

HETEROGENEITY OF HELPER/INDUCER T LYMPHOCYTES

II. Effects of Interleukin 4- and Interleukin 2-producing T Cell Clones on Resting B Lymphocytes

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Murine class II MHC-restricted helper/inducer T lymphocytes can be divided into at least two nonoverlapping subsets, based on patterns of lymphokine gene transcription and secretion. One subset, provisionally called Th1, secretes IL-2 and IFN- γ upon stimulation by antigen or lectin and uses IL-2 as its autocrine growth factor. The other subset, called Th2, produced IL-4 and IL-5 and used IL-4 as its growth factor (1-5). Cloned lines representing Th1 and Th2 cells also vary in their proliferative responses to exogenous cytokines (6). It has been postulated that these subsets perform fundamentally different functions, Th1 cells being primarily involved in cell-mediated immune responses, such as delayed hypersensitivity, and Th2 cells in humoral immunity (7, 8).

Studies aimed at analyzing the requirements for helper T cell-dependent antibody responses have produced contradictory results. Thus, both murine and human B cells proliferate and differentiate in response to IL-2 and IFN- γ (9-11), and Th1 clones have been reported to induce antibody responses as a result of cognate interactions with B cells (12). In contrast, more recent experiments have emphasized the helper function of Th2 cells (13-15), and have even suggested that only IL-4-producing T cells are capable of helping resting B lymphocytes (4). A fundamental problem with such experiments is that they do not include formal comparisons of Th1 and Th2 cells of the same antigen specificity. Moreover, these analyses do not take into account recent data indicating that different lymphokines may be uniquely responsible for the production of different antibody isotypes, e.g., IL-4 for IgG1 and IgE (16, 17), IL-5 for IgA (18), and IFN- γ for IgG2a (17).

In the present experiments, we have compared the ability of murine Th1 and Th2 clones to induce the proliferation and differentiation of resting B cells. The system we have chosen involves isolation of B cells without an affinity purification step in order to avoid possible activation. We have used, as a model antigen, rabbit anti-mouse Ig and examined the ability of rabbit Ig-specific, Ia-restricted T cell clones to help B cells (13, 14). Such an experimental model resembles, to the extent possible, the physiologic *in vivo* situation of cognate help mediated

This work was supported by grants AI-22802 and AI-25022, and by a National Research Scientist Award to W. H. Boom from the National Institutes of Health, U.S. Public Health Service.

1350 J. EXP. MED. © The Rockefeller University Press · 0022-1007/88/04/1350/14 \$2.00

Volume 167 April 1988 1350-1363

by Ia-restricted T cells at low antigen concentrations. Moreover, we have assayed in parallel cultures the proliferation of B cells and the secretion of IgM, IgG1, and IgG2a in order to determine the relative contributions of Th1 and Th2 cells to different aspects of B cell activation. Our results establish striking differences in the helper activity of these two T cell subsets, demonstrate an obligatory role of IL-4 and IL-5 in the proliferation and differentiation of resting B cells, and show that IFN γ is both an inhibitor of B cell activation and the major or only inducer of a switch to IgG2a secretion.

Materials and Methods

Antigens, Cytokines, and Antibodies. F(ab')₂ fragments of rabbit anti-mouse Ig (RAMG)¹ were purchased from Cappel Laboratories, Malvern, PA. F(ab')₂ fragments of rabbit Ig (RGG) were made by pepsin digestion and purified by chromatography on protein A-Sepharose. These two reagents were used as the antigens in all experiments with resting B cells. Rabbit Ig was purchased from Sigma Chemical Co., St. Louis, MO, and used as antigen for weekly passage of T cell clones. *Escherichia coli* LPS was purchased from Difco Laboratories, Detroit, MI.

Murine rIL-4 (expressed in yeast) was generously provided by Dr. A. Gillis, Immunex Corp., Seattle, WA. The activity of this preparation was determined at Immunex Corp. and expressed in units as defined (19). Murine and human rIL-2 were purchased from Genzyme Corp., Boston, MA, and used according to units as defined by the supplier. Murine rIFN- γ was generously given by Genentech, Inc., South San Francisco, CA. Concentrations are expressed as units defined by the supplier.

The neutralizing mAb for murine IL-4, 11B11, has been described (20) and was used as a hybridoma culture supernatant at final dilutions of 1:10 to 1:50. Anti-IL-2 antibody, S4B6, was a gift of Dr. T. Mosmann, DNAX, Palo Alto, CA, and was used at a 1:20 dilution of an (NH₄)₂SO₄ precipitated 20 \times concentrate of hybridoma supernatant. Anti-IL-5 antibody was a generous gift from Dr. K. Takatsu, Kumamoto University Medical School, Kumamoto, Japan, and was used as an ascites fluid at a dilution of 1:1,000 to 1:10,000 (21). The hamster anti-murine IFN- γ mAb, H22.10.30.22, was the generous gift of Dr. R. Schreiber, Washington University School of Medicine, St. Louis, MO (22). The affinity-purified antibody was used at concentrations of 0.1 to 1.0 μ g/ml. Additional mAbs used for T cell depletion in the preparation of small resting B cells were anti-Thy-1.2 and anti-Lyt-1.2 (New England Nuclear, Boston, MA) and anti-Lyt-2, kindly provided by Dr. F. Fitch, University of Chicago, Chicago, IL.

T Cell Clones and Lymphokine Assays. The following four RGG-specific T cell clones were used in studies described in Results. CDC25 and CDC35 were obtained from Dr. D. Parker, University of Massachusetts Medical School, Worcester, MA, and belong to the Th2 subset of helper/inducer T cells (IL-4 producers) (6, 13). D1.1 and D1.6 were generated in our own laboratory and belong to the Th1 subset of helper/inducer T cells (IFN- γ , IL-2 producers) (6). CDC35, D1.1, and D1.6 are I-A^d restricted, and CDC25 is I-A^{(k \times d)^{F1} restricted. Clones were maintained by weekly stimulation with irradiated spleen cells and antigen, as described (6). Spleen cells were isolated from BALB/c (for CDC35, D1.1., D1.6) and (BALB/c \times A/J) F₁ (CAF1) mice (for CDC25). 8–12-wk-old mice were purchased from The Jackson Laboratory, Bar Harbor, ME, and maintained in accordance with guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. [NIH] FS-23).}

For all experiments with resting B lymphocytes, viable T cells were purified by centrifugation over Ficoll-Isopaque 8–10 d after stimulation with antigen, and treated with mitomycin C (Sigma Chemical Co.) (50 μ g/ml final concentration) for 45 min at 37°C.

¹ Abbreviations used in the paper: RAMG, rabbit anti-mouse Ig; RGG, F(ab')₂ fragments of rabbit Ig; SN, supernatant.

To assure that mitomycin C treatment had inhibited proliferation, T cells were cultured with 100 U of IL-2 in each experiment and shown to not incorporate [³H]thymidine. For measuring IL-4 and IL-2 secretion, supernatants (SN) were collected on day 1 of culture and assayed at 50% vol/vol for stimulation of HT-2 cells in the presence and absence of anti-IL-4 (1:40 dilution) or anti-IL-2 (1:20) antibodies, and previously described (2). For assays of IFN- γ secretion, day 1 SN were assayed at 25% vol/vol for growth inhibition of 2×10^4 WEHI-279 cells, in the absence and presence of anti-IFN- γ antibody, as described (23). For all assays, standard curves were generated using recombinant lymphokines present in test SN.

Purification and Culture of B Cells. Small resting B cells were purified from the spleens of unprimed BALB/c or CAF1 mice, according to methods described by Kreiger et al. (24). Spleen cells were treated with anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2 for 45 min at 4°C, followed by a 30-min incubation at 37°C in low tox rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario). The remaining cells were washed and placed on a discontinuous Percoll gradient composed of 2.5 ml each of 50, 60, 65, and 72% Percoll (Pharmacia, Uppsala, Sweden). Approximately 10^8 cells were placed on the gradient and centrifuged (2,000 g) at 4°C for 12 min. Cells recovered at the 65/72% interface were considered to be small resting B cells, corresponding to a density of 1.081–1.089 g/ml. These cells did not respond to the anti-IgM mAb, Bet2 (1:4 dilution of hybridoma supernatant), of IL-4 alone, but did proliferate when Bet2 and IL-4 were present in a co-stimulation assay (25). For the experiments described under results, 10^5 B cells were cultured with antigen, mitomycin C-treated T cells, LPS, or other stimuli in 0.2 ml of RPMI 1640 supplemented with 2 mM L-glutamine, penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 10 mM Hepes, 5×10^{-5} M 2-ME and 10% heat-inactivated FCS in flat-bottomed microculture plates at 37°C in 5% CO₂. In addition, all experiments included the following controls: B cells alone, and B cells with IL-2 or Con A to measure residual T cell contamination. No lymphokine secretion or proliferation was detected in these groups (not shown).

Assays for B Cell Activation. For all experiments with resting B cells, two parallel sets of cultures were established. The first was used to measure B cell proliferation and lymphokine secretion. Supernatants (50 μ l) were harvested after 24 h in all experiments for lymphokine assays, as described above, to demonstrate that the mitomycin C-treated T cells had been activated. Cultures were pulsed for 6 h with 1 μ Ci [³H]thymidine per well on day 3, harvested in a PHD cell harvester, and incorporated radioactivity was measured by scintillation counting. The second set of cultures was used to measure IgM, IgG1, and IgG2a and in the SN, by an ELISA. In brief, flexible microtiter plates were coated overnight at 4°C with polyvalent goat anti-mouse Ig (5 μ g/ml) (Southern Biotechnology Associates, Inc., Birmingham, AL), washed and blocked with 1% BSA (Sigma Chemical Co.) in PBS (pH 7.2). Culture supernatants were added to microtiter wells for 4 h at room temperature and washed. The presence of IgM, IgG1, and IgG2a was detected after incubation with alkaline phosphatase-labeled affinity-purified goat anti-mouse antibodies specific for each isotype (Southern Biotechnology Associates, Inc.), the addition of substrate (*p*-nitrophenyl phosphate-disodium; (Sigma Chemical Co.), and measurement of the optical density at 405 nm. 4-day SN were used for IgM and IgG1 measurement, at a 1:4 dilution, and 7-d SN were used for IgG2a measurement at a 1:2 dilution. Standard curves were obtained for each experiment with purified myeloma proteins of known isotypes (ICN, Lisle, IL), which were run in parallel. Results are expressed as the mean nanograms/milliliter of antibody present in SN of duplicate cultures. There was no cross-reaction of the alkaline phosphatase-labeled affinity-purified goat antibodies with the inappropriate myeloma proteins in the 10,000 to 0.1 ng/ml range. Positive control cultures for IgM, IgG, and IgG2a secretion were B cells incubated with LPS, LPS + IL-4 (100 U/ml), and LPS + IFN- γ (10 U/ml), respectively (17).

Results

Presentation of RAMG by Small Resting B Cells to RGG-specific Th1 and Th2 Clones. Initial studies were aimed at establishing the feasibility of our experi-

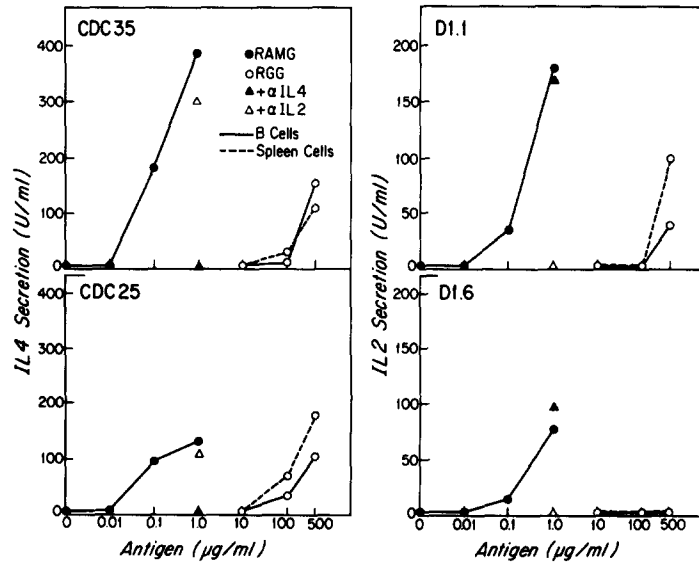


FIGURE 1. Presentation of antigen by B cells to Th1 and Th2 clones. B cells (10^5) (—) or 2,500 rad irradiated spleen cells (10^6) (---) were cultured in duplicate with mitomycin C-treated T cells (10^4) and $F(ab')_2$ RAMG (●) or $F(ab')_2$ RGG (○). BALB/c B cells were used for CDC35, D1.1, and D1.6, and CAF1 B cells for CDC25. SN were removed at 24 h and assayed for IL-2 or IL-4 by stimulation of DNA synthesis in the HT2 indicator line. Data are expressed as units/milliliter, using human r-IL-2 or murine rIL-4 as standards. In some groups, SN were also tested in the presence of anti-IL2 (Δ) or anti-IL-4 antibody (\blacktriangle) to confirm the nature of the HT2-stimulating lymphokine.

mental system for analyzing cognate T-B interactions, by determining the ability of small resting B cells to present the model antigen, $F(ab')_2$ RAMG, to RGG-specific T cells. As shown in Fig. 1, both Th1 (D1.1 and D1.6) and Th2 (CDC25 and CDC35) cells secreted lymphokine when cultured with purified B cells and $F(ab')_2$ RAMG at concentrations of 0.1–1 $\mu\text{g}/\text{ml}$. Antibody blocking confirmed that the lymphokine measured in these assays was IL-2 or IL-4, respectively. Moreover, both purified B cells and irradiated splenocytes presented $F(ab')_2$ RGG at 10^3 – 10^4 -fold higher concentrations than $F(ab')_2$ RAMG, again confirming the efficiency of Ig-mediated antigen presentation (26, 27). Finally, only the Th1 clones secreted IFN- γ in response to antigen presented by B cells, and the antigen dose-response was identical to that seen for IL-2 secretion (Fig. 2).

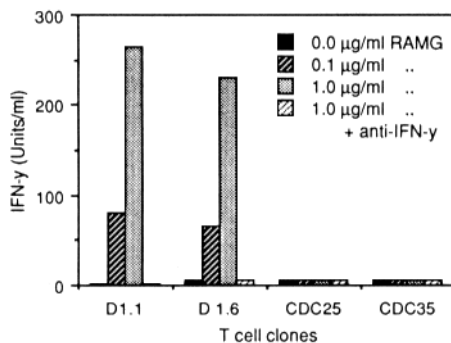


FIGURE 2. IFN- γ production by Th1 and Th2 cells. Cell clones and B cells were cultured with and without $F(ab')_2$ RAMG as in Fig. 1. SN collected at 24 h were assayed for IFN- γ by inhibition of proliferation of WEHI279 cells; in some groups, anti-IFN- γ antibody was also added. Data are expressed as units/milliliter using murine rIFN- γ as the standard.

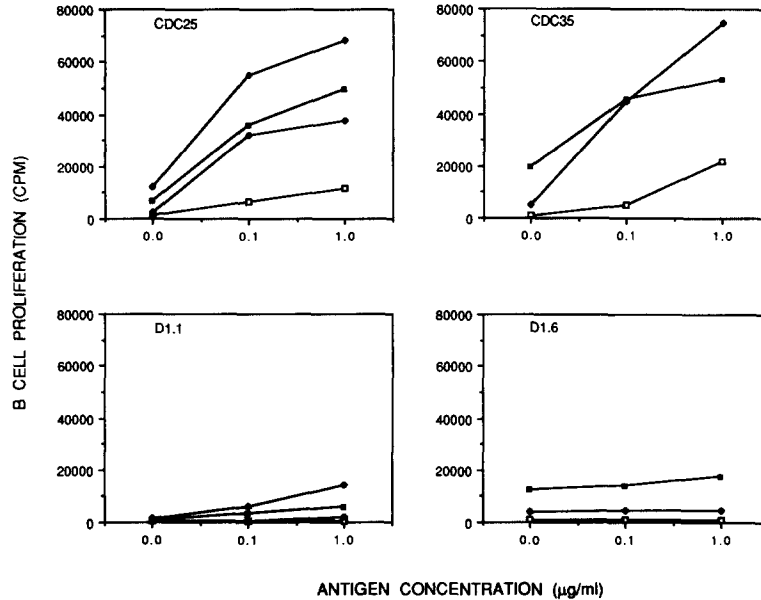


FIGURE 3. B lymphocyte proliferation induced by T cell clones: T cell dose-response. Purified B cells (10^5) were cultured with and without $F(ab')_2$ RAMG and mitomycin C-treated T cell clones, 10^3 (□), 5×10^3 (◆), 20×10^3 (■), 50×10^3 (◇, for CDC25) or 100×10^3 (◇, for D1.1). Proliferation was measured on day 3 by [3 H]thymidine incorporation.

These experiments showed that both subsets of T cells gave equivalent responses to antigen presented by purified small resting B lymphocytes. We could, therefore, use this system for studying the activation of B cells as a consequence of cognate interactions with Th1 and Th2 clones.

Induction of B Cell Proliferation and Differentiation by Th1 and Th2 Clones. To first determine the optimal cell numbers for measuring helper function, we examined the ability of mitomycin C-treated T cell clones to activate B cells to proliferate in the presence of antigen. Increasing numbers (10^3 – 10^5) of T cells were added to 10^5 B cells with 0–1 µg/ml of $F(ab')_2$ RAMG, and proliferation was assayed on day 3. As few as 10^3 Th2 cells (CDC25 and CDC35) induced significant, antigen-dependent B cell proliferation, and 5×10^3 T cells gave near-maximal responses. In contrast, the Th1 clones induced little or no B cell proliferation at any cell number (Fig. 3), even though lymphokine secretion was readily detectable. Moreover, the mitomycin-treated T cells did not proliferate in response to Con A or IL-2, and no DNA synthesis was detectable if the B cells were also treated with mitomycin C (not shown).

Based on these results, in subsequent experiments we used 5×10^3 T cells and 10^5 B cells for studying antigen-specific help. Parallel cultures were assayed for B cell proliferation and secretion of IgM, IgG1, and IgG2a. Only the Th2 clones, CDC25 and CDC35, induced both proliferation and secretion of IgM and IgG1 (Fig. 4). The Th1 clones stimulated neither B cell growth nor antibody secretion. Moreover, neither subset of T cells was capable of eliciting an IgG2a response, although B cells cultured with LPS and 10 U/ml of IFN- γ secreted 182 and 310 ng/ml of IgG2a in two experiments.

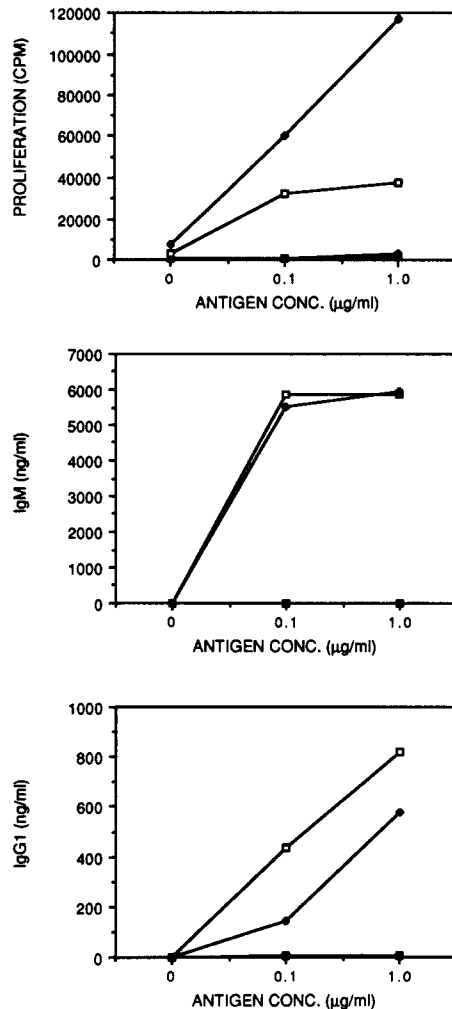


FIGURE 4. Induction of B lymphocyte proliferation and Ig secretion by T cell clones. B cells (10^5) were cultured in triplicate with or without $F(ab')_2$ RAMG and 5×10^3 mitomycin C-treated T cells. Proliferation was measured on day 3 by [3 H]thymidine incorporation. SN were collected from parallel cultures on day 4 and assayed for IgM and IgG1 by ELISA (25% vol/vol). All four T cell clones secreted lymphokine (IL-2 or IL-4), and no IgG2a secretion was detected up to 7 d (not shown). (■) D1.1, (◇) D1.6, (□) CDC25, (◆) CDC35.

Role of Lymphokines in Th2-mediated B Cell Help. The relative contributions of different lymphokines to Th2-mediated B cell proliferation and antibody secretion were evaluated using neutralizing mAbs. As shown in Table I, anti-IL-4 significantly inhibited proliferation and IgG1 production, especially in the presence of 0.1 µg/ml of $F(ab')_2$ RAMG, when the levels of lymphokines secreted are lower than at higher antigen concentrations. This is consistent with the view that IL-4 is primarily involved in proliferation of and IgG1 secretion by B cells (16). It is noteworthy that proliferation is only partly inhibited by anti-IL-4, suggesting that other pathways may also be operative (e.g., reference 28). Anti-IL-4 also significantly inhibited IgM secretion, especially at RAMG concentrations of 0.1 µg/ml. Antibody to IL-5 had no effect on B cell proliferation, but inhibited both IgM and IgG1 secretion. Even more striking was the result obtained using a combination of the two antibodies, which completely abrogated secretion of both IgM and IgG1. These experiments demonstrate different but

TABLE I
Role of IL-4 and IL-5 in B Cell Activation by the Th2 Clone CDC35

Antigen/ mitogen	Amount	Lymphokine/antibody	B cell proliferation	Antibody secretion	
				IgM	IgG1
	<i>μg/ml</i>			<i>ng/ml</i>	
—		—	6,880	<20	<1
RAMG	0.1	—	53,453	824	400
	1.0	—	104,224	1,952	1,126
RAMG	0.1	Anti-IL-4 (1:10)	9,052	<20	<1
	1.0	Anti-IL-4	57,929	190	48
	0.1	Anti-IL-4 (1:50)	16,953	<20	<1
	1.0	Anti-IL-4	95,914	824	339
RAMG	0.1	Anti-IL-5 (1:1,000)	42,567	<20	<1
	1.0	Anti-IL-5	106,724	190	296
	0.1	Anti-IL-5 (1:5,000)	37,163	<20	<1
	1.0	Anti-IL-5	102,124	965	670
		Anti-IL-4 (1:50) + anti-IL-5 (1:5,000)	64,892	<20	<1
RAMG	0.1	IFN- γ (10 U/ml)	20,022	<20	<1
	1.0	IFN- γ (10 U/ml)	70,948	1,670	732
	0.1	IFN- γ (100 U/ml)	13,310	<20	<1
	1.0	IFN- γ (100 U/ml)	46,131	1,036	276
		—	849	<20	<1
LPS	10	—	111,151	6,178	<1
		IL-4 (100 u/ml)	140,228	3,220	876
LPS		Anti-IL-4 (1:50)	123,297	6,741	<1
LPS		Anti-IL-5 (1:5,000)	104,696	4,698	<1
LPS		Anti-IL-4 + anti-IL-5	115,112	4,769	<1

BALB/c B cells (10^5) were cultured in duplicate with mitomycin C-treated CDC35 (5×10^3) and F(ab')₂ RAMG. Certain cultures had anti-IL-4, anti-IL-5, or IFN- γ added to them. In addition, B cells were stimulated with LPS (10 μ g/ml) alone, or with either IL-4 (100 U/ml), anti-IL-4, or anti-IL-5 added. B cell proliferation was measured on day 3 by [³H]thymidine incorporation. Supernatants (SN) from parallel cultures were assayed on day 4 for IgM and IgG1 secretion, by ELISA. CDC35 T cells not added to cultures of B cells with LPS.

complimentary roles of IL-4 and IL-5 in B cell activation, and suggest that the helper function of Th2 cells can be attributed to these lymphokines. Moreover, LPS-induced B cell activation was not affected by anti-IL-4 or anti-IL-5 antibody, demonstrating that the antibodies do not inhibit B cell responses nonspecifically.

Finally, IFN- γ , which is known to block IL-4-dependent B cell activation (29, 30), also inhibited proliferation of and IgG1 secretion by B cells in response to antigen and Th2 cells (Table I). The extent of inhibition was comparable to that observed with anti-IL-4 antibody. There was, however no IgG2a response under these conditions. The addition of 10 U/ml of IFN- γ to LPS-stimulated B cells led to 272 ng/ml of IgG2a secretion in this experiment.

Inhibitory Effects of Th1 Cells and the Role of IFN. Numerous studies have shown that IFN- γ has an antiproliferative effect on activated B lymphocytes (23, 28, 29). To determine if such inhibition was also observed in cognate T-B interactions, BALB/c B cells were cultured with F(ab')₂ RAMG and mixtures of RGG

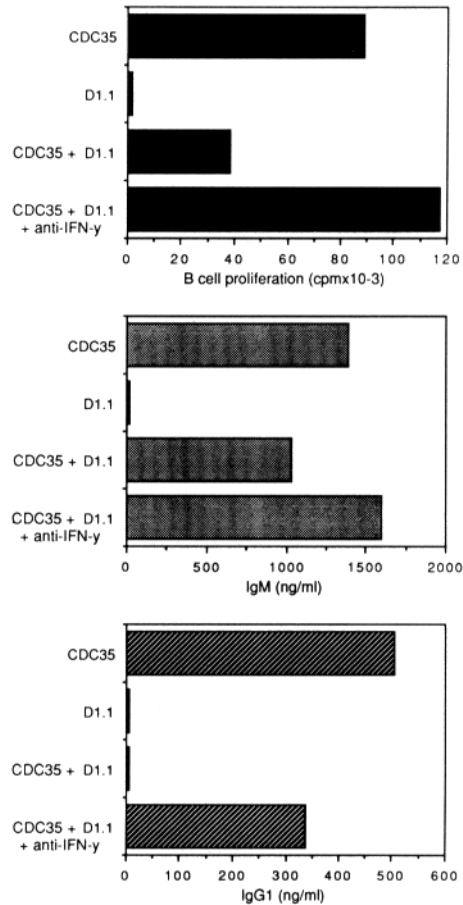


FIGURE 5. Inhibition of Th2-induced B cell activation by Th1 cells. BALB/c B cells (10^5) were cultured with F(ab')₂ RAMB ($1 \mu\text{g/ml}$) and 5×10^3 mitomycin C-treated T cells, as shown, without and with anti-IFN- γ antibody ($1 \mu\text{g/ml}$). Proliferation and Ig secretion were measured as in Fig. 4. CDC35 cells did not induce proliferation of Ig secretion in the absence of F(ab')₂ RAMG, and both clones secreted their appropriate lymphokines (IL-2 or IL-4) in the presence of RAMG (not shown).

+ I-A^d-specific Th1 and Th2 clones. As shown in Fig. 5, the Th2 clone CDC35 induced proliferation and Ig secretion. In contrast, the Th1 clone D1.1 failed to activate B cells. When both clones were added together, D1.1 markedly inhibited the two B cell responses that are most dependent on IL-4, i.e., proliferation and IgG1 secretion, and this inhibition was reversed by anti-IFN- γ antibody.

These results raised the possibility that the apparent inability of Th1 cells to help B lymphocytes may be due to a dominant inhibitory effect of IFN- γ . To test this possibility, a saturating concentration of anti-IFN- γ antibody was added to cultures of B cells, antigen, and Th1 clones. As shown in Table II, no proliferation or antibody secretion was detected. It is also noteworthy that when the B cells were stimulated by LPS, the addition of antigen and Th1 cells did lead to IgG2a secretion. This result is consistent with the view that IFN- γ inhibits early events in B cell activation that are induced by IL-4, but mediates a switch to IgG2a under conditions where the inhibitory effect is not dominant (e.g., in LPS-stimulated cultures).

Bystander Help by Th1 and Th2 Cells. Finally, we considered the possibility that Th1 and Th2 cells may differ in their ability to help B cells in cognate inter-

TABLE II
Inhibition of IFN- γ Does Not Restore Helper Function of D1.1 (TH-1) for Resting B Cells

T cells	Antigen/mitogen ($\mu\text{g/ml}$)	Lymphokine/ antibody ($\mu\text{g/ml}$)	B cell proliferation	Antibody secretion		
				IgM	IgG1	IgG2a
			<i>cpm</i>		<i>ng/ml</i>	
—	—	—	489	<20	<1	<0.1
—	LPS (10)	—	73,599	7,800	<1	<0.1
—	LPS	IL-4	105,593	3,872	640	<0.1
—	LPS	IFN- γ	67,265	6,560	<1	49
D1.1	—	—	1,471	<20	<1	<0.1
	RAMG (0.1)	—	2,294	<20	<1	<0.1
	(1.0)	—	4,278	<20	<1	<0.1
	(1.0)	Anti-IFN- γ (0.1)	3,356	<20	<1	<0.1
	(1.0)	(0.3)	2,858	<20	<1	<0.1
	(1.0)	(1.0)	3,301	<20	<1	<0.1
↓	RAMG (1.0) + LPS	—	64,679	1,391	<1	26

BALB/c B cells (10^5) were cultured in duplicate with mitomycin C-treated D1.1 (5×10^3) and F(ab')₂ RAMG. Certain cultures had either anti-IFN- γ (0.1, 0.3, or 1 $\mu\text{g/ml}$) or LPS (10 $\mu\text{g/ml}$) added to them. In addition, B cells were stimulated with LPS alone, or LPS with either IL-4 (100 U/ml) or IFN- γ (10 U/ml) added. B cell proliferation was measured on day 3. Supernatants from parallel cultures were assayed on day 4 for IgM and IgG1, and on day 7 for IgG2a secretion, by ELISA. All concentrations of anti-IFN- γ completely neutralized the IFN- γ secreted by D1.1 cells cultured with B cells and RAMG.

actions as opposed to bystander cells, which are stimulated only by secreted lymphokines. To test this, mixtures of A/J(H-2^a) and mitomycin C-treated BALB/c (H-2^d) small resting B cells were stimulated with F(ab')₂ RAMG and RGG + I-A^d-specific Th1 and Th2 clones. Only the Th2 clone, CDC35, induced proliferation of and antibody secretion by the bystander B cells (Table III). Controls showed that neither the A/J(I-A^k) nor the mitomycin C-treated BALB/c B cells cultured separately could be activated under these conditions.

Discussion

Despite a vast amount of work, the requirements for antigen-specific, helper T cell-dependent activation of B lymphocytes remain poorly defined. Thus, many cytokines, including IL-1, IL-2, IFN- γ , IL-4, and IL-5, have been shown to influence B cell growth and/or differentiation in vitro (9–11, 16–18), but the critical question of which are obligatory for antibody responses to low (physiologic) concentrations of protein antigens is not yet resolved. The recent identification of Ia-restricted helper T cell subsets that differ in lymphokine secretion profiles, and the development of neutralizing antibodies specific for lymphokines, provide two approaches for addressing this question. In the studies described in this paper, we have used both approaches to analyze the activation of resting B lymphocytes by Ia-restricted T cells. In particular, we have compared the helper functions of a panel of T cell clones with the same antigenic specificity but belonging to the Th1 and Th2 subsets. Several aspects of the experimental system used in these studies are noteworthy. First, we have used unselected small, resting B cells and anti-Ig as a model antigen, so that activation

TABLE III
Bystander Help by Th1 and Th2 Cells

T cells	B cells	F(ab') ₂ RAMG	B cell proliferation	Antibody secretion	
				IgM	IgG1
			<i>cpm</i>	<i>ng/ml</i>	
D1.1 (Th1)	BALB/c	—	1,148	<20	<1
		+	26,858	<20	<1
	A/J	+	926	<20	<1
	mitomycin C BALB/c	+	1,523	<20	<1
	A/J + mitomycin C BALB/c	+	4,275	<20	<1
CDC35 (Th2)	BALB/c	—	10,478	<20	<1
		+	123,986	3,599	270
	A/J	+	1,179	<20	<1
	mitomycin C BALB/c	+	7,080	<20	<1
	A/J + mitomycin C BALB/c	+	102,872	1,808	486

Mitomycin C-treated T cells (5×10^3) were cultured with either BALB/c, A/J, mitomycin C-treated BALB/c, or A/J + Mitomycin C-treated BALB/c B cells (10^5) and $1 \mu\text{g/ml}$ of F(ab')₂ RAMG. B cell proliferation and Ig secretion were measured as in Table I. D1.1. cells secreted $> 60 \text{ U/ml}$ IL-2 in response to RAMG and untreated or mitomycin C-treated BALB/c B cells.

of the maximum possible number of cells can be assayed without the bias imposed by affinity purification and variations in the avidity of antigen-receptor interactions (13, 14). Second, we have assayed the same or parallel cultures for stimulation of the helper T cell clones (by measuring IL-2 or IL-4 secretion) and for different aspects of the B cell response (proliferation and antibody production). Finally, we have measured the secretion of three different antibody isotypes, IgM, IgG1, and IgG2a, whose production may depend on different lymphokines (17).

The major conclusion from these studies is that Th2 clones (CDC25 and CDC35) induce both proliferation of and IgM and IgG1 secretion by resting B cells. Th2 cells stimulate B lymphocytes that they interact with directly as well as bystander B cells. In contrast, Th1 clones of the same specificity (D1.1. and D1.6) fail to activate B cells in any of the assays used (Figs. 3 and 4, Table III). In fact, IFN- γ produced by Th1 cells inhibits B cell activation, its major effect being on the IL-4-dependent responses, i.e., proliferation and IgG1 secretion (Fig. 5). The failure of Th1 cells to help resting B lymphocytes cannot, however, be attributed to a dominant inhibitory effect of IFN- γ , since helper function cannot be restored to Th1 cells by the addition of anti-IFN- γ antibody (Table II). These results indicate that Th1 cells do not produce the factors necessary for activating resting B lymphocytes, and that neither IL-2 nor IFN- γ is obligatory for such responses. Such a conclusion contradicts a number of earlier studies showing that IL-2 and IFN- γ are important helper factors for B cells (9–12). Many of these earlier studies used erythrocytes as antigens (9, 12), and it is possible that this represents a unique situation that cannot be extrapolated to soluble proteins. Moreover, we and others have shown that Th1 cells (and their secreted mediators) can help previously activated B cells (23) or B cells stimu-

lated polyclonally in the presence of high concentrations of antigen (4), particularly if the inhibitory effect of IFN- γ is neutralized. This suggests that different mechanisms may lead to stimulation of resting and activated B cells, and studies are currently under way to formally address this possibility.

The stimulation of resting B cells by Th2 clones is dependent on both IL-4 and IL-5. Thus, antibody inhibition experiments indicate that IL-4 is the major lymphokine involved in IgG1 secretion and plays a role in proliferation, although other mechanisms may also contribute to increased DNA synthesis (30). On the other hand, IL-5 is not a growth factor for B cells under these conditions, but is necessary for maximal IgM production and may also contribute to the IgG1 response (Table I). The observation that combinations of the two antibodies completely inhibit differentiation of resting B cells indicates that cognate help mediated by Th2 cells can be attributed to these two lymphokines.

Despite the potent helper activity of the Th2 clones, these cells are incapable of inducing IgG2a responses. Controls done in parallel using polyclonally (LPS) stimulated B cells confirmed the findings of Snapper and Paul (17) that IFN- γ is both necessary and sufficient for the switch to IgG2a. Thus, IFN- γ has two opposing effects: it partially inhibits B cell proliferation and production of some antibody isotypes (most notably IgG1), but is required for the secretion of IgG2a. This raises the obvious question of how IgG2a responses are induced in physiologic situations, especially when both subsets are likely to be activated. It is possible that the production of this isotype may require a balance between IL-4 and IFN- γ , such that high concentrations of IL-4 may override the inhibitory influence of IFN- γ and initiate responses that culminate in B cell proliferation and secretion of all Ig isotypes when both Th1 and Th2 cells are present and activated. Alternatively, IgG2a secretion may depend on sequential stimulation of B cells, first with a Th2 helper cell that will induce clonal proliferation and IgM and IgG1, and subsequently with a Th1 cell that will selectively mediate the switch to IgG2a. We are currently testing these possibilities using the panel of clones and recombinant lymphokines described above.

Finally, we have also shown that both Th1 and Th2 clones respond maximally to antigen presented by highly purified B cells, as measured by lymphokine secretion (Fig. 1). Moreover, B cells present ligands that bind to their Ig receptors at much lower concentrations than antigens taken up by receptor-independent pathways or antigens presented by irradiated splenocytes (26), and this is true for both subsets of T cells. Thus, at least in the short-term stimulation assays used in these experiments, Th1 and Th2 subsets do not respond preferentially to antigen presented by different presenting cells (B cells or irradiated whole splenocytes). One might predict that the most efficient APC for Th1 cells are macrophages, since IFN- γ induces Ia expression on macrophages, and for Th2 clones are B cells, since IL-4 enhances B cell Ia expression. This may occur under limiting conditions, but is clearly not the case at the cell numbers and antigen concentrations that we have used. It is also possible that different T cell subsets vary in proliferative responses to antigen presented by different accessory cells, and this reflects the requirement for costimulators such as IL-1. Thus, it is known that Th2 cells are IL-1 sensitive whereas Th1 cells are not (6). To what extent the activation, proliferation, and function of Th1 and Th2 cells are

dependent on selective pressures imposed by different APCs is a question that remains to be resolved.

Summary

To compare the helper function of murine T cell clones that secrete IL-2 and IFN- γ (Th1 cells) or IL-4 and IL-5 (Th2), purified resting B cells were stimulated with F(ab')₂ rabbit anti-mouse Ig (RAMG) and rabbit Ig-specific, class II MHC-restricted cloned T cells belonging to the two subsets. Both Th2 clones examined induced strong proliferative responses of B cells in the presence of RAMG, as well as the secretion of IgM and IgG1 antibodies. In contrast, the Th1 clones tested failed to stimulate B cell growth or antibody secretion. Th2-mediated B cell activation was dependent on IL-4 and IL-5, and was also inhibited by IFN- γ or IFN- γ produced by Th1 cells present in the same cultures. However, the failure of Th1 cells to help resting B cells could not be reversed with neutralizing anti-IFN- γ antibody. In addition to this inhibitory effect, IFN- γ was required for the secretion of IgG2a antibody, particularly when B cells were stimulated with polyclonal activators such as LPS. Finally, both sets of T cell clones secreted lymphokines when stimulated with purified B cells and RAMG. These experiments demonstrate that T cells that differ in lymphokine production also differ in their ability to help B cells as a result of cognate interactions at low concentrations of antigens. Moreover, IL-4, IL-5, and IFN- γ serve different roles in the T cell-dependent proliferative and differentiative responses of resting B lymphocytes.

We thank the following for generous gifts of reagents: Drs. W. E. Paul and J. Ohara (11B11 antibody), Dr. S. Gillis (IL-4), Dr. K. Takatsu (anti-IL-5), and Dr. R. Schreiber (Anti-IFN- γ). Virginia Hamel provided outstanding secretarial help.

Received for publication 24 November 1987 and in revised form 28 December 1987.

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