ROLE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN T CELL ACTIVATION OF B CELL SUBPOPULATIONS

A Single Monoclonal T Helper Cell Population Activates Different B Cell Subpopulations by Distinct Pathways*

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It has recently been shown (1, 2) that distinct pathways exist for the T cell-dependent activation of defined B cell subpopulations. In these studies, it was demonstrated that conventional heterogeneous helper T (T_H)[1] cell populations could only activate Lyb-5$^-$ B cells via a pathway that required *H-2*-restricted T cell recognition of accessory cells as well as B cells, whereas the same heterogeneous T_H cell populations were able to activate B cell populations containing Lyb-5$^+$ B cells through a different pathway, requiring T cell recognition of accessory cells but not B cells. These earlier studies did not establish, however, whether identical T_H cells activate both Lyb-5$^-$ and Lyb-5$^+$ B cell subpopulations by distinct mechanisms or whether different subpopulations of T_H cells activated each B cell subpopulation.

The nature of the T_H cell requirements in T-dependent (TD) antibody responses has previously been studied in a number of experimental settings. Findings from several laboratories (3–7) have indicated that the interaction of two or more distinct T cell subpopulations may be required for the generation of optimal TD antibody responses. The use of monoclonal T cell populations has provided a recent addition to the available approaches for the study of T cell functions, however, and several recent reports have established the ability of monoclonal T cell populations to function as highly efficient antigen-specific and major histocompatibility complex (MHC)-restricted T_H cells (8–11). In addition, these reports have suggested differences in the mechanisms by which cloned T_H cell populations can function. In particular, certain cloned T_H cells have been reported to be *H-2* restricted for recognition of B cells as well as accessory cells (9), whereas others have been shown to be restricted for recognition of accessory cells alone (11). Similarly, responses mediated by cloned T_H cells have either demonstrated a requirement for carrier-hapten linkage (10) or have not (11).

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[1] *Abbreviations used in this paper:* ATS, anti-mouse thymocyte serum; C, complement; Con A, concanavalin A; FGG, fowl gamma globulin; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; SRBC, sheep erythrocytes; TD, T dependent; T_H, T helper; TNP, trinitrophenyl.

Therefore, in the present report, experiments were undertaken to investigate the ability of monoclonal T helper cells to activate either Lyb-5$^-$ or Lyb-5$^+$ B cell subpopulations. Using antigen-specific, *H-2*-restricted monoclonal T_H cells, it was demonstrated that the same monoclonal T_H cells were capable of activating each B cell subpopulation, but through distinct mechanisms. The activation of Lyb-5$^-$ B cells by cloned T_H cells required MHC-restricted T_H cell-B cell interactions as well as carrier-hapten linkage. In contrast, the activation of Lyb-5$^+$ B cells by the same cloned T_H cells was MHC unrestricted and did not require carrier-hapten linkage. Thus, a single cloned T_H cell population was capable of activating different B cell subpopulations through two distinct activation pathways.

Materials and Methods

Animals. C57BL/10 (B10), B10.BR, (B10 × B10.BR)F_1, and (C57BL/6 × C3H/HeJ)F_1 (B6C3F_1) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. CBA/N and CBA/CaHN mice were obtained from the Small Animal Section, National Institutes of Health.

Antigens. Keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) and fowl gamma globulin (FGG) (N. L. Cappel Laboratories, Cochranville, PA) were conjugated with 2,4,6-trinitrobenzene sulfate (Pierce Chemical Co., Rockford, IL) as previously described (12). The degrees of substitution were 20 trinitrophenyl (TNP) residues per 100,000 daltons KLH (TNP-KLH) and 9 TNP residues per 100,000 daltons FGG (TNP-FGG).

Immunization. Mice were immunized with 100 μg of TNP-KLH in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally 3–8 wk before use.

Derivation of KLH-reactive Clones. Preparation of immune lymph node cells and cell culture were carried out as previously described (13). Cloning of KLH-reactive T cells was accomplished by limiting dilution methods. Clones were then maintained in the presence of syngeneic filler cells, alternating 4 d of culture in the presence of KLH and 0.5–1% concanavalin A (Con A) supernatant (13) with 10 d of (resting) culture in the absence of antigen or Con A supernatant. The antigen specificity and genetic restriction of these cloned T cells were assessed by assaying the proliferative responses of these lines to antigen in the presence of irradiated parental strain accessory cells. As previously demonstrated (14), B6C3F_1-derived clone 14 T cells and clone 16 T cells are KLH specific and *H-2* restricted to recognizing antigen presented in the context of *H-2*^{k} and *H-2*^{b}, respectively.

Preparation of Cells for In Vitro Antibody Responses

CLONED T CELLS. Cloned T cells were assayed for helper activity after 10 d of resting culture in the absence of antigen.

(B + ACCESSORY) CELLS. T-depleted TNP-KLH-primed spleen (B + accessory) cells were prepared by treatment with a T cell-specific cytotoxic rabbit anti-mouse brain serum (RAMB) plus complement (C) (12). In selected experiments, more rigorous T cell depletion was accomplished by pretreating in vivo with 0.5 ml of a 1/10 dilution of rabbit anti-mouse thymocyte serum (ATS) (M. A. Bioproducts, Walkersville, MD) and then treating spleen cells sequentially in vitro with RAMB + C, monoclonal anti-Thy-1.2 (14), and monoclonal anti-Lyt-1.2 (New England Nuclear, Boston, MA) plus C. In certain experiments as noted, populations were further treated by passing through Sephadex G-10 columns to deplete of accessory cells (12). These populations, depleted of both T cells and accessory cells, are referred to as B cells.

ACCESSORY CELLS. Unprimed spleen cells were T depleted by treatment with RAMB + C followed by 2,000 rad irradiation, and were used as a source of accessory cells.

Culture Conditions for In Vitro Antibody Response. Cultures were performed as previously described in 2-ml wells incubated for 5 d at 37°C. in 5% CO_2-humidified air (2). For assays of cloned T_H cell activity, titrated numbers of T cells were added to 3 × 10^6 TNP-primed (B + accessory) cells per culture. In experiments using Sephadex G-10-passed B cell populations, previously described culture conditions were modified to use 2-mercaptoethanol at a concentration of 10^{-7} (instead of 5 × 10^{-5}) M. Cells were harvested, washed, and assayed for plaque-

forming cells (PFC) on TNP-conjugated sheep erythrocytes (TNP-SRBC) (2, 14). In particular experiments, the TNP specificity of PFC was confirmed either by blocking with 1×10^{-5} M TNP (2, 14) or by testing on unconjugated SRBC. Cells cultured in the absence of antigen uniformly generated <80 PFC/culture.

Results

The Same Cloned T_H Cells Can Activate Different B Cell Subpopulations. It was recently demonstrated (1, 2) in studies using heterogeneous T cell populations that at least two pathways exist for TD activation of the B cells participating in antibody responses. To test the possibility that a single population of cloned T cells can function in more than one B cell activation pathway, KLH-specific B6C3F$_1$-cloned T_H cells were studied under experimental conditions that had previously been shown (2) to result in the activation of different B cell subpopulations. These cloned T_H cells were assayed for their ability to cooperate with TNP-primed B10, CBA/CaHN (Lyb-5$^+$ and Lyb-5$^-$ B cells) or CBA/N (Lyb-5$^-$ B cells only) B cells for responses to either 0.001 μg/ml TNP-KLH or 20 μg/ml TNP-KLH (Table I, experiment 1). At high antigen concentration, cloned T_H cells cooperated in an *H-2*-restricted fashion with B10 and CBA/CaHN, but not with CBA/N (B + accessory) cells, to generate predominantly IgM PFC (Table I, experiment 1), consistent with a requirement for Lyb-5$^+$ B cells in these responses (2), and demonstrating that an isolated population of Lyb-5$^-$ B cells is not activated under these conditions. In contrast, predominantly IgG responses to 0.001 μg/ml TNP-KLH were generated by all three strains of B cells in the presence of appropriately *H-2*-restricted T_H cells, demonstrating that Lyb-5$^-$ B cells were activated in such responses (Table I, experiment 1). To minimize the possibility that residual T cells present in the (B + accessory) cell populations were playing an essential role in these responses, similar experiments were carried out using in vivo pretreatment of hapten-primed mice with ATS, followed by sequential in vitro treatment with RAMB, anti-Thy-1.2, and anti-Lyt-1.2 + C. The results of such experiments were identical to those presented above (Table I, experiment 2). These results demonstrate that the same cloned T_H cells can, under appropriate conditions,

TABLE I

The Same MHC-restricted Cloned T Helper Cells Can Activate Different B Cell Subpopulations

TNP-KLH	Cloned T cells*	Anti-TNP PFC/culture (B + accessory) cells			
		Experiment 1			Experiment 2
		B10‡	CBA/CaHN‡	CBA/N‡	B10§
0.001 μg/ml‖	Clone 14	9 ± 9	2,770 ± 380	1,529 ± 240	0
	Clone 16	1,320 ± 91	0	168 ± 61	1,400 ± 83
20 μg/ml¶	Clone 14	0	1,630 ± 141	51 ± 35	192 ± 41
	Clone 16	943 ± 21	137 ± 80	0	1,728 ± 76

* 3×10^3 cells/culture. Clone 14 is specific for KLH plus *H-2*^{k} and clone 16 is specific for KLH plus *H-2*^{b}.
‡ 3×10^6 RAMB + C-treated TNP-primed spleen cells/culture.
§ TNP-primed spleen cells were pretreated in vivo with ATS, followed by sequential in vitro treatment with RAMB, anti-Thy-1.2, and anti-Lyt-1.2 + C. 3×10^6 cells/culture.
‖ Responses are presented as mean indirect (IgG) PFC/culture.
¶ Responses are presented as mean direct (IgM) PFC/culture.

function through at least two distinct pathways of B cell activation that differ in the identity of the B cell subpopulations being activated.

The Same Cloned T_H Cells Can Activate Lyb-5$^-$ B Cells by an MHC-restricted Interaction and Can Activate Lyb-5$^+$ B Cells by an MHC-unrestricted Mechanism. Because the same cloned T_H cells could be shown under different response conditions to activate either Lyb-5$^-$ or Lyb-5$^+$ B cells, it was next determined whether these T_H cells provided identical activation signals for the responses of these two B cell subpopulations. Experiments were carried out to determine whether the MHC restrictions demonstrated for the activation of either Lyb-5$^-$ or Lyb-5$^+$ B cells reflected requirements for T_H cell recognition of B cells, accessory cells, or both. Cloned T_H cells were assayed for their ability to cooperate with TNP-primed B10 or B10.BR (B + accessory) cell populations for responses to TNP-KLH at concentrations of either 20 μg/ml or 0.001 μg/ml in the presence or absence of (B10 × B10.BR)F_1 accessory cells (Table II, experiment 1). In the absence of F_1 accessory cells, T_H function was MHC restricted at both low and high antigen concentrations, with clone 14 T cells cooperating with B10.BR (*H-2^k*) but not B10 (*H-2^b*) (B + accessory) cells and clone 16 T cells showing the reciprocal restriction pattern. In the presence of F_1 accessory cells, both clone 14 and clone 16 T cells cooperated efficiently with either B10 or B10.BR B cells for responses to 20 μg/ml TNP-KLH (Table II, experiment 1), demonstrating that under these conditions, cloned T_H cells were restricted only in their recognition of accessory cell MHC determinants, but not B cell MHC determinants. In contrast, for the response to 0.001 μg/ml TNP-KLH, the restriction of cloned T_H cell activity was not reversed by F_1 accessory cells, demonstrating that under the conditions of low antigen concentration, the ability of the same T_H cell to activate B cells required recognition of the MHC products expressed by B cells (Table II, experiment 1). These findings

TABLE II

Cloned T Helper Cells Can Function through Pathways that Are Either Restricted or Unrestricted for T Cell Recognition of B Cells

TNP-KLH	Cloned T cells*	F_1 accessory cells‡	Anti-TNP PFC/culture (B + accessory) cells§			
			Experiment 1		Experiment 2	
			B10	B10.BR	CBA/CaHN	CBA/N
0.001 μg/ml‖	Clone 14	−	0	940 ± 50	3,048 ± 134	1,603 ± 120
	Clone 14	+	38 ± 19	662 ± 201	2,991 ± 458	1,267 ± 33
	Clone 16	−	2,832 ± 398	19 ± 19	0	24 ± 20
	Clone 16	+	2,438 ± 166	38 ± 25	120 ± 93	91 ± 50
20 μg/ml¶	Clone 14	−	29 ± 23	413 ± 74	1,553 ± 241	15 ± 5
	Clone 14	+	480 ± 78	336 ± 50	1,620 ± 119	ND**
	Clone 16	−	595 ± 69	0	252 ± 62	0
	Clone 16	+	451 ± 62	365 ± 47	1,356 ± 324	0

* 1×10^4 cells/culture. Clone 14 is specific for KLH plus *H-2^k* and clone 16 is specific for KLH plus *H-2^b*

‡ 1×10^6 RAMB + C treated, 2,000-rad irradiated, unprimed (B10 × B10.BR)F_1 spleen cells/culture.

§ 3×10^6 RAMB + C treated TNP-primed spleen cells/culture.

‖ Responses are presented as mean indirect (IgG) PFC/culture.

¶ Responses are presented as mean direct (IgM) PFC/culture.

** Not done.

indicate that, under different conditions of antigenic stimulation, the same cloned T_H cells function through two distinct B cell activation pathways, one of which requires MHC-restricted T_H recognition of B cells and one of which does not. To identify the B cell subpopulations activated by T_H cells in these T_H-B-restricted or -unrestricted pathways, TNP-primed CBA/CaHN (Lyb-5^+ and Lyb-5^- B cells) and CBA/N (Lyb-5^- B cells only) (B + accessory) cells were similarly cultured with cloned T_H cells and assayed for responses to either 20 μg/ml or 0.001 μg/ml TNP-KLH. At high antigen concentrations, CBA/CaHN but not CBA/N B cells were responsive to the help provided by Clone 14 T cells (Table II, experiment 2), confirming the requirement for Lyb-5^+ B cells under these conditions. The addition of (B10 × B10.BR)F_1 accessory cells to these responding populations also permitted responses by CBA/CaHN B cells in cooperation with *H-2*^{b}-restricted clone 16 T_H cells. In contrast, for responses to 0.001 μg/ml TNP-KLH, CBA/CaHN (Lyb-5^+ + Lyb-5^-) and CBA/N (Lyb-5^-) B cells were both responsive in the presence of clone 14 but not clone 16 T_H cells, and this restriction in the activation of Lyb-5^- B cells was not overcome by addition of F_1 accessory cells.

Further experiments were carried out to determine whether T_H cell recognition of accessory cells as well as B cells is required for the responses to low concentrations of TNP-KLH. TNP-primed B6C3F_1 (B + accessory) cells were depleted of accessory cells by passage through G-10 Sephadex columns (14). The resulting B cells were then co-cultured with cloned T_H cells in the presence of either B10, B10.BR, or a mixture of B10 and B10.BR accessory cells and stimulated with 0.001 μg/ml TNP-KLH (Table III). When B10.BR accessory cells were present in culture, clone 14 but not clone 16 T cells were efficient helpers of responses by B6C3F_1 B cells. Moreover, the coexistence of inappropriate haplotype accessory cells did not interfere with T_H activity (Table III). Clone 16 T cells showed a reciprocal *H-2* restriction pattern in their recognition of B10 but not B10.BR accessory cells (Table III).

These results demonstrate that the same monoclonal T_H cell population can activate B cells by at least two distinct mechanisms. These cloned T_H cells activate Lyb-5^- B cells by a pathway requiring *H-2*-restricted T cell recognition of both B cells and accessory cells, whereas the same cloned T_H cells activate Lyb-5^+ B cells by a distinct

TABLE III

Cloned T Helper Cells are H-2 Restricted in Their Recognition of Accessory Cells

Cloned T helper cells	Anti-TNP IgG PFC/culture*		
	Accessory cells‡		
	B10	B10.BR	B10 + B10.BR
Clone 14	67 ± 33	1,065 ± 59	969 ± 182
Clone 16	417 ± 43	43 ± 21	475 ± 21

* 1×10^4 cloned T helper cells were co-cultured with 3×10^6 TNP-primed, RAMB + C-treated, G-10 Sephadex column-passed B6C3F_1 spleen cells in the presence of 0.001 μg/ml TNP-KLH. Clone 14 is specific for KLH plus *H-2*^{k} and clone 16 is specific for KLH plus *H-2*^{b}.

‡ 1×10^6 unprimed, RAMB + C-treated, 2,000-rad irradiated spleen cells were added to cultures as accessory cells.

pathway requiring *H-2*-restricted T cell recognition of only accessory cells but not B cells.

Monoclonal T_H Cell Activation of Lyb-5$^-$ B Cells via Genetically Restricted T_H-B Cell Interactions Requires Linked Carrier-Hapten Recognition, whereas the Activation of Lyb-5$^+$ B Cells via Nonrestricted T_H-B Cell Interactions Does Not. To determine whether the two distinct B cell activation pathways described above differ in their requirements for covalent carrier-hapten linkage, the following experiments were carried out. TNP-primed CBA/CaHN and CBA/N (B + accessory) cells were co-cultured with clone 14 (*H-2^k*-restricted) T_H cells (Table IV). Clone 14 T_H cells supported the responses of CBA/CaHN (Lyb-5$^+$ + Lyb-5$^-$) and CBA/N (Lyb-5$^-$) (B + accessory) cells to 0.001 μg/ml TNP-KLH (Table IV, group A), but no significant responses were stimulated by 0.001 μg/ml KLH or TNP-FGG (itself immunogenic in the presence of FGG-specific T_H cells [data not shown]). In addition, responses were not induced by a mixture of 0.001 μg/ml KLH and 0.001 μg/ml TNP-FGG (Table IV, groups B, C, and D), demonstrating a requirement for covalent carrier-hapten linkage in the activation of Lyb-5$^-$ B cells under these conditions. For responses to high dose (20 μg/ml) TNP-KLH, clone 14 T_H cells cooperated with CBA/CaHN but not CBA/N (B + accessory) cells (group E), confirming a requirement for Lyb-5$^+$ B cells in these responses. Although the responses were not stimulated either by 20 μg/ml KLH alone (group F) or by 20 μg/ml TNP-FGG alone (group G), a mixture of high dose KLH and high dose TNP-FGG generated responses in CBA/CaHN (B + accessory) cells

TABLE IV
Activation of Lyb-5$^+$ B Cells Does Not Require Linked Carrier-Hapten Recognition

Groups	Antigens (μg/ml)		Anti-TNP PFC/culture* (B + accessory) cells‡ CBA/CaHN (Lyb-5$^+$ + Lyb-5$^-$)	Anti-TNP PFC/culture* (B + accessory) cells‡ CBA/N (Lyb-5$^-$)
A	TNP-KLH	(0.001)	768 ± 126	566 ± 41
B	KLH	(0.001)	28 ± 21	9 ± 4
C	TNP-FGG	(0.001)	0	0
D	TNP-FGG	(0.001)	57 ± 16	0
	+ KLH	(0.001)		
E	TNP-KLH	(20)	508 ± 25	14 ± 8
F	KLH	(20)	67 ± 34	0
G	TNP-FGG	(20)	0	0
H	TNP-FGG	(20)	456 ± 81	43 ± 29
	+ KLH	(20)		
I	TNP-FGG	(20)	0	0
	+ KLH	(0.001)		
J	TNP-FGG	(0.001)	854 ± 9	33 ± 20
	+ KLH	(20)		

* Responses in groups A–D represent IgG PFC/culture. Responses in groups E–J represent IgM PFC/culture.

‡ 2×10^6 RAMB + C-treated TNP-primed spleen cells were co-cultured with 1×10^3 clone 14 T_H cells. Clone 14 is specific for KLH plus *H-2^k*.

(group H). Responses under these conditions, requiring the presence of Lyb-5$^+$ B cells, thus demonstrated no requirement for carrier-hapten linkage. The fact that Lyb-5$^+$ (CBA/CaHN) but not Lyb-5$^-$ (CBA/N) B cells are activated by high concentrations of TNP-KLH or by high concentrations of TNP-FGG plus KLH could result from differences in the ability of activated T_H cells to trigger each of these B cell subpopulations. Alternatively, these findings could result from the ability of high concentrations of specific hapten (TNP) to differentially inhibit the response of Lyb-5$^-$ B cells. To further evaluate these possibilities, responses were assessed to a mixture of 0.001 μg/ml TNP-FGG plus 20 μg/ml KLH (Table IV, group J). Under these conditions, where the concentration of hapten was low and the concentration of carrier was high, CBA/CaHN and not CBA/N B cells were activated. These findings suggest that the selective activation of Lyb-5$^+$ B cells resulted from differences in the activation state of T_H cells exposed to either high or low concentrations of carrier, and not from the direct effects of hapten dose on different B cell subpopulations.

The results presented above demonstrated that the TD activation of Lyb-5$^+$ B cells can occur either in the absence of MHC-restricted T_H cell-B cell interaction or in the absence of covalent carrier-hapten linkage. Additional studies were designed to further determine whether Lyb-5$^+$ B cells are activated by a pathway which is simultaneously both T_H-B unrestricted and carrier-hapten unlinked. It was again demonstrated that *H-2*^{b}-restricted and KLH-specific clone 16 T cells cooperated with CBA/CaHN (*H-2*^{k}) (B + accessory) cells only in the presence of B6C3F$_1$ accessory cells for responses to 20 μg/ml TNP-KLH (Table V). In the same experiment, Lyb-5$^-$ CBA/N (B + accessory) cells were unresponsive under these conditions (data not shown). Under these conditions of T_H-B-unrestricted response, neither 20 μg/ml free KLH nor 0.001 μg/ml TNP-FGG induced significant responses, whereas a mixture of KLH and TNP-FGG induced a response equal in magnitude to that generated by covalently linked TNP-KLH. Thus, the same cloned T_H cells that activate Lyb-5$^-$ B cells by a pathway that requires both MHC-restricted T_H-B cell interaction and carrier-hapten linkage can activate populations containing Lyb-5$^+$ B cells through a strikingly different

TABLE V

Responses Stimulated via Nonrestricted T_H-B Cell Interactions Do Not Require Linked Carrier-Hapten Recognition

Antigens (μg/ml)		F$_1$ accessory cells*	Anti-TNP IgM PFC/culture
TNP-KLH	(0.001)	−	28 ± 16
TNP-KLH	(0.001)	+	0
TNP-KLH	(20)	−	0
TNP-KLH	(20)	+	384 ± 25
KLH	(20)	+	38 ± 9
TNP-FGG	(0.001)	+	0
TNP-FGG + KLH	(0.001) (20)	+	403 ± 66

* 2 × 10^6 RAMB + C-treated TNP-primed CBA/CaHN (*H-2*^{k}) spleen cells were co-cultured with 3 × 10^3 clone 16 T_H cells in the presence or absence of 1 × 10^6 B6C3F$_1$ (*H-2*^{b} × *H-2*^{k}) accessory cells. Clone 16 cells are specific for KLH plus *H-2*^{b}.

pathway that requires neither restricted T_H-B cell interaction nor carrier-hapten linkage.

Discussion

It has recently been demonstrated (1, 2) that alternate pathways exist for B cell activation in TD antibody responses. It was shown that Lyb-5^- B cells can be activated by heterogeneous T_H cell through an activation pathway requiring *H-2*-restricted interaction between T_H cells and B cells. In contrast, a second pathway exists that requires the participation of Lyb-5^+ B cells, and for which T_H-B cell interaction is unrestricted. The results presented here demonstrate that the same monoclonal T_H cells can function through both activation pathways to trigger in vitro B cell responses to the soluble antigen TNP-KLH. At low concentrations of TNP-KLH, cloned T_H cells cooperated with TNP-primed (B + accessory) cell populations to generate predominantly IgG responses, responses that did not require the presence of Lyb-5^+ B cells. For these IgG responses, T_H cells were *H-2* restricted in their cooperation with both Lyb-5^- B cells and accessory cells, and they additionally required covalently linked carrier-hapten presentation. At high concentration of TNP-KLH, the same cloned T cells also cooperated with (B + accessory) cells to generate predominantly IgM responses, which did require the participation of Lyb-5^+ B cells. In these responses, T_H cells were *H-2* restricted only in their interaction with accessory cells, and demonstrated no requirement for recognition of B cell *H-2* products. In addition, no requirement for carrier-hapten linkage was observed, suggesting that once T_H cells were activated by high concentration of specific antigen, the helper activity generated was antigen nonspecific. For this pathway of response, T_H cells are thus antigen specific and *H-2* restricted only at the level of T cell activation. After activation, T_H cells or their soluble products provide nonspecific helper activity to populations containing Lyb-5^+ B cells, with no apparent requirement for direct T_H-B cell interaction in this pathway of B cell triggering.

The present results are consistent with previous reports (6–9, 14) establishing the ability of monoclonal T cell populations to provide efficient help for both IgM and IgG antibody responses in vitro. Although these results do not exclude a physiologic role for interactions among different T cell subpopulations in the generation of helper activity, they do argue that a single T cell effector population is sufficient to provide such activity. In addition, the results presented here demonstrate that a single monoclonal T_H cell population can function either through a pathway that requires both MHC-restricted T_H-B cell interaction and covalent carrier-hapten linkage, or through a pathway requiring neither of these elements. These findings suggest that a single T_H cell can exert helper activity through at least two distinct mechanisms. The ability of T_H cells to provide help that requires neither restricted T_H-B interaction nor carrier-hapten linkage suggests a mechanism that requires antigen specificity and MHC restriction only at the level of T_H cell activation, with the subsequent generation of antigen-nonspecific and MHC-unrestricted helper activity. In the absence of evidence for direct T_H-B cell interaction in this pathway, these findings are consistent with the existence of nonspecific T_H cell factors that are released after the MHC-restricted stimulation of T_H cells by high concentrations of specific antigen, and that mediate the helper activity observed under these conditions. The existence of such nonspecific helper factors generated by the stimulation of cloned T_H cells has in fact

been demonstrated previously (14). In contrast to this pathway of T cell help, requirements for restricted T_H-B interaction and carrier-hapten linkage suggest the existence of a mechanism that involves direct cell contact between carrier-specific T_H cells and hapten-specific B cells, a mechanism originally formulated by Mitchison (15). To observe such a specific pathway of B cell activation under appropriate experimental conditions, the form of nonspecific help described above must not exist under these conditions. Thus, two distinct activation states may exist for the same monoclonal T_H cell population. Low concentrations of carrier-hapten conjugate activate these T_H cells to a state at which they are able to provide MHC-restricted help for activation but do not produce functionally significant quantities of nonspecific helper factors. In contrast, these same cloned T_H cells may be activated by higher concentrations of specific carrier to produce helper factors that are both antigen nonspecific and genetically unrestricted.

The results of these studies using monoclonal T_H cell populations also confirm the existence of differences in the activation requirements of different B cell subpopulations. Thus, even when help is provided by the same T_H cell population, the signals which function to activate Lyb-5^- and Lyb-5^+ B cells differ. From the present studies alone, this conclusion cannot be formally differentiated from the alternative interpretation that activation requirements differ, not between Lyb-5^- and Lyb-5^+ B cells per se, but rather between IgG- and IgM-producing B cells. The strongest evidence against this latter interpretation was provided by previously reported (1) in vivo adoptive transfer experiments, in which it was shown that Lyb-5^- and Lyb-5^+ B cells differ in their activation requirements even when all B cell populations were unprimed and generated exclusively IgM PFC responses (1). In the present studies, as well as in previous reports (1, 2), it was shown that the Lyb-5^- B cells present in the defective mutant CBA/N can be activated by MHC-restricted T_H-B cell interaction in the presence of linked carrier and hapten. These same Lyb-5^- B cells were not activated in the absence of restricted T-B interaction or carrier-hapten linkage. Consistent with this inability to trigger Lyb-5^- B cells by an unrestricted pathway, it has previously been observed (1, 14) that soluble T cell products isolated from either specifically or nonspecifically stimulated T cells trigger only populations containing Lyb-5^+ B cells. The possibility that Lyb-5^+ B cells can also be activated through MHC-restricted T_H-B interaction cannot be clearly evaluated, because purified populations of Lyb-5^+ B cells have not yet been isolated. It has been demonstrated, however, that nonspecific help mediated by T cells or their soluble products activates only those B cell populations containing Lyb-5^+ B cells (1, 14). The simplest interpretation of these findings is that the Lyb-5^+ and Lyb-5^- subsets differ in their ability to be activated by the antigen-nonspecific and MHC-unrestricted products of activated T_H cells, such that only Lyb-5^+ B cells are activated by such factors.

The findings presented here demonstrate that monoclonal T_H cells are not only sufficient to provide help for B cell activation, but that the same monoclonal T_H cells are competent to function through two distinct pathways of B cell activation. These pathways appear to be mediated by different activation states of the same T_H cells and function to trigger responses by distinct B cell subpopulations. Such findings emphasize the necessity that studies of immune cell interactions consider both the identities and the activation states of the T cell, B cell, and accessory cell subpopulations participating in these responses.

Summary

It has recently been demonstrated that the Lyb-5$^+$ and Lyb-5$^-$ B cell subpopulations differ in their requirements for major histocompatibility complex (MHC)-restricted activation by T helper (T_H) cells. To determine whether these MHC-restricted and -unrestricted pathways of B cell activation result from differences in the participating T_H cell populations or reflect differences exclusively in the responding B cell subpopulations, experiments were carried out using cloned T_H cells for in vitro antibody responses to trinitrophenyl-keyhole limpet hemocyanin. The same cloned T helper cells were able to activate both CBA/N (Lyb-5$^-$) B cells and CBA/CaHN (Lyb-5$^+$ + Lyb-5$^-$) B cells under different experimental conditions. The activation of Lyb-5$^-$ B cells by cloned T helper cells required both MHC-restricted T_H cell-B cell interaction and carrier-hapten linkage. In contrast, the activation of Lyb-5$^+$ B cells required only MHC-restricted T helper cell interaction with accessory cells, while T-B interaction was MHC unrestricted and did not require carrier-hapten linkage. Thus, the differences in activation requirements observed for the Lyb-5$^-$ and Lyb-5$^+$ B cell subsets do not result from differences in the T_H cell populations activating these B cells, but rather reflect differences in the ability of these B cells to respond to signals from the same T_H cells.

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