gene and contain Lyb-5<sup>+</sup> B cells. Homozygous CBA/N mice of either sex express the *xid* gene and are deficient in Lyb-5<sup>+</sup> B cells, whereas homozygous CBA/J and CBA/CaHN mice of either sex do not possess the *xid* gene and contain normal numbers of Lyb-5<sup>+</sup> B cells. Adult males 2-5 mo old were used in all experiments.

*Long-Term-Radiation Bone Marrow Chimeras.* Chimeras are designated as bone marrow donor → irradiated recipient. Recipient mice were irradiated with either 950 rad x-ray or 1,000 rad cesium and reconstituted 2-6 h later with 15 × 10<sup>6</sup> bone marrow cells that had been depleted of T cells by pretreatment with rabbit anti-mouse brain serum (RAMB) and complement (C). Spleen cells were obtained from each chimera no earlier than 2 mo after irradiation and were individually typed by indirect immunofluorescence using H-2-specific reagents as previously described (11). By such testing, spleen cells from each chimera were of donor origin without detectable (<5%) cells of host origin. All chimeric cell populations were tolerant to both donor and host MHC determinants as assayed by cell-mediated lympholysis or by mixed lymphocyte reactions.

*Antigens.* Trinitrophenyl (TNP) conjugates of keyhole limpet hemocyanin (KLH; lot 530195, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and lipopolysaccharide (LPS), provided by Dr. John Ryan, Yale University, were prepared as previously described (17). Sheep erythrocytes (SRBC) were obtained weekly from a single sheep, 1245. The final concentration of each antigen used in vitro was the optimal concentration for that antigen and was 5 µg/ml TNP-KLH, 2 µg/ml TNP-LPS, and 0.05% SRBC. The amount of antigen used in vivo in adoptive transfer was 50 µg TNP-KLH per mouse and 20 µl packed SRBC per mouse.

*Preparation of Anti-Lyb-5.1 Alloantisera.* Anti-Lyb-5.1 serum was prepared according to the procedure of Ahmed et al. (15). Briefly, C57BL/6 mice were hyperimmunized with DBA/2 spleen cells. The hyperimmune serum was then exhaustively absorbed with DBA/2 thymocytes

until no anti-H-2<sup>d</sup> activity remained. The serum was then further absorbed with spleen cells from abnormal CBD2F<sub>1</sub> ♂ mice until no cytotoxicity remained against such cells. This absorbed antiserum, although inactive against abnormal CBD2F<sub>1</sub> ♂ mice and Lyb-5.1<sup>-</sup> strains, retained the capacity to kill 20–25% of spleen cells from normal D2CBF<sub>1</sub> mice and from other Lyb-5.1<sup>+</sup> strains with cytotoxic titers of 1:16–1:32. Control antiserum was obtained by further absorbing anti-Lyb-5.1 serum with DBA/2 spleen cells until it was no longer cytotoxic for DBA/2 or other Lyb-5.1<sup>+</sup> cells.

#### *Preparation of Cells*

**T CELLS.** T cells were prepared by passage of spleen cells over nylon fiber columns and collection of the nylon nonadherent eluate (17).

**“B” CELLS.** “B” cells were prepared by depleting spleen cells of T cells by pretreatment with either RAMB plus C or with a hybridoma monoclonal anti-Thy-1.2 plus C that was the gift of Dr. P. Lake, University College, London, England. Although depleted of T cells, “B” cells were not depleted of accessory cells.

**DEPLETION OF ADHERENT SPLEEN CELLS.** Spleen cells were depleted of adherent accessory cells by passage over G-10 Sephadex columns as previously described (17).

**B CELLS.** B cells were prepared by depleting spleen cells of adherent accessory cells by G-10 Sephadex passage and then depleting the remaining lymphocyte population of T cells by treatment with anti-Thy-1.2 plus C.

**SPLEEN ADHERENT CELLS (SAC).** 2-h glass-adherent SAC populations were pretreated with RAMB plus C, irradiated with 1,000 rad, and precultured at 10<sup>7</sup>/ml in a roller drum overnight before addition to the antibody cultures as previously described (18). The accessory cell activity of such cell populations has been shown to reside in radiation-resistant, non-T, non-B, glass-adherent cells that express I region-encoded MHC determinants (19).

**ACCESSORY CELLS.** As a source of added accessory cells for adoptive transfer experiments, spleen cells were pretreated with anti-Thy-1.2 plus C and irradiated with 1,500 rad (11).

*Cytotoxic Treatment of Spleen Cells with Anti-Lyb-5.1 plus C.* Spleen cells were treated with anti-Lyb-5.1 serum by a two-stage cytotoxicity procedure (15, 16). Briefly, spleen cells were suspended to a density of 5 × 10<sup>6</sup>/ml in a 1:8 dilution of anti-Lyb-5.1 in RPMI-1640 medium with 5% fetal calf serum and incubated for 30 min at 4°C. The cells were then washed and resuspended to the same volume in rabbit C (1:10) in 0.1% gelatin-RPMI-1640 and incubated 30 min at 37°C. The cells were then washed, counted, and resuspended.

*Supernate from Concanavalin A-stimulated Spleen Cells.* Spleen cells were cultured for 18–20 h at 10<sup>7</sup> cells/ml with 2.5 μg/ml concanavalin A (Con A) as previously described (20). After harvesting and filtering the supernate (SN), the SN was supplemented with 0.2 M alpha-methyl-D-mannoside to functionally inactivate any remaining Con A. Con A SN was used at a final concentration in culture of 25%.

*In Vivo Assay for Cell Collaboration.* 4–6 h after irradiation with 850 rad cesium, recipient animals were injected intravenously with the indicated unprimed spleen cell subpopulations and either 50 μg TNP-KLH or 20 μl packed SRBC. Each mouse received 0–2 × 10<sup>6</sup> unprimed T cells, 10<sup>7</sup> unprimed B cells, and 10<sup>7</sup> accessory cells, or 15 × 10<sup>6</sup> unfractionated spleen cells. 5 d after transfer, the spleens from these mice were assayed for the number of either anti-TNP or anti-SRBC plaque-forming cells (PFC).

*In Vitro Assay for Cell Collaboration.* 0.5 × 10<sup>6</sup> unprimed spleen cells for responses to soluble antigens and 1 × 10<sup>6</sup> unprimed spleen cells for responses to SRBC were cultured in a volume of 200 μl per flat-bottomed well of microtiter plates for 4 d in a 5% CO<sub>2</sub>-humidified air atmosphere as previously described (17). Cells were harvested by repeated pipetting, washed, resuspended in balanced salt solution, and individual cultures were assayed for number of anti-TNP PFC.

*PFC Assay.* Anti-TNP and anti-SRBC PFC were assayed by the slide modification of the Jerne hemolytic plaque technique (21). All points shown in each experiment represent the geometric mean responses of three replicate cultures or three to five adoptively transferred mice.

## Results

*Assessment of the B Cell Subpopulations Required in Primary T<sub>H</sub> Cell-dependent PFC Responses in Microculture.* Normal unprimed spleen cells are able to generate primary T<sub>H</sub> cell-

dependent PFC responses in microculture to a variety of antigens including TNP-KLH and SRBC (17). To identify the B cell subpopulations that are activated by  $T_H$  cells in microculture, the in vitro responses of Lyb-5<sup>-</sup> plus Lyb-5<sup>+</sup> B cell populations were compared with the responses of B cell populations that were devoid of the Lyb-5<sup>+</sup> subset and those that consisted entirely of Lyb-5<sup>-</sup> cells.

First, the in vitro  $T_H$  cell-dependent responses of spleen cells from D2CBF<sub>1</sub> male mice that contained both Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cell subpopulations were compared with those of CBD2F<sub>1</sub> male mice, which differ genetically only in the origin of their X chromosome and as a consequence are devoid of the Lyb-5<sup>+</sup> B cell subpopulation. D2CBF<sub>1</sub> male spleen cells, which contained both Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cell subpopulations, responded in microculture to both TNP-KLH and SRBC (Table I, experiment 1); in contrast, CBD2F<sub>1</sub> male spleen cells, which contained only Lyb-5<sup>-</sup> B cells, failed to respond in microculture to either TNP-KLH or SRBC (Table I, experiment 1). The failure of spleen cells from mice that express the *xid* gene and that are devoid of Lyb-5<sup>+</sup> B cells to generate primary  $T_H$  cell-dependent responses in microculture has previously been shown to result entirely from the failure of their Lyb-5<sup>-</sup> B cells to be triggered in microculture by either  $T_H$  cell or accessory cell activation signals and not from any functional incompetence on the part of either their accessory cells or  $T_H$  cells (22). Also, the failure of their Lyb-5<sup>-</sup> B cells to be activated in microculture in  $T_H$  cell-dependent responses was not due to their failure to survive in culture because they could be triggered under the same conditions to respond to TNP-LPS (Table I).

To test the conclusion that  $T_H$  cell-dependent responses in microculture do in fact require the participation of the Lyb-5<sup>+</sup> B cell subpopulation, similar experiments were performed assessing the ability of Lyb-5<sup>-</sup> B cells isolated from genetically normal mice to generate  $T_H$  cell-dependent responses in microculture (Table I, experiment 2). It has previously been demonstrated that treatment of spleen cells with anti-Lyb-5.1 plus C does not functionally affect either accessory cell or  $T_H$  cell populations, but only eliminates the Lyb-5<sup>+</sup> B cell subpopulation (23). As can be seen in Table I, experiment 2, the cytotoxic elimination of the Lyb-5<sup>+</sup> B cell subset from the B cell population of genetically normal mice resulted in the abrogation of their  $T_H$  cell-dependent response to TNP-KLH, but did not affect the  $T_H$  cell-independent response to TNP-LPS.

Thus, experiments using B cell populations deprived either genetically or serologically of the Lyb-5<sup>+</sup> B cell subpopulation demonstrated that Lyb-5<sup>+</sup> B cells are

TABLE I  
*T<sub>H</sub> Cell-dependent Primary Responses in Microculture Require Lyb-5<sup>+</sup> B Cells*

Experiment	Spleen cells	Treatment of spleen cells	B cell subpopulations present	PFC/10 <sup>6</sup> cultured cells*			
				TNP-KLH	SRBC	TNP-LPS	No antigen
1	D2CBF <sub>1</sub> ♂	—	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	165 (1.11)	107 (1.25)	386 (1.07)	1 (1.0)
	CBD2F <sub>1</sub> ♂	—	Lyb-5 <sup>-</sup>	0	2 (1.60)	322 (1.31)	1 (1.25)
2	D2CBF <sub>1</sub> ♂	—	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	138 (1.18)	—	470 (1.11)	4 (1.82)
	D2CBF <sub>1</sub> ♂	Anti-Lyb-5.1 + C	Lyb-5 <sup>-</sup>	4 (1.26)	—	402 (1.11)	0
	D2CBF <sub>1</sub> ♂	Absorbed anti-Lyb-5.1 + C	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	118 (1.27)	—	410 (1.08)	0

\* Geometric mean (SE) of triplicate cultures.

required for the generation of primary  $T_H$  cell-dependent responses in microculture to TNP-KLH and SRBC.

*Participation of Lyb-5<sup>+</sup> B Cells in  $T_H$  Cell-dependent Responses Is Not MHC Restricted.* Because Lyb-5<sup>+</sup> B cells were necessary for the generation of  $T_H$  cell-dependent responses in microculture, it was possible to determine whether the participation of Lyb-5<sup>+</sup> B cells in these responses was MHC restricted. It has previously been demonstrated that  $(A \times B)F_1 T_H$  cells that differentiate in a parent<sub>A</sub> environment, such as those from an  $A \times B \rightarrow A$  chimera, are restricted to the self-recognition of parent<sub>A</sub> MHC determinants (11, 24-26). Consequently, in experiments in which  $T_H$  cells, B cells, and antigen-presenting accessory cells are physically separated into individual and functionally distinct cell populations, it is possible to determine whether the restricted self-recognition of parental MHC determinants by  $A \times B \rightarrow A$  chimeric T cells is restricted to the self-recognition of parent<sub>A</sub> MHC determinants expressed on accessory cells, B cells, or both.

In the experiment displayed in Table II, the functional purity of the T cell, accessory cell, and B cell populations was demonstrated by the fact that no one cell population, nor any combination of two cell populations, was competent to respond in microculture to TNP-KLH. Upon the addition of normal B10  $\times$  B10.A ( $H-2^{b/a}$ ) T cells, responses were obtained with either parental accessory cell population and with either parental B cell population. In contrast, upon the addition of B10  $\times$  B10.A  $\rightarrow$  B10 ( $H-2^{b/a} \rightarrow H-2^b$ ) chimeric T cells, responses were obtained only in the presence of B10 ( $H-2^b$ ) accessory cells, demonstrating that the chimeric T cells were restricted in their self-recognition of MHC determinants expressed by accessory cells. However, in the presence of B10 accessory cells, B10  $\times$  B10.A  $\rightarrow$  B10 chimeric T cells activated both parental B cell populations equally well. Similarly, upon the addition of B10  $\times$

TABLE II  
Primary  $T_H$  Cell-dependent Responses to TNP-KLH in Microculture Do Not Require  $T_H$  Cell Recognition of B Cell-expressed MHC Determinants

T cells*	Accessory cells‡	B cells§		
		B10	B10.A	None
—	—	6 (1.60)	2 (1.00)	—
—	B10	2 (1.25)	3 (1.63)	—
—	B10.A	2 (1.22)	3 (1.22)	—
B10 $\times$ B10.A	—	5 (2.00)	3 (1.44)	0
B10 $\times$ B10.A	B10	213 (1.16)	239 (1.04)	0
B10 $\times$ B10.A	B10.A	177 (1.88)	154 (1.62)	0
B10 $\times$ B10.A $\rightarrow$ B10	—	6 (1.15)	7 (1.70)	0
B10 $\times$ B10.A $\rightarrow$ B10	B10	209 (1.43)	213 (1.53)	0
B10 $\times$ B10.A $\rightarrow$ B10	B10.A	9 (1.75)	7 (1.30)	0
B10 $\times$ B10.A $\rightarrow$ B10.A	—	0	3 (1.58)	0
B10 $\times$ B10.A $\rightarrow$ B10.A	B10	6 (1.84)	7 (1.70)	0
B10 $\times$ B10.A $\rightarrow$ B10.A	B10.A	151 (1.16)	200 (1.39)	0

\*  $1 \times 10^5$  T cells.

‡  $4 \times 10^4$  SAC.

§ PFC/ $10^5$  cultured cells,  $4 \times 10^5$  B cells.

B10.A  $\rightarrow$  B10.A (H-2<sup>b/a</sup>  $\rightarrow$  H-2<sup>a</sup>) chimeric T cells responses were obtained only in the presence of B10.A (H-2<sup>a</sup>) accessory cells; however, in the presence of B10.A accessory cells, responses were obtained with either parental B cell population. Because primary T<sub>H</sub> cell-dependent responses in microculture require the participation of Lyb-5<sup>+</sup> B cells, it can be concluded from this experiment that their participation in these responses is not MHC restricted. Because it is not yet technically possible to select a population of Lyb-5<sup>+</sup> B cells that is devoid of Lyb-5<sup>-</sup> B cells, it cannot be determined whether B cells other than Lyb-5<sup>+</sup> B cells are also activated in these responses. Nevertheless, the most straightforward interpretation of these experiments is that in microculture, T<sub>H</sub> cells activate only Lyb-5<sup>+</sup> B cells and their activation is not MHC restricted.

*Assessment of the B Cell Subpopulations Activated in Primary T<sub>H</sub> Cell-dependent PFC Responses In Vivo.* Even though Lyb-5<sup>-</sup> B cells did not appear to be triggered by T<sub>H</sub> cells in microculture, we considered the possibility that Lyb-5<sup>-</sup> B cells might be activated by T<sub>H</sub> cells to respond in vivo in short-term adoptive transfer experiments (Table III). As before, spleen cell populations containing B cells that consisted entirely of the Lyb-5<sup>-</sup> B cell subset failed to respond in vitro to either TNP-KLH or SRBC; however, in contrast to their failure to respond in microculture, the same spleen cell populations did respond to TNP-KLH and SRBC in vivo with a response equal in magnitude to that of cells that contained both Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations. Thus, the failure of Lyb-5<sup>-</sup> B cells to respond in in vitro microculture to TNP-KLH and SRBC did not reflect the absence of TNP-KLH- and SRBC-specific Lyb-5<sup>-</sup> B cells, but rather reflected the failure of these B cells to be triggered in microculture. Because Lyb-5<sup>-</sup> B cells were successfully triggered in vivo, it was possible to assess in short-term adoptive transfer experiments whether or not their activation was MHC restricted.

*Activation of Lyb-5<sup>-</sup> B cells by T<sub>H</sub> Cells is MHC Restricted.* In contrast to the relative ease in distinguishing MHC-restricted T<sub>H</sub> cell-B cell interactions from MHC-restricted T<sub>H</sub> cell-accessory cell interactions in vitro, this discrimination is more difficult in adoptive transfer experiments because there is potentially more than one source of functionally relevant accessory cells. Because accessory cell function is to some extent radiation resistant, the irradiated adoptive host may be one source of functional accessory cells; and, because of the relative potency of accessory cells and the relatively large number of B cells required in adoptive transfer, even small numbers of accessory cells contaminating the transferred B cell population may be another source of

TABLE III  
*Lyb-5<sup>-</sup> B Cell Subpopulations Are Activated in Primary T<sub>H</sub> Cell-dependent Responses In Vivo in Short-Term Adoptive Transfer but Not In Vitro in Microculture*

Spleen cells	B cell subpopulations present	In vitro microculture PFC/10 <sup>6</sup> cultured cells*			In vivo adoptive transfer PFC/spleen‡		
		TNP-KLH	SRBC	No antigen	TNP-KLH	SRBC	No antigen
CBA/J	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	264 (1.47)	284 (1.03)	6 (1.95)	1,500 (1.05)	1,012 (1.02)	<20
CBA/N	Lyb-5 <sup>-</sup>	0	4 (1.84)	0	1,984 (1.09)	956 (1.12)	<20

\* Geometric mean (SE) of triplicate cultures.

‡ Geometric mean (SE) of five individual mice in each group.

functional accessory cells. Nevertheless, it is possible to distinguish MHC-restricted T cell-accessory cell interactions from MHC-restricted T cell-B cell interactions *in vivo* by again making use of the MHC-restricted self-recognition repertoire of  $A \times B \rightarrow A$  chimeric  $T_H$  cells. Specifically, the uncertainty regarding the haplotype origin of the accessory cells functioning in adoptive transfer can be avoided by assaying the ability of  $T_H$  cells from  $A \times B \rightarrow A$  chimeras to activate strain B "B" cells in a strain B adoptive host (Fig. 1). Because  $A \times B \rightarrow A$  chimeric  $T_H$  cells are restricted to the self-recognition of parent<sub>A</sub> accessory cell MHC determinants (11), the strain B accessory cells resident in the strain B-irradiated host will not be recognized by the chimeric  $T_H$  cells. Similarly, because the responding "B" cell population is also of strain B origin, the  $A \times B \rightarrow A$  chimeric  $T_H$  cells will also not recognize the accessory cells contaminating the strain B "B" cell population. Thus, because activation of  $T_H$  cells requires recognition of accessory cell MHC determinants,  $T_H$  cell-dependent immune responses will not be initiated in a strain B-irradiated host that was adoptively transferred with  $A \times B \rightarrow A$  chimeric T cells and strain B "B" cells unless strain A accessory cells are specifically transferred as well. Consequently, in these experimental animals, the MHC haplotypes of the interacting T cells ( $A \times B \rightarrow A$ ), accessory cells (strain A), and B cells (strain B) are clearly identified.

In the first short-term adoptive transfer experiment of this sort, unprimed B10  $\times$  B10.A ( $H-2^{b/a}$ ) and B10  $\times$  B10.A  $\rightarrow$  A/J ( $H-2^{b/a} \rightarrow H-2^a$ ) chimeric T cells were assayed for their ability to activate SRBC-specific C.SW ( $H-2^b$ ) "B" cells in an irradiated B10 ( $H-2^b$ ) host (Fig. 2). C.SW mice were used as the source of  $H-2^b$  "B" cells rather than B10 because C.SW mice are Lyb-5.1, whereas B10 mice are Lyb-5.2. As can be seen in Fig. 2, no response was observed in the absence of added T cells, demonstrating that the anti-SRBC response was indeed  $T_H$  cell dependent (Fig. 2 A, D, and G). Upon the addition of normal  $F_1$  ( $H-2^{b/a}$ ) T cells, C.SW ( $H-2^b$ ) "B" cell responses were obtained in the presence of either B10 ( $H-2^b$ ) or B10.A ( $H-2^a$ ) added accessory cells (Fig. 2 B and E). Upon the addition of  $F_1 \rightarrow A/J$  ( $H-2^{b/a} \rightarrow H-2^a$ ) chimeric T cells, C.SW ( $H-2^b$ ) "B" cell responses were only obtained in the presence of B10.A ( $H-2^a$ ) added accessory cells but not B10 ( $H-2^b$ ) added accessory cells (Fig. 2 C and F). Because only  $H-2^a$  but not  $H-2^b$  accessory cells were able to activate the  $F_1$  chimeric  $T_H$  cells, it can be concluded that the activation of  $T_H$  cells by accessory cells is MHC restricted. However, because the chimeric  $T_H$  cells were restricted to the recognition of  $H-2^a$  MHC determinants, the fact that they were able to trigger

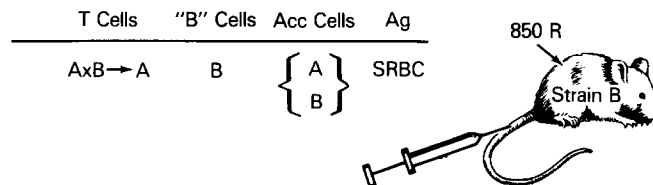


FIG. 1. Schematic of experimental protocol in which  $T_H$  cell recognition of accessory cell MHC determinants can be distinguished from  $T_H$  cell recognition of B cell MHC determinants *in vivo*.  $A \times B \rightarrow A$  chimeric  $T_H$  cell populations are restricted to the self-recognition of parent<sub>A</sub> MHC determinants and so do not recognize the MHC determinants of either the strain B adoptive host or the strain B "B" cell population. Thus, activation of the  $T_H$  cells should require the specific addition of strain A accessory cells.

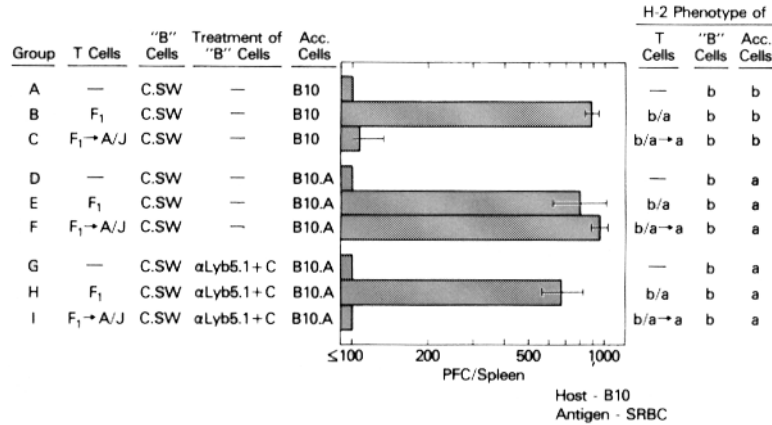


FIG. 2. In vivo activation of unfractionated ( $\text{Lyb-5}^+$  plus  $\text{Lyb-5}^-$ ) B cells by  $\text{T}_\text{H}$  cells is not MHC restricted, whereas activation of  $\text{Lyb-5}^-$  B cells by  $\text{T}_\text{H}$  cells is MHC restricted.  $2 \times 10^6$  unprimed T cells from either normal ( $\text{B10} \times \text{B10.A}$ ) $\text{F}_1$  or chimeric ( $\text{B10} \times \text{B10.A}$ ) $\text{F}_1 \rightarrow \text{A/J}$  mice were injected into irradiated B10 hosts along with  $10^7$  untreated or Lyb-5.1 plus C-treated C.SW B cells,  $10^7$  B10 or B10.A irradiated accessory cells, and SRBC. The number of anti-SRBC PFC/spleen were assayed 5 d after transfer. Less than 20 PFC/spleen were obtained in the no-antigen controls.

unfractionated  $\text{H-2}^b$  B cells demonstrates that the activation of unfractionated ( $\text{Lyb-5}^-$  plus  $\text{Lyb-5}^+$ ) B cells by  $\text{T}_\text{H}$  cells is not MHC restricted.

However, it was possible that this only reflected the MHC-unrestricted participation of the  $\text{Lyb-5}^+$  B cell subset. Consequently, this result did not necessarily preclude the possibility that the activation by  $\text{T}_\text{H}$  cells of the  $\text{Lyb-5}^-$  B cell subset might be MHC restricted. To assess this possibility, the  $\text{Lyb-5}^+$  B cell subpopulation was eliminated from the responding C.SW ( $\text{H-2}^b$  and  $\text{Lyb-5.1}$ ) "B" cell population by treatment with anti-Lyb-5.1 and C. That the remaining  $\text{Lyb-5}^-$  B cells could be triggered by  $\text{T}_\text{H}$  cells to respond to SRBC under these adoptive transfer conditions was shown by the fact that they were triggered by normal, unrestricted  $\text{F}_1$  T cells (Fig. 2H). In contrast to the activation of isolated  $\text{Lyb-5}^-$  B cells by unrestricted  $\text{F}_1$  T cells, restricted  $\text{F}_1 \rightarrow \text{A/J}$  chimeric  $\text{T}_\text{H}$  cells failed to activate the  $\text{Lyb-5}^-$  B cell subpopulation, even in the presence of appropriate B10.A accessory cells (Fig. 2I). Because the only difference between the two  $\text{T}_\text{H}$  cell populations was their ability to recognize  $\text{H-2}^b$  MHC determinants, this experiment demonstrates that the activation of  $\text{Lyb-5}^-$  B cells requires the recognition by  $\text{T}_\text{H}$  cells of the MHC determinants that  $\text{Lyb-5}^-$  B cells express.

To confirm these results, other short-term adoptive transfer experiments of this sort were performed with MHC-different cell combinations and with a "B" cell population that was genetically, rather than serologically, deprived of  $\text{Lyb-5}^+$  B cells (Fig. 3). The ability of unrestricted  $\text{B10} \times \text{B10.BR}$  ( $\text{H-2}^{b/k}$ ) and restricted  $\text{B10} \times \text{B10.BR} \rightarrow \text{B10}$  ( $\text{H-2}^{b/k} \rightarrow \text{H-2}^b$ ) T cells to activate SRBC-specific B cells from CBA/J mice ( $\text{H-2}^k$ , containing  $\text{Lyb-5}^-$  plus  $\text{Lyb-5}^+$  B cell subsets) and CBA/N mice ( $\text{H-2}^k$ , containing only the  $\text{Lyb-5}^-$  B cell subset) was assessed in CBA/J ( $\text{H-2}^k$ ) irradiated hosts. In the absence of added T cells, no responses were observed (Fig. 3A, D, and G). Upon the addition of normal  $\text{F}_1$  T cells, CBA/J "B" cells responses were obtained in the presence of either  $\text{H-2}^k$  or  $\text{H-2}^b$  added accessory cells (Fig. 3B and E). In contrast, upon the addition of chimeric  $\text{F}_1 \rightarrow \text{B10}$  T cells, CBA/J "B" cell responses



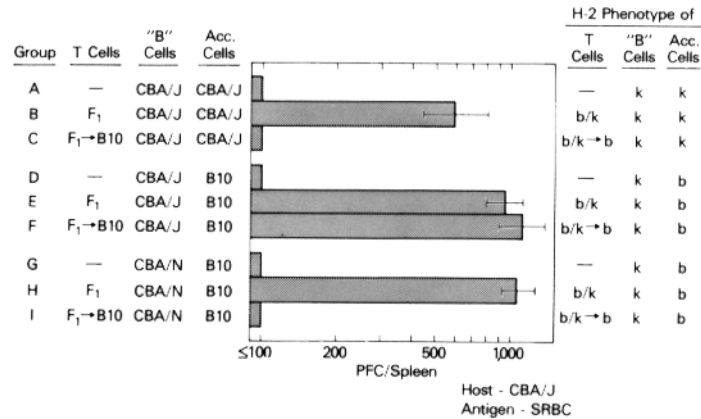


FIG. 3. In vivo activation of CBA/J (Lyb-5<sup>+</sup> plus Lyb-5<sup>-</sup>) B cells by T<sub>H</sub> cells is not MHC restricted, whereas activation of CBA/N (Lyb-5<sup>-</sup>) B cells by T<sub>H</sub> cells is MHC restricted.  $2 \times 10^6$  unprimed T cells from either normal (B10  $\times$  B10.BR)F<sub>1</sub> or chimeric (B10  $\times$  B10.BR)  $\rightarrow$  B10 mice were injected into irradiated CBA/J hosts along with  $10^7$  untreated CBA/J or CBA/N B cells,  $10^7$  B10 or CBA/J irradiated accessory cells, and SRBC. The number of anti-SRBC PFC/spleen were assayed 5 d after transfer. Less than 20 PFC/spleen were obtained in the no-antigen controls.

were obtained only in the presence of H-2<sup>b</sup> but not H-2<sup>k</sup> added accessory cells (Fig. 3C and F), again demonstrating that the activation of chimeric F<sub>1</sub> T<sub>H</sub> cells by added accessory cells is MHC restricted, but that the activation of Lyb-5<sup>-</sup> plus Lyb-5<sup>+</sup> B cell populations by T<sub>H</sub> cells is not MHC restricted. However, CBA/N "B" cells (also H-2<sup>k</sup> but containing only Lyb-5<sup>-</sup> B cells) were activated only by unrestricted F<sub>1</sub> T<sub>H</sub> cells (Fig. 3H) and not by restricted F<sub>1</sub>  $\rightarrow$  B10 chimeric T<sub>H</sub> cells, even in the presence of appropriate H-2<sup>b</sup> added accessory cells (Fig. 3I).

Thus, it can be concluded from these experiments that activation of unprimed Lyb-5<sup>-</sup> B cells by unprimed T<sub>H</sub> cells requires T<sub>H</sub> cell recognition of the MHC determinants that B cells express, whereas activation of unprimed Lyb-5<sup>+</sup> B cells by unprimed T<sub>H</sub> cells does not.

*Lyb-5<sup>+</sup> B Cells, but Not Lyb-5<sup>-</sup> B Cells, Can Be Triggered to Respond in Microculture to SRBC by Con A SN.* The results presented thus far provide strong evidence that Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cell subsets differ in their cellular interaction requirements for activation. Indeed, the apparent ability of T<sub>H</sub> cells to activate Lyb-5<sup>+</sup> B cells without recognizing their MHC determinants is consistent with the possibility that T<sub>H</sub> cells can activate Lyb-5<sup>+</sup> B cells without physically interacting with them. Thus, one difference between Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cells might derive from their responsiveness to soluble T<sub>H</sub> signals. Because Con A SN has been reported to possess T<sub>H</sub> cell replacing function for SRBC-specific responses (14), the ability of Con A SN to activate SRBC-specific Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cells was assessed.

First, the ability of Con A SN to activate SRBC-specific B cells directly, rather than via a non-B cell intermediary, was ascertained. As can be seen in Table IV, spleen cells depleted of accessory cells were unable to respond to SRBC unless SAC were specifically added to the cultures, demonstrating that the responding lymphocyte population was functionally deprived of accessory cells (Table IV, parts A and B). Anti-Thy-1.2 plus C treatment of the already accessory cell-depleted responding lymphocyte population abolished their ability to respond to SRBC upon the addition

TABLE IV  
*Con A SN Directly Activates SRBC-specific B Cells*

Group	Treatment of spleen cells*	Lymphocyte populations present	Accessory cells	Con A SN	PFC/10 <sup>6</sup> cultured cells‡	
					SRBC	No anti-gen
A	G-10	T and B	—	—	0	0
B	G-10	T and B	SAC	—	405 (1.06)	0
C	G-10 + anti-Thy-1.2 + C	B	SAC	—	6 (2.46)	3 (1.33)
D	G-10 + anti-Thy-1.2 + C	B	—	+	634 (1.04)	7 (1.74)

\* Geometric mean (SE) of triplicate cultures.

‡ Strain of spleen cells was B10.

TABLE V  
*Con A SN Does Not Directly Activate SRBC-specific Lyb-5<sup>-</sup> B Cells*

Spleen cells	Treatment of spleen cells	B cell subpopulations present	Con A SN	PFC/10 <sup>6</sup> cultured cells*	
				SRBC	No anti-gen
CBA/CaHN	Anti-Thy-1.2 + C	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	—	5 (1.08)	1 (1.23)
CBA/CaHN	Anti-Thy-1.2 + C	Lyb-5 <sup>-</sup> + Lyb-5 <sup>+</sup>	+	>100	1 (1.11)
CBA/N	Anti-Thy-1.2 + C	Lyb-5 <sup>-</sup>	—	0	0
CBA/N	Anti-Thy-1.2 + C	Lyb-5 <sup>-</sup>	+	6 (1.18)	0

\* Geometric mean (SE) of triplicate cultures.

of SAC, demonstrating that the responding lymphocyte population was now functionally deprived of both T cells and accessory cells (Table IV, part C). Thus, the responding lymphocyte population after G-10 passage and anti-Thy-1.2 plus C treatment functionally consisted entirely of B cells. The addition of Con A SN to this functionally isolated B cell population was sufficient to trigger them to respond to SRBC (Table IV, part D). Although the possibility cannot be entirely excluded that Con A SN acts by enhancing the activity of a non-B cell that was present in small numbers in the functionally isolated B cell population, the most straightforward conclusion that can be drawn from this experiment is that Con A SN is capable of directly activating SRBC-specific B cells.

The SRBC-specific B cell subpopulation that can be directly activated in microculture by Con A SN was next determined. As can be seen in Table V, CBA/CaHN B cells that contained both Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cells subsets were triggered by the Con A SN to respond to SRBC. In contrast, CBA/N B cells, which consisted entirely of the Lyb-5<sup>-</sup> B cell subset, were not triggered by the Con A SN to respond to SRBC. Similar results were obtained with anti-Lyb-5 plus C-treated B cells from genetically normal mice (data not shown). It should be emphasized that the failure of Lyb-5<sup>-</sup> B cells to be activated in microculture by the nonspecific activating factors contained within the Con A SN contrasts sharply with their ability to be nonspecifically activated under the same experimental conditions by LPS (Table I). Thus, although these experiments do not reveal the reasons for the differences in response between Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations to Con A SN, they do demonstrate that

Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cells differ in their ability to respond to the soluble activating signals, which are contained within the Con A SN.

These results are consistent with the concept that Lyb-5<sup>-</sup> B cells fail to be activated in T<sub>H</sub> cell-dependent responses in microculture because the direct, genetically restricted interaction between carrier-specific T<sub>H</sub> cells and hapten-specific Lyb-5<sup>-</sup> B cells is an unlikely event with the small numbers of unprimed T<sub>H</sub> cells and B cells used. Rather, these results suggest that T<sub>H</sub> cell-dependent primary responses in microculture that are obtained are likely to be mediated by soluble T<sub>H</sub> cell signals which, like Con A SN, predominantly activate Lyb-5<sup>+</sup> B cells.

### Discussion

This study has demonstrated that subpopulations of B cells that are distinct in their cell surface antigens and in their ontogeny are also distinct in their genetic requirements for activation by T<sub>H</sub> cells. Specifically, these experiments demonstrated that activation of the developmentally late-appearing Lyb-5<sup>+</sup> B cell subpopulation in primary T<sub>H</sub> cell-dependent responses did not require T<sub>H</sub> cell recognition of B cell-expressed MHC determinants, whereas activation of the developmentally early-appearing Lyb-5<sup>-</sup> B cell subpopulation in the same responses did require T<sub>H</sub> cell recognition of B cell-expressed MHC determinants. In addition, it was demonstrated that the ability of Lyb-5<sup>+</sup> B cells to be activated in a genetically unrestricted manner by T<sub>H</sub> cells paralleled their responsiveness to soluble nonspecific T<sub>H</sub> cell factors.

The results of this study further emphasize the functional differences between the Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations. It had previously been shown that only Lyb-5<sup>+</sup> B cells were activated by antigen-presenting accessory cells, even when the antigen being presented was one that would otherwise activate both Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cells, e.g., TNP-*Brucella abortus* (23, 24). These results were interpreted to demonstrate that Lyb-5<sup>+</sup> B cells were responsive to accessory cell-derived activation signals, whereas Lyb-5<sup>-</sup> B cells were not. The present results extend these observations to include soluble nonspecific T<sub>H</sub> signals, which activate Lyb-5<sup>+</sup> B cells but not Lyb-5<sup>-</sup> B cells. Although the striking functional differences observed between Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations support the concept that these two subpopulations derive from two distinct B cell lineages, these results are also compatible with the possibility that the further differentiation of Lyb-5<sup>-</sup> B cells into Lyb-5<sup>+</sup> B cells results not only in the expression of the Lyb-5 determinant but in the expression of receptors that bind additional T<sub>H</sub> and accessory signals as well.

Whether or not Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> B cell subpopulations develop from independent B cell lineages, the two subpopulations clearly differ in their responsiveness to several cellular activation signals (23). Because different experimental conditions promote different cellular interactions, it is likely that responses against the same antigen might be mediated by different B cell subpopulations, depending upon the particular experimental conditions employed. Indeed, only Lyb-5<sup>+</sup> anti-SRBC B cells appeared to be activated in primary responses in microculture, whereas both Lyb-5<sup>+</sup> and Lyb-5<sup>-</sup> anti-SRBC B cells were activated in primary responses in vivo in short-term adoptive transfer; other experimental conditions appear primarily to promote the activation of Lyb-5<sup>-</sup> B cells.<sup>2</sup> Thus, the controversy about whether T<sub>H</sub> cell

<sup>2</sup> Asano, Y, A. Singer, and R. J. Hodes. Role of the major histocompatibility complex (MHC) in T cell activation of B cell subpopulations. MHC-restricted and -unrestricted B cell responses are mediated by distinct B cell subpopulations. Manuscript submitted for publication.

recognition of B cell-expressed MHC determinants is required for B cell activation might well result from the fact that those assays in which genetically restricted  $T_H$ -B cell interactions were observed primarily activated Lyb-5<sup>-</sup> B cells, whereas those assays in which no MHC restrictions were observed between  $T_H$  and B cells primarily activated Lyb-5<sup>+</sup> B cells. In this regard, it might be suggested that the in vivo experiments of Sprent (5, 6), in which the activation of anti-SRBC B cells was MHC restricted, were primarily mediated by Lyb-5<sup>-</sup> B cells, in contrast to the in vivo anti-SRBC responses observed in the present report, which were mediated by both Lyb-5<sup>-</sup> and Lyb-5<sup>+</sup> B cells.

It is also of interest that in experiments in which Marrack and co-workers (14) found the activation of B cells by  $T_H$  cells to be MHC restricted, the MHC restriction was overcome by the addition to culture of Con A SN. Although such results have been interpreted as evidence for two independent  $T_H$  cell subpopulations, one which was MHC restricted in its interaction with B cells and one which was not, it is now possible to suggest a different interpretation of these data. On the basis of the present study, it can be hypothesized that in the absence of Con A SN, the MHC-restricted responses observed were primarily due to the selective activation of Lyb-5<sup>-</sup> B cells; however, upon the addition of Con A SN, genetically unrestricted Lyb-5<sup>+</sup> B cells were activated. Thus, the loss of MHC restriction was not merely due to the addition of genetically unrestricted activating factors, but was due to the activation of a genetically unrestricted B cell subpopulation. Although the heterogeneity of the responding B cell populations is probably sufficient to explain many of the previous observations that suggested the existence of functionally distinct  $T_H$  cell subpopulations, it is nevertheless likely that both  $T_H$  cell populations and B cell populations are functionally heterogeneous. Indeed, it is even conceivable that distinct  $T_H$  cell subpopulations only interact with particular B cell subpopulations.

It has recently been proposed that  $T_H$  cell activation of B cells progresses through two distinct phases such that activation by  $T_H$  cells of small resting B cells to a blast state is MHC restricted, whereas activation by  $T_H$  cells of B cell blasts to secrete antibody is MHC unrestricted (27). Although the present experiments do not address these issues directly, it seems unlikely that the controversy over MHC-restricted  $T_H$ -B cell interactions can be entirely resolved by such an hypothesis. For example, normal B cell populations, some of which were probably in a blast state just from their exposure to environmental antigens, were always used in the present experiments, but  $T_H$  cell activation of B cells from CBA/N mice (which contained only Lyb-5<sup>-</sup> B cells) was MHC restricted, whereas activation of B cells from CBA/J mice (which contained Lyb-5<sup>-</sup> plus Lyb-5<sup>+</sup> B cells) was not MHC restricted. Furthermore, in experiments previously reported by others (28), the activation of B cells obtained from TNP-LPS-immunized animals, which would be expected to contain many blasts and few resting cells, was highly MHC restricted. Although apparently contrary to the hypothesis that activation of B cell blasts is MHC unrestricted, these latter results are compatible with the data presented in this report insofar as TNP-LPS activates and would be expected to prime Lyb-5<sup>-</sup> B cells. Nonetheless, it is possible that MHC-restricted activation of small resting B cells to blasts, followed by MHC-unrestricted activation of the blast, represents one pathway of B cell activation. It would, of course, be of interest to ascertain the Lyb-5 phenotype of the B cells identified as "resting cells" and "blasts" in these studies.

Because MHC restrictions generally represent a developmentally advanced mechanism of regulating cellular interactions, it may seem somewhat surprising that it is the interaction of  $T_H$  cells with the B cell subset that appears early in ontogeny, i.e.,  $Lyb-5^-$  B cells, that is MHC restricted. However, the ability to observe MHC-restricted  $T_H$  cell activation of  $Lyb-5^-$  B cells implies that  $T_H$  cell recognition of B cell MHC determinants is the only interaction that triggers  $Lyb-5^-$  B cells to secrete antibody in T cell-dependent antibody responses. Thus,  $Lyb-5^-$  B cells appear to be unresponsive to nonspecific soluble T cell signals and accessory cell signals that are elaborated in the course of a  $T_H$  cell-dependent immune response. Indeed, in contrast to  $Lyb-5^+$  B cells,  $Lyb-5^-$  B cells are neither triggered by Con A SN nor by antigen-presenting accessory cells (22, 23). The failure to observe MHC-restricted  $T_H$  cell activation of the developmentally late-appearing  $Lyb-5^+$  B cell subset does not necessarily imply that these cells cannot be triggered by a genetically restricted interaction with  $T_H$  cells, but rather suggests that  $Lyb-5^+$  B cells have developed additional mechanisms for responding to additional cellular activation signals. One possible schematic of the diverse interaction pathways by which  $Lyb-5^+$ , as opposed to  $Lyb-5^-$ , B cells can be triggered is shown in Fig. 4. It must be emphasized that it is conceivable that the  $T_H$  cells that activate  $Lyb-5^-$  B cells might be distinct from those that activate  $Lyb-5^+$  B cells, although this possibility is not explicitly shown in Fig. 4. Similarly, it is also conceivable that the accessory cells that activate  $T_H$  cells might be distinct from those that directly interact with  $Lyb-5^+$  B cells. Thus, although the scheme outlined in Fig. 4 must be considered a minimal one for the cell interactions that can lead to the activation of B cells, it emphasizes that B cell activation, at least activation of the  $Lyb-5^+$  subset, is not simply a linear sequence of cell interactions from accessory cells  $\rightarrow$   $T_H$  cells  $\rightarrow$  B cells, but that there exists a number of different cellular interaction pathways that can lead to the activation of  $Lyb-5^+$  B cells.

In conclusion, whether or not these speculations are correct, the present experiments

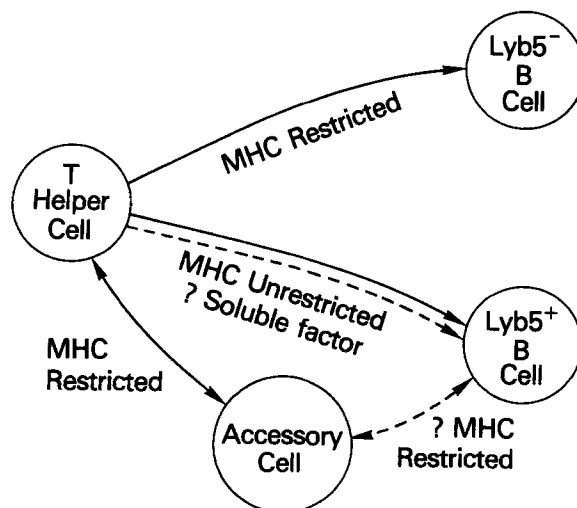


FIG. 4. Schematic of possible cell interaction pathways leading to activation of  $Lyb-5^-$  and  $Lyb-5^+$  B cell subpopulations. Dashed lines indicate activation pathways that might be mediated by soluble factors.

demonstrate that ontogenetically distinct subpopulations of B cells have genetically distinct cellular activation requirements, even for a response in which both subpopulations are activated simultaneously.

### Summary

This report has examined the requirements for T helper ( $T_H$ ) cell recognition of major histocompatibility complex (MHC) determinants expressed by B cells for the activation of unprimed  $Lyb-5^+$  and  $Lyb-5^-$  B cell subpopulations. The generation of primary  $T_H$  cell-dependent plaque-forming cell responses in vitro microculture required the presence of  $Lyb-5^+$  B cells because B cell populations that were deprived, either genetically or serologically, of the  $Lyb-5^+$  subpopulation were not activated in these responses. Cell-mixing experiments in which  $A \times B \rightarrow A$  chimeric  $T_H$  cells were mixed with purified populations of parental accessory cells and parental B cells demonstrated that the in vitro activation of  $Lyb-5^+$  B cells did not require  $T_H$  cell recognition of B cell MHC determinants, although it did require  $T_H$  cell recognition of accessory cell MHC determinants.

In contrast to the failure of  $Lyb-5^-$  B cells to be activated in primary  $T_H$  cell-dependent responses in vitro microculture, isolated populations of  $Lyb-5^-$  B cells were triggered by  $T_H$  cells in vivo in short-term adoptive transfer experiments. By the use of  $A \times B \rightarrow A$  chimeric  $T_H$  cells and parental strain B adoptive hosts, it was possible in vivo to distinguish genetically restricted  $T_H$  cell recognition of B cells from genetically restricted  $T_H$  cell recognition of accessory cells. Similar to the results obtained in vitro, the activation in vivo of unfractionated ( $Lyb-5^+$  plus  $Lyb-5^-$ ) B cell populations did not require  $T_H$  cell recognition of B cell MHC determinants. In contrast, in the same in vivo responses activation of isolated populations of  $Lyb-5^-$  B cells did require  $T_H$  cell recognition of B cell MHC determinants. The most straightforward interpretation of these experiments is that  $T_H$  cell recognition of B cell MHC determinants is required for the activation of  $Lyb-5^-$  B cells but is not required for the activation of  $Lyb-5^+$  B cells.

To better understand why  $T_H$  cell activation of one B cell subpopulation is genetically restricted, whereas activation of another subpopulation is not, the response of  $Lyb-5^+$  and  $Lyb-5^-$  B cells to the soluble activating factors present in concanavalin A-induced spleen cell supernates (Con A SN) was examined. It was observed that  $Lyb-5^-$  B cells, as opposed to  $Lyb-5^+$  B cells, were unable to respond in microculture to the nonspecific  $T_H$  cell-activating factors present in Con A SN, even though they were able to nonspecifically respond under the same conditions to trinitrophenyl-lipopolysaccharide. It was observed that the ability of B cell subpopulations to respond to nonspecific soluble T cell factors paralleled their ability to be activated by  $T_H$  cells in a genetically unrestricted manner.

Thus, the present experiments demonstrate that activation by  $T_H$  cells of  $Lyb-5^-$  B cells is MHC restricted, whereas activation of  $Lyb-5^+$  B cells is not. These experiments suggest that one possible explanation for such differences is that activation of  $Lyb-5^+$  B cells does not require direct interaction with  $T_H$  cells because they can be activated by soluble activation signals that  $T_H$  cells secrete.

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