UCSF
UC San Francisco Previously Published Works

Title
Polyclonal stimulation of resting B lymphocytes by antigen-specific T lymphocytes.

Permalink
https://escholarship.org/uc/item/86c0w9xg

Journal
Journal of Experimental Medicine, 159(3)

ISSN
0022-1007

Authors
DeFranco, AL
Ashwell, JD
Schwartz, RH
et al.

Publication Date
1984-03-01

DOI
10.1084/jem.159.3.861

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

POLYCLONAL STIMULATION OF RESTING B LYMPHOCYTES BY ANTIGEN-SPECIFIC T LYMPHOCYTES

BY ANTHONY L. DEFRANCO, JONATHAN D. ASHWELL, RONALD H. SCHWARTZ, AND WILLIAM E. PAUL

From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

B lymphocyte responses appear to involve a number of distinct, separately controlled steps. Strong evidence now exists for at least three events: (*a*) stimulation of a resting B cell to enter the G_1 phase of the cell cycle (activation[1]), (*b*) commitment of this cell to proliferate and expand clonally, and (*c*) differentiation of this cell to the antibody secretory cell stage (1–5). The first two steps can be stimulated by anti-μ or anti-δ antibodies, presumably acting as surrogates for antigen (6–8). The proliferative step requires high concentrations of these antibodies, whereas the entry into G_1 phase can be stimulated by low concentrations[2] (4). In this polyclonal system, B cell growth factor (now designated BSF-p1),[3] a T cell–derived lymphokine, can stimulate proliferation of cells incubated with this lower dose of anti-μ (9). Progression of cells to the antibody-secreting stage can be induced by addition of other T cell–derived factors (3, 5, 10, 11).

B cell responses can also be obtained through the action of major histocompatibility complex (MHC)[4]-restricted, antigen-specific helper T cells. Several groups have reported achieving polyclonal stimulation of B cells by using either antigen-primed, MHC-restricted lymph node T cells (12–14) or in vitro–propagated T cell lines (15–17). We wished to determine the stage in the B cell response at which such T cells expressed this function and to examine the MHC-restriction of B cell activation and proliferation. The use of in vitro propagated T cell lines of known specificity provided an attractive approach to this end because large numbers of specific T cells could be added to B cells, with the expectation that optimal conditions for B cell stimulation could be achieved. We

A. L. DeFranco was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation. His present address is: Dept. of Microbiology and Immunology, UCSF, San Francisco, CA 94143.

[1] In this paper we will use the term "activation" to specifically allude to the stimulation of resting B cells to enter the G_1 phase of the cell cycle.

[2] DeFranco, A. L., E. S. Raveche, and W. E. Paul. Manuscript in preparation.

[3] A working group on B cell stimulatory factors met in Kyoto on August 21, 1983, under the Chairmanship of Professor Tadamitsu Kishimoto and the sponsorship of the U.S.–Japan Cooperative Medical Sciences Program. This group reviewed the status of various T cell–drived factors that act on B cells, with the goal of developing a standard nomenclature. The group proposed that the factor previously designated B cell growth factor now be referred to as B cell stimulatory factor (BSF)-p1.

[4] *Abbreviations used in this paper:* AEF, allogeneic effector factor; APC, antigen-presenting cells; B10 etc., C57BL/10 etc.; Con A, concanavalin A; cyt. c, pigeon cytochrome *c*; Fl-15-5-5, fluoresceinated 15-5-5; GAT, poly($Glu^{60}Ala^{30}Tyr^{10}$)n; IL-2, interleukin 2; LPS, lipopolysaccharide; MHC, major histocompatibility complex; mIg, membrane immunoglobulin.

J. EXP. MED. © The Rockefeller University Press · 0022-1007/84/03/861/20 $1.00 861
Volume 159 March 1984 861–880

report here polyclonal stimulation of resting B cells by T cell lines, by T cells cloned from such lines, and by cloned T cell hybridomas. T cells stimulated by antigen and antigen-presenting cells (APC) of the appropriate MHC type were capable of activating essentially *all* resting B cells to enter G_1 phase in the absence of antigens for which the B cells were specific. A large fraction (~35%) of these cells entered S phase and substantial amounts of antibody were secreted. This polyclonal T cell stimulation of resting B cells was found to be MHC-unrestricted at the level of B cell–T cell interaction. It appears to represent a mechanism through which resting B cells may be activated by the action of T cells without occupancy of mIg and without cognate MHC-restricted T cell–B cell interactions.

Materials and Methods

Reagents. Goat anti-μ antibodies were purified by positive and negative affinity chromatography procedures as described previously (8). GAT (poly-[$Glu^{60}Ala^{30}Tyr^{10}$]n) was purchased from Vega Biochemicals, Tucson, AZ, and pigeon cytochrome *c* was purchased from Sigma Chemical Co., St. Louis, MO, purified over carboxymethyl cellulose as previously described (18) and generously provided to us by Dr. Lawrence Samelson of this laboratory.

Mice. B10 MHC-congenic strains of mice were bred as described (19) and were generally 2–6 months of age at time of use. B10.*xid* mice were obtained from Dr. Carl Hansen, NIH. These mice were derived from B10.ScN mice, a lipopolysaccharide (LPS) unresponsive strain, into which the X chromosome from CBA/N mice was introduced as previously described (20).

Cell Preparations. Spleen cells were incubated with monoclonal anti–Thy-1.2 (New England Nuclear, Boston, MA), monoclonal anti–Lyt-1 (53.7.3) and monoclonal anti–Lyt-2 (53.6.7) (21) on ice for 30–45 min, centrifuged, resuspended in guinea pig complement (1:4 dilution; Flow Laboratories, Rockville, MD) containing the monoclonal mouse anti-rat kappa chain antibody MAR 18.5 (22), which increases the cytotoxicity of the anti–Lyt-1 and anti–Lyt-2 reagents, and incubated at 37°C for 30 min. Cells prepared in this way completely lacked proliferative Con A or mixed leukocyte responses. Following T cell depletion, spleen cells were fractionated on a Percoll density gradient as described (23). The low density fraction (density <1.062 g/ml) was previously found to be enriched for macrophages (as assessed by latex bead phagocytosis) and for radioresistant APC. The high density fraction (density between 1.074 and 1.086 g/ml) was >80% mIg^+-cells and these were shown to be resting B cells by several criteria (23). Except where specified, this high density fraction from the Percoll gradient was used and is referred to as "B cells." For some experiments, B cells were additionally purified by passage through columns containing Sephadex G-10 (24) or passage through such columns followed by purification by adherence to and recovery from anti-μ–coated petri plates (Corning 100-mm tissue culture–treated plates, coated with 10 μg/ml of affinity-purified goat anti-μ, 7 ml per plate) (25, 26). Flow microfluorometric analysis of these populations indicated that after passage over a column of Sephadex G-10, 95% of the cells were mIg^+; after recovery from anti-μ–coated plates >99% of the cells were mIg^+.

Long-term T Cells and Hybridomas. The T cell lines were grown as described in detail elsewhere (27, 28). The T cell hybridomas used in this study have been previously reported (29). Interleukin 2 (IL-2) secretion by the hybridomas was tested to ensure that the cells had not lost specificity after recovery from liquid nitrogen storage.

Tissue Culture and Analysis. Cells were cultured in Iscove's/F12 medium (30) with 10% fetal bovine serum. Generally, 2×10^5 B cells were cultured with $\sim 2 \times 10^4$ T cells from T cell lines or with 1×10^5 hybridoma T cell in 96-well flat-bottom plates (Costar #3596) in duplicate. The T cells were irradiated sufficiently to reduce their proliferation and incorporation of [^{3}H]thymidine to insignificant levels: for 11.4 T cells, 2,000 rads, for the T cell hybridomas, 10,000 rads, for other T cell clones, 3,300 rads. For Coulter counter (Coulter Electronics, Inc., Hialeah, FL) analysis of cell size, cells were harvested ~24 h

after the initiation of the culture and analyzed as described previously (23). For proliferation assays, 1 μCi of [^{3}H]thymidine was added to the cultures at 24 h after the start of culture and the incorporation of radioactivity was measured by collecting the cells, after 42–48 h of culture, with an automated harvester (Ph.D. Harvester, Cambridge Technology, Cambridge, MA) and counting the filters in a liquid scintillation counter. The period of time chosen for the incorporation of thymidine corresponds closely to the first round of replication of resting B cells stimulated with anti-μ antibody (23). Similar results were obtained in several experiments with either a 4-h pulse of thymidine on day 2 or a longer pulse on day 3. [^{3}H]Thymidine incorporation decreased considerably by day 4. Cell cycle analysis by propidium iodide staining of intact nuclei from hypotonically lysed cells was performed as described (23) except that a FACS 2 (Bectin-Dickinson, Mountain View, CA) was used to measure fluorescence of individual nuclei. The cell population was divided into cells in G_0 or G_1 phases (diploid amounts of DNA), cells in G_2 or M phases (tetraploid amounts of DNA) and cells in S phase (content of DNA between diploid and tetraploid amounts). Colcemid (25 ng/ml) was added at 24 h of culture to prevent cells from completing M phase, thus ensuring that only cells entering S phase for the first time would be counted. IgM secretion was measured after 4 d of culture by an ELISA method (31) using goat anti-μ (50 μg/ml) to coat flat-bottom tissue culture plates (Costar #3596), and developing with Biotinylated-Bet 2 (a monoclonal rat anti-mouse IgM [32]) and avidin-horseradish peroxidase (Vector Laboratories, Inc., Burlingame, CA). Optical density of the wells was measured with a Dynatech plate reader. Standard curves with known amounts of an IgM myeloma protein were used to convert absorbance into concentration of IgM.

Results

Antigen-stimulated T Cell Lines Can Activate Resting B Cells. Resting B cells were prepared from mouse spleen cells by treatment with monoclonal antibodies to T cell antigens and complement and by separation of the remaining cells by Percoll density gradient centrifugation. The most dense cells collected (>1.074 g/ml) have a median volume of 110 μm^3 and are quite homogeneous in size. When resting B cells from B10.A mice were cultured with the syngeneic cloned B10.A T cell line, 11.4, and with GAT (100 μg/ml), the antigen for which 11.4 T cells are specific, a prompt and dramatic increase in cell volume occurred. By 21 h, virtually all of the cultured B cells had increased in size, typically attaining a mean cell volume of ~200 μm^3 in the presence of 1/10 as many T cells as B cells (Fig. 1). This size enlargement, which appears to reflect entry of G_0 cells into the G_1 phase of the cell cycle (23), was dependent upon the presence of 11.4 T cells; without 11.4 T cells, no B cell enlargement was observed. B10.S(9R) B cells, which do not possess the A^k_β:A^k_α restriction element, for which 11.4 T cells are "co-specific," did not show the dramatic enlargement that syngeneic B10.A B cells underwent in the presence of 11.4 T cells and GAT. In the absence of GAT, 11.4 T cells caused a slight size enlargement of B cells from either B10.A or B10.S(9R) mice.

B10.A B cells also entered S phase in the presence of 11.4 T cells and GAT. Incorporation of [^{3}H]thymidine by B10.A B cells in the presence of irradiated 11.4 T cells and GAT was considerable and often comparable to that stimulated by lipopolysaccharide (Table I). The magnitude of the response of B10.A B cells was a function of the number of 11.4 cells present and had not reached maximal plateau values even at a ratio of 4 B cells for each T cell (i.e., 2×10^5 B10.A B cells and 5×10^4 11.4 T cells). Analysis of the fraction of B cells entering S phase for the first time, using propidium iodide staining of isolated nuclei,

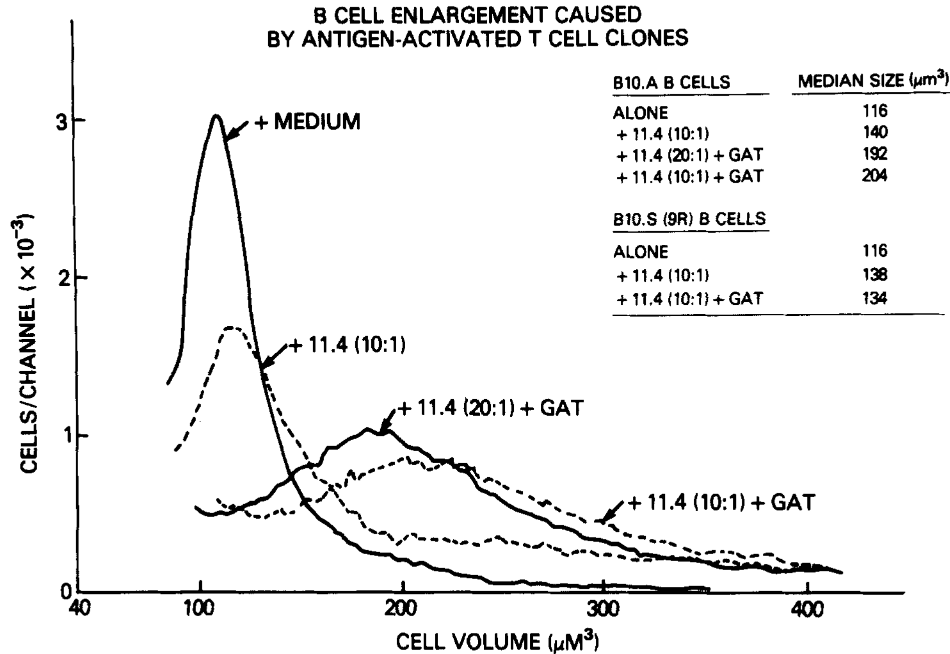

FIGURE 1. B cell enlargement caused by antigen-stimulated T cells. B10.A and B10.S(9R) resting B cells (1×10^6 in 1 ml) were co-cultured for 21 h alone, with 11.4 T cells or with 11.4 T cells and GAT (100 μg/ml) and the cell size distribution of the population and median size was measured with a Coulter Counter and channelyzer. Ratios refer to the relative number of B to T cells. Cell size distributions are shown for B10.A B cells; median cell volume measurements are presented, in tabular form, for B10.A and B10.S(9R) B cells. Only 5–10% of the cells in culture were 11.4 T cells; thus, most of the profile is due to B cells.

TABLE I

B Cell Proliferation Induced by Antigen-stimulated T Cells

B cells	Stimulant	[³H]Thymidine incorporation (cpm)	
		Expt. 1	Expt. 2
None	11.4 T cells	500	—
	11.4 T cells + GAT	350	—
B10.A B cells	0	4,200	2,500
	LPS	130,000	128,000
	11.4 T cells	12,800	14,500
	11.4 T cells plus GAT	69,500	101,000
B10.RIII B cells	0	3,900	6,900
	LPS	115,000	124,000
	11.4 T cells	38,100	50,300
	11.4 T cells plus GAT	29,400	69,700

B10.A or B10.RIII B cells (2×10^5/well), were cultured with nothing, LPS (50 μg/ml), or 2×10^4 11.4 T cells (irradiated at 2,000 rads) with or without GAT (100 μg/ml). [³H]Thymidine incorporation was measured as described in Materials and Methods.
—, not done.

indicated that 35% of cells surviving 48 h in cultures containing colcemid (25 ng/ml) had entered S phase in response to 11.4 T cells and GAT at a ratio of 1 T cell for every 10 B cells (Table II). These results demonstrate the polyclonal nature of the B cell proliferation.

Other T cell lines and T cell hybridomas also expressed the capacity to stimulate resting B cells to enter S phase. These included B10.A-drived lines specific for antigen in association with syngeneic Ia molecules and lines specific for allogeneic Ia molecules. Table III presents results from experiments with four B10.A T cell lines specific for cytochrome *c* and the E^k_β:E^k_α restriction element and one additional line specific for GAT and the A^k_β:A^k_α restriction element. We have also observed that three of four antigen-specific T cell

TABLE II
Entry into S Phase of Resting B Cells Cultured with 11.4 T Cells and GAT

No. added T cells	No stimulant: % of cells entering S	Δ % of cells entering S	
		GAT	GAT + anti-μ
0	(10.6)	—	6.0
2×10^4	(8.8)	5.6	47.8
5×10^4	(11.4)	11.2	49.6
1×10^5	(10.2)	35.0	58.1

B10.A B cells (1×10^6) were cultured with varying numbers of 11.4 T cells in the presence or absence of GAT (100 μg/ml) and anti-μ (2 μg/ml). Colcemid (25 ng/ml) was added at 24 h and nuclei stained at 48 h with propidium iodide. Fluorescence measurements of nuclear DNA content were made on the FACS II; percent of cells in S, G_2, and M phases were summed to give percent of cells entering S phase for the first time (23). Results in parentheses indicate absolute percent of cells entering S phase in the absence of GAT or anti-μ. Other entries represent the increment in percent of cells in S phase (Δ percent).
—, not done.

TABLE III
Stimulation of Proliferation of Allogeneic and Syngeneic B Cells By Antigen-activated T Cell Clones

Expt.	T cells	Syngeneic B cells			Allogeneic B cells + irrad. syngeneic APC			
		No.	Antigen: –	Antigen: +	MHC type and no. of B cells	APC	Antigen: –	Antigen: +
1	A.5.1	1×10^5	240	16,900	v; 1×10^5	2×10^4	200	19,100
2	PC.3.3	2×10^5	12,400	29,300	b; 2×10^5	2×10^5	16,300	36,000
3	PC.1B	2×10^5	5,500	38,300	i18; 2×10^5	2×10^5	8,300	17,100
4	PC.3L	1×10^5	1,100	15,200	i18; 1×10^5	1×10^5	1,600	6,700
	PC1.K	1×10^5	1,100	11,700	t4; 1×10^5	1×10^5	1,300	6,600

Responding syngeneic (B10.A) or allogeneic (B10.Sm, H-2^v; B10, H-2^b; B10.A(18R), H-2^{i18}; B10.S(9R), H-2^{t4}) B cells (1×10^5 or 2×10^5) were cultured with 2×10^4 T cells (3,300 rads) with no antigen, with GAT (100 μg/ml; A.5.1) or with pigeon cytochrome *c* (PC.3.3, PC.1B, PC.3L, PC1.K). Antigen-presenting cells added to allogeneic B cells were Sephadex G-10–passed B cells (1,000 rads) in each case except in Expt. 1 were 3,300 rad-treated low density cells, obtained from Percoll separation, were used. Results are expressed as cpm of [^{3}H]thymidine incorporation at 48 h of culture.

hybridomas (see reference 19) are capable of causing polyclonal responses by syngeneic resting B cells when the T cells are stimulated by antigen.

11.4 T cells cultured with GAT and B10.A B cells also caused B10.A B cells to differentiate into IgM-secreting cells (Table IV). This was assessed by measuring the amount of IgM in the supernatant on day 4 of such cultures. Maximal IgM secretion was obtained in the presence of 10^3 11.4 T cells, which is far less than the number of 11.4 T cells that elicited maximal proliferation of B10.A B cells (see below). The failure to observe significant amounts of IgM secretion when numbers of 11.4 T cells optimal for B cell proliferation were used might represent a depletion of nutrients from culture since the degree of thymidine incorporation on day 4, the day when IgM synthesis was measured, was much less than at days 2 or 3. Alternatively, it may reflect an inability of rapidly proliferating cells to differentiate.

It is very unlikely that the activation of resting B cells in these cultures and their entry into S phase depends upon the presence of antigen(s) capable of binding to their mIg receptors, since virtually all the resting cells enter G_1 phase and a considerable proportion enter S phase. It can hardly be expected that antigens complementary to receptors of all B cells are present in these cultures.

Stimulation of Resting B Cells Does Not Require That They Participate in MHC-

TABLE IV
IgM Secretion of B Cells Stimulated by Antigen-activated 11.4 T Cells

Addition	IgM secreted by day 4 (ng/culture)			
	B10.A B cells:		B10.S(9R) B cells:	
	–	+GAT	–	+GAT
Expt. 1				
None	100		150	
LPS	>5,850		2,000	
11.4,				
1×10^2	190	190	140	220
3×10^2	170	780	200	530
1×10^3	200	1,900	300	520
3×10^3	240	1,700	620	1,300
1×10^4	240	160	510	8
3×10^4	200	30		
Expt. 2				
None	25		95	
LPS	≥4,400		≥4,400	
11.4,				
5×10^3	560	1,600	220	≥4,400
1×10^4	110	570	300	3,500
2×10^4	230	200	230	1,300
5×10^4	250	40	1,170	770

B cells were incubated in triplicate at 2×10^5 cells/well with additions noted above. Culture fluids were analyzed for IgM content on day 4 of culture by the ELISA method (31). In every case, the standard deviation was <50% of the value. In Expt. 1 B10.A B cells, 7×10^4, irradiated at 1,000 rads, were added to cultures containing B10.S(9R) B cells as a source of antigen-presenting cells; in Expt. 2, B10.A low density cells 5×10^4, irradiated at 1,000 rads, were added. When these irradiated cells were incubated alone with either LPS or with 11.4 and GAT, they did not secrete significant levels of IgM.

restricted Interactions with T Cells. B cells from B10.S(9R), B10, B10.D2, or other non A^k_β:A^k_α bearing stains of mice cultured with 11.4 T cells and GAT failed to enlarge or to incorporate [^{3}H]thymidine. This might reflect a failure of 11.4 T cells to activate these B cells because stimulated 11.4 T cells cannot collaborate with allogeneic B cells and/or because 11.4 T cells are not stimulated by GAT in the absence of a source of A^k_β:A^k_α-bearing APC. To examine this issue, we studied B10.S(9R) B cell activation in cultures containing B10.BR (A^k_β:A^k_α-bearing) B cells, 11.4 T cells, and GAT. To measure B cell activation, we assessed the enlargement of both the B10.S(9R) and B10.BR B cells, using flow microfluorometry, by measuring the degree of forward light scatter of cells that were fluorescence positive or that were fluorescence negative when stained with fluoresceinated (Fl) 15-5-5, a monoclonal antibody that binds to cells bearing H-2K^d or H-2D^k gene products (33) (i.e., binds to B10.BR but not B10.S(9R) cells). When B10.S(9R) B cells were cultured with 11.4 T cells and GAT for 24 h, no increase in forward light scatter was observed, while B10.A B cells cultured with 11.4 T cells and GAT showed substantial increase in their forward light scatter histograms. This is illustrated in Fig. 2 *B*, in which the two cell types were mixed after culture and the forward light scatter histograms of the fluorescence-positive (B10.BR) and fluorescence-negative (B10.S(9R)) B cells in the mixture were measured. By contrast, when the B10.A and B10.S(9R) B cells were mixed and then cultured with 11.4 T cells and GAT, both fluorescence-positive (B10.BR) and fluorescence-negative (B10.S(9R)) B cells displayed equivalent increases in the magnitude of their forward light scatter (Fig. 2 *A*). This increase in forward light scatter was dependent upon 11.4 T cells and GAT (data not

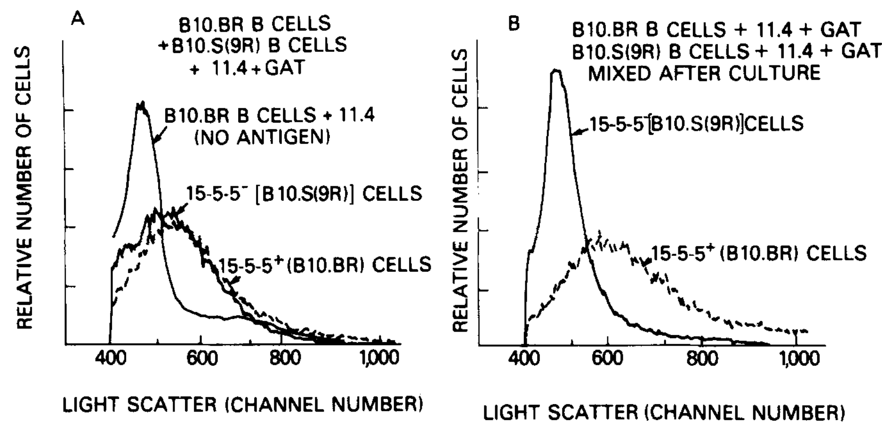

FIGURE 2. Stimulation of enlargement of allogeneic B cells by 11.4 T cells in the presence of syngeneic B cells and GAT. B10.BR (15-5-5$^+$) B cells were mixed with B10.S(9R) (15-5-5$^-$) B cells either before (*A*) or after (*B*) culture for 20 h in the presence of 11.4 T cells (B cell/T cell ratio 10:1) and GAT (100 μg/ml). B cells were cultured at a total density of 2 × 10^6/ml in each case. Cells were stained with Fl-15-5-5. Forward light scatter and fluorescence data were collected on each cell using a FACS 2. Forward light scatter profiles of the fluorescence-positive (B10.BR) cells and of the fluorescence-negative (B10.S[9R]) cells were obtained. Cells were also stained with fluorescence-labeled anti-μ to demonstrate that the profiles were primarily due to B cells (not shown). The forward light scatter profiles of B10.S(9R) and B10.A B cells mixed after culture (*B*) were equivalent to the profiles of similarly cultured B10.S(9R) and B10.A B cells analyzed separately (data not shown).

shown). Since forward light scatter is a function of cell volume, this result indicates that B10.S(9R) B cells enlarge in the presence of B10.BR B cells, 11.4 T cells, and GAT. Presumably, B10.A B cells present GAT to 11.4 T cells, stimulate them, and they in turn activate B10.S(9R) B cells in an MHC-unrestricted manner.

Although B10.S(9R) B cells do not proliferate in the presence of irradiated 11.4 T cells and GAT, the addition of B10.A B cells irradiated at 1,000 rads, as a source of APC, resulted in [^{3}H]thymidine incorporation by the allogeneic B cells. The magnitude of this response was dependent upon the number of irradiated B10.A B cells added and was quite substantial in the presence of 1–3 $\times 10^5$ irradiated B10.A B cells (Fig. 3). Comparison of [^{3}H]thymidine incorporation by B10.A and B10.S(9R) B cells in the presence of different numbers of 11.4 T cells revealed that the B10.S(9R) cells required two- to threefold more T cells than did syngeneic cells to achieve equivalent responses (Fig. 4).

Incorporation of [^{3}H]thymidine by B10.S(9R) B cells also occurred in the presence of 11.4 T cells stimulated with GAT in the presence of T cell–depleted unfractionated B10.A spleen cells irradiated at 3,300 rads or in the presence of a macrophage- and dendritic cell-rich low density (<1.062 g/ml) cell fraction. A detailed comparison of the relative effectiveness of irradiated resting B cells, macrophage-rich cell populations, and whole spleen cells to stimulate 11.4 T cells for "recruitment" of B10.S(9R) B cell activation and proliferation and for stimulation of proliferation of 11.4 T cells, themselves, is presented in the companion paper (19). We note here that, in the presence of GAT, highly purified high density B10.A B cells are at least as effective, on a per cell basis, as either of the other cell populations for causing 11.4 T cell–dependent B10.S(9R) B cell proliferation provided the B cells are irradiated at 1,000 rads or less.

The ability to cause proliferation of allogeneic B cells when examined in this

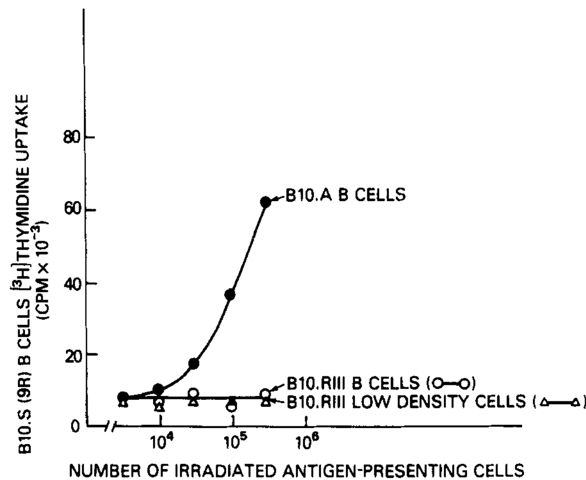

FIGURE 3. Incorporation of [^{3}H]thymidine by B10.S(9R) B cells stimulated by 11.4 T cells as function of the number of APC added to activate the T cells. 2×10^5 B10.S (9R) B cells were cultured with 2×10^4 11.4 T cells and various numbers of irradiated B10.A B cells, B10.R III B cells, or B10.R III low density cells as antigen-presenting cells. Incorporation of [^{3}H]thymidine was measured between 24 and 48 h of culture.

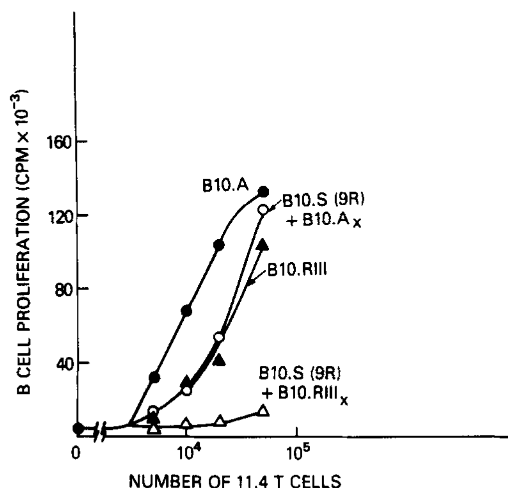

FIGURE 4. Incorporation of [^{3}H]thymidine by B10.S(9R) B cells is stimulated by 11.4 T cells cultured with irradiated B10.A B cells (B10.A$_x$; 1,000 rads) and GAT but not by 11.4 T cells cultured with irradiated B10.R III B cells (B10.R III$_x$; 1,000 rads). Incorporation of [^{3}H]-thymidine by 2 × 10^5 resting B cells isolated from B10.A, B10.R III or B10.S(9R) mice was measured between 24 and 48 h of culture. 2 × 10^5 irradiated B cell stimulators were used as indicated. In cultures containing B10.A or irradiated B10.A B cells, GAT (100 μg/ml) was added to culture.

way was not a unique property of 11.4 T cells, but rather was found to be a general property of antigen-specific T cell clones that could stimulate proliferation of syngeneic B cells. Experiments with other B10.A T cell clones are shown in Table III. As already described, all five lines, when irradiated, caused considerable proliferation of syngeneic B cells in the presence of the antigen for which the T cells were specific. Addition of T cells and antigen caused proliferation of allogeneic B cells, provided that irradiated B10.A APC were added. In this situation, the presence of antigen and functional APC stimulated each of the cloned T cells to cause significant [^{3}H]thymidine uptake by allogeneic B cells.

B10.S(9R) B cells, co-cultured with irradiated B10.A B cells, 11.4 T cells, and GAT also differentiate into Ig-secreting cells by day 4 (Table IV). The optimal number of 11.4 T cells for this differentiation is 3 × 10^3 cells, considerably less than the optimal number for B cell proliferation, just as was true of the differentiation of syngeneic B10.A B cells.

B Cells from "xid" Mice Can Stimulate and Respond to 11.4 T Cells. B cells from mice with the *xid*-determined defect (xid B cells) have been reported to be capable of interacting with T cells in an MHC-restricted manner in order to differentiate into Ig-secreting cells, but to fail to respond in non–MHC-restricted T cell–B cell interactions (34). We tested the ability of xid B cells to proliferate when co-cultured with 11.4 T cells and GAT, both when these cells bore the A^k_β:A^k_α-restriction element and when they lacked it. B cells from (CBA/N × DBA/2)F_1 male mice, which have the *xid*-determined defect and express A^k_β:A^k_α, showed excellent [^{3}H]thymidine incorporation in response to 11.4 T cells and GAT (Table V). These responses were greater than those stimulated by LPS and were not further enhanced by addition of 1,000-rad irradiated B10.A B cells. Although

TABLE V
Proliferation of xid B Cells in Response to Antigen-stimulated T Cells

Addition	GAT:	$(DBA/2 \times CBA/N)F_1$		$(CBA/N \times DBA/2)F_1$		B10		B10.xid	
		−	+	−	+	−	+	−	+
		[^{3}H]Thymidine incorporation (cpm)							
None		4,300		3,000		2,900		2,000	
LPS		114,000		61,700		32,200		7,000	
Con A		4,000		1,500		1,800		2,000	
11.4 T		9,200	141,000	2,600	95,200	5,600	4,600	4,400	4,700
$B10.A_x$ B Cells + 11.4 T Cells		8,200	136,000	2,600	87,300	3,600	60,100	5,400	37,000

B cells were incubated at 2×10^5 cells/well with additions as shown and their incorporation of [^{3}H]thymidine measured after 48 h of culture. 2×10^4 2,000 rad-treated 11.4 T cells were added where indicated. 7×10^4 irradiated (1,000 rads) B10.A ($B10.A_x$) B cells were added to cultures as indicated as a source of APC. These irradiated B cells did not incorporate significant amounts of [^{3}H]thymidine. $(DBA/2 \times CBA/N)F_1$ and $(CBA/N \times DBA/2)F_1$ B cells were derived from male donors. The former are phenotypically normal; the latter express the *xid*-determined defect.

the response of B cells from $(DBA/2 \times CBA/N)F_1$ male mice, which are phenotypically normal, was somewhat greater than those of the xid B cells, this may simply reflect the superior survival of the normal cells in culture.

The capacity of xid B cells that lacked the A^k_β:A^k_α restriction element to be stimulated by 11.4 T cells was examined by using B10.*xid* (H-2^b) B cells and comparing them to C57BL/10 SgSn B cells. Neither B cell population incorporated [^{3}H]thymidine in the presence of 11.4 T cells and GAT; addition of irradiated B10.A B cells as a source of APC caused substantial proliferation on the part of both cell populations. As with the syngeneic B cells, the response of xid B cell was about one-half of the response of normal B cells (Table V). The meager response of the B10.*xid* B cells to LPS is, at least in part, due to the fact that they are derived from the LPS-unresponsive strain C57BL/10 ScN. The control mice, C57BL/10 SgSn, are normally responsive to LPS.

These results indicate that B cells from xid mice are competent to stimulate 11.4 T cells in the presence of GAT and capable of being recruited, in an MHC-unrestricted manner, to enter S phase.

Anti-IgM Antibodies Synergize with 11.4 T Cells in B Cell Activation. Thus far we have shown that essentially all B10.A B cells will enter the G_1 phase of the cell cycle in the presence of 11.4 T cells and GAT. Antigen(s) capable of binding to the B cell's mIg do not appear to be required for this activation. Similarly, many of these B cells also enter S phase in such cultures. We have previously shown that low concentrations of anti-μ (1 or 2 μg/ml) cause virtually all resting B cells to enter G_1 phase but very few to enter S phase (9). Some of these G_1 B cells enter S phase in the presence of BSF-p1 (10, unpublished observations).

We next examined the effect of addition of 2 μg/ml of anti-μ to cultures containing B10.A B cells, 11.4 T cells, and GAT (Table II). Our results indicate that when limiting numbers of 11.4 T cells are present, anti-μ causes a striking enhancement in entry of B cells into S phase. Thus, co-culture of 10^6 B10.A B cells with 2×10^4 11.4 T cells (50:1 ratio) and GAT caused only 5.6% of the B cells to enter S phase. In other experiments, it was shown that 2×10^4 11.4 T cells caused very limited B cell size enlargement (median cell volume at 24 h of 124 μm^3 with control of 120 μm^3). Culturing 1×10^6 B10.A B cells with 2 μg of anti-IgM caused striking size enlargement but stimulated only 6.0% of the cells

to enter S phase. When 2 μg of anti-IgM alone was added to cultures of 10^6 B cells, 2×10^4 11.4 T cells and GAT, 47.8% of the cells were stimulated to enter S phase; this is 36.2% more than the sum of the numbers of B cells that entered S phase in the separate cultures. The results suggest that anti-μ enhanced entry of B10.A cells into G_1 and that stimulated 11.4 T cells, perhaps by secreting BSF-pI, caused the G_1 cells to enter S phase. This also suggests that 11.4 T cells use separate signals to initiate entry of B10.A B cells into the G_1 and S phases, with the activation signal being limiting under these culture conditions.

Stimulation of 11.4 T Cells by B10.RIII Cells Leads to an Apparent MHC-restricted B Cell Stimulation. We have shown that 11.4 T cells stimulated with GAT in the presence of a source of A^k_β:A^k_α-bearing cells caused the enlargement, entry into S phase, and differentiation of both syngeneic (B10.A) and allogeneic (B10.S(9R)) B cells. It would seem reasonable to conclude that the T cell–B cell interaction leading to B cell stimulation is not MHC-restricted, while the stimulation of the T cells is restricted. However, further examination of B cell responses mediated by 11.4 T cells revealed a situation in which B cell stimulation did appear to be restricted.

In addition to proliferating in response to GAT and the A^k_β:A^k_α restriction element, 11.4 T cells can be stimulated to proliferate by allogeneic H-2^r-encoded molecules expressed on B10.RIII cells, although this alternative stimulation is considerably weaker (data not shown). Furthermore, B10.RIII B cells co-cultured with 11.4 T cells, without GAT, are stimulated to enlarge (mean volume at 24 h of 152 μm^3) and to incorporate [^{3}H]thymidine (Table I). Although B10.RIII B cells require approximately threefold more 11.4 T cells than do B10.A B cells (in the presence of 100 μg/ml of GAT) for equivalent B cell–proliferative responses, the maximal response obtained with B10.RIII B cells is only slightly less than with B10.A B cells cultured with the same number of 11.4 T cells in the presence of GAT (Fig. 4). In contrast, 11.4 T cells stimulated either with 1,000-rad irradiated B10.RIII B cells (1×10^4 to 5×10^5) or with 3,300-rad irradiated B10.R III low density cells were ineffective at stimulating [^{3}H]thymidine incorporation by the B10.S(9R) B cells (Fig. 3). In the same experiment, B10.S(9R) B cells co-cultured with irradiated B10.A B cells, 11.4 T cells, and GAT were strikingly stimulated. Indeed, 1×10^4 irradiated B10.A B cells were superior to 5×10^5 irradiated B10.RIII B cells in causing 11.4 T cells to stimulate B10.S(9R) B cells to enter S phase (Fig. 3). This difference in the capacity to cause 11.4 T cell–dependent stimulation of B10.S(9R) B cells is much greater than the two- to threefold difference in the proliferative responses of B10.A and B10.R III B cells stimulated by 11.4 T cells (Fig. 4).

This discrepancy might reflect a qualitative or a quantitative difference in stimulation of 11.4 T cells by B10.RIII B cells as compared with B10.A B cells and antigen. That is, B10.RIII B cells might only be able to stimulate 11.4 T cells to engage in MHC-restricted B cell responses, while B10.A B cells, together with GAT, might cause 11.4 T cells to produce nonspecific soluble factors capable of stimulating B cells without reference to their MHC type. Alternatively, B10.RIII B cells and B10.A B cells plus GAT might both stimulate production of nonspecific activating factors by 11.4 T cells, but the former might cause production of smaller amounts, sufficient to stimulate only those B cells in close

proximity to the 11.4 T cells. This would markedly favor the activation of B10.RIII B cells, which in the course of presenting Ia^r molecules to 11.4 T cells are physically linked to them, over the activation of B10.S(9R) B cells, which would be randomly distributed in regard to 11.4 cells. If this explanation is correct, we might anticipate that diminishing the degree of stimulation of individual 11.4 T cells by B10.A B cells plus GAT might lead to an advantage in the stimulation of B10.A over B10.S(9R) B cells. We tested this by comparing the concentrations of GAT required to cause [^{3}H]thymidine incorporation by B10.A B cells, when cultured with irradiated 11.4 T cells only, with those required to stimulate B10.S(9R) B cells co-cultured with irradiated B10.A B cells and 11.4 T cells. We found that two- to fourfold higher concentrations of GAT were required to cause degrees of [^{3}H]thymidine incorporation by B10.S(9R) B cells comparable to that of B10.A B cells and that at 50 μg GAT/ml, a very substantial preference for stimulating syngeneic B cells was observed (Fig. 5).

Discussion

These experiments demonstrate the ability of T cell lines to cause many, if not all, resting B cells to enter G_1 phase, proliferate, and secrete large amounts of antibody. These T cell–mediated responses were displayed by B cells of different MHC genotypes, provided that the T cells were optimally stimulated by antigen in the context of APC bearing syngeneic Ia molecules. For this purpose, syngeneic resting B cells were as efficient at antigen presentation as were populations enriched for macrophages and dendritic cells (19). The response of allogeneic B cells were comparable to these of syngeneic B cells, provided substantial numbers

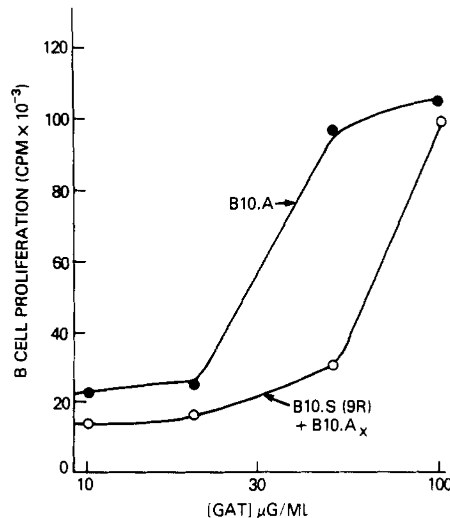

FIGURE 5. Concentration of GAT required to stimulate proliferation of syngeneic and allogeneic B cells. 1 × 10^5 B10.A B cells or 1 × 10^5 irradiated B10.A_x B cells (B10.A_x; 1,000 rads) and 2 × 10^5 B10.S(9R) B cells were cultured with 2 × 10^4 11.4 T cells and varying concentrations of GAT. Proliferation was assessed by incorporation of [^{3}H]thymidine between 24 and 48 h of culture.

of syngeneic B cells or macrophages were present, presumably to serve as APC for the T cells, and provided that high concentrations of antigen were used to stimulate the T cells (Fig. 5).

Resting B cells could be driven to proliferate by most of the IL-2–secreting T cell hybridomas tested, by all of the tested T cell lines including cloned lines propagated by alternating cycles of growth and rest, and by one T cell line isolated by cloning in soft agar, followed by continuous growth on IL-2 without antigen or accessory cells (28). Therefore, the ability of in vitro T cells lines and hybridomas to stimulate B cells as reported here is not a rare or uncommon property of T cells, but rather seems to be typical of many in vitro T cells maintained by different growth techniques.

Recent work with T cell–derived lymphokines such as BSF-p1 (9, 35) and other growth and differentiation factors (36–40) suggests possible mechanisms by which the T cells used in this work might have caused some of the responses observed. Previous experiments had not revealed, however, the existence of a T cell–derived factor capable of acting on resting B cells and stimulating them to enter and progress through G_1 phase of the cell cycle. Anti-μ previously has been shown to perform this activation step (4), suggesting that some antigens with the ability to cross-link mIg might be able to do this as well. The experiments presented in this paper clearly demonstrate that T cells can stimulate this activation step under the appropriate circumstances, without requirement for occupancy of B cell antigen receptors or interaction with B cell Ia molecules. Synergy was seen between antigen-stimulated 11.4 T cells and anti-μ in the stimulation of B cell proliferation (Table II). At suboptimal numbers of 11.4 T cells, B cell enlargement was slight, suggesting little if any movement of the resting B cells from G_0 to G_1. Low concentrations of anti-μ caused excellent size enlargement of resting B cells, but induced few cells to enter S phase (23). Adding both small numbers of 11.4 T cells and low concentrations of anti-μ caused very substantial numbers of B cells to enter S phase. This result suggests that the 11.4 T cells are more efficient at causing G_1 phase B cells to proliferate than at causing resting B cells to enter G_1 phase. If this is a common property of helper T cells, then under suboptimal conditions, any agent that caused a resting B cell to enter G_1 phase, such as anti-μ or, possibly an antigen capable of cross-linking mIg on the B cell surface, would promote the B cell response to the antigen-stimulated helper T cells.

MHC-restricted Vs. MHC-unrestricted Activation. The conclusion that antigen-stimulated T cells can polyclonally stimulate resting B cells to enlarge, proliferate, and differentiate to antibody-secreting cells in an MHC-unrestricted manner is surprising in view of most previous in vitro studies of helper T cell stimulation of B cells. The bulk of those studies, both examining specific antibody production (2, 17, 34, 41, 42) and polyclonal stimulation of [^{3}H]thymidine incorporation and Ig secretion with either T cells obtained from antigen-primed donors (12–14) or more recently with antigen-specific T cell lines (15–17, 42), demonstrated a requirement for an MHC-restricted step. Some stimulants, such as sheep erythrocytes or high concentrations of hapten-carrier conjugates are capable of stimulating "bystander" B cells in the presence of T cells (43) or can stimulate responses in the apparent absence of T cells if T cell–replacing factors are

provided (3). However, whether resting B cells are the responsive cells in these circumstances has not been shown. We believe that the results presented here conclusively demonstrate that helper T cells can activate and stimulate proliferation of B cells by an MHC-unrestricted mechanism, even in the absence of the antigen recognized by the B cell.

There are several possible explanations for the differences between our results and those results emphasizing the need for MHC-restricted T cell–B cell interaction. One possibility is that all helper cell activation of B cells is unrestricted and that apparent MHC restriction is the result of suboptimal T cell activation or of the use of helper cell populations that are limited in the amount of factor(s) they can produce. For example, 11.4 T cells in the presence of 100 μg/ml GAT and lightly irradiated B10.A B cells stimulated the proliferation of allogeneic B cells. However, at suboptimal concentrations (e.g. 50 μg/ml) of GAT, B10.A B cells were preferentially stimulated to a striking extent. This effect was even more dramatic when 11.4 T cells were stimulated with B10.RIII cells. B10.RIII antigen-presenting cells are 10–30-fold poorer at stimulating proliferation of 11.4 T cells compared with B10.A APC in the presence of 100 μg/ml GAT (data not shown). 11.4 T cells were capable of causing B10.RIII stimulator B cells to proliferate vigorously. However, bystander B10.S(9R) B cells were not induced to proliferate in the presence of 11.4 and lightly irradiated B10.RIII B cells. This is in striking contrast to the observation that the same bystander B10.S(9R) B cells proliferated quite well in the presence of 11.4 T cells and lightly irradiated B10.A B cells plus GAT. A possible explanation for these results is that B10.RIII cells stimulate the 11.4 T cells less intensely than do B10.A B cells and GAT and that the stimulation by B10.RIII cells leads to release of B cell activating factor(s) by 11.4 T cells in correspondingly lower amounts than would be produced in response to stimulation by B10.A B cells and high concentrations of GAT. These lower amounts of factor(s) might be sufficient only to activate B cells that are quite close to the 11.4 T cells. B10.RIII B cells would be activated, according to this explanation, because they have presented antigen (i.e., Ia^r) to 11.4 T cells by physically interacting with them and therefore are very close to the source of the B cell activating factor(s). The bystander cells are, on the average, farther away, and therefore are not activated nearly as well. Similarly, when B10.A B cells and lower concentrations of GAT are used, 11.4 T cells are stimulated relatively poorly and a similar preference for syngeneic responses occurs. This explanation suggests that polyclonal stimulation of B cells by helper T cells such as that reported from our laboratory by Tse et al. (12) and by Marrack and Kappler (13) or by T cell lines such as seen by Coutinho (15) and Ratcliffe et al. (16) may be the result of an unrestricted mechanism that only operates at a short distance from the helper T cell. In such cases, syngeneic B cell responses would be favored because T cells would recognize and bind to antigen and class II molecules on the surface of syngeneic B cells.

A similar argument could explain apparent MHC restriction in antigen-specific antibody responses. When high doses of antigen are used (e.g. 100 μg/ml), many cells in the culture can present antigen and the magnitude of T cell response is great, leading to production of large amounts of activating factor(s) and to nonrestricted B cell activation. On the other hand, when lower antigen concen-

trations are used one might expect that only the antigen-specific B cell would bind sufficient antigen, via its membrane immunoglobulin, to act as an antigen-presenting B cell. Such a possibility has been graphically illustrated by Hedrick and Schwartz (44), who found that T cells specific for IgG2a allotypes could respond to a 1,000-fold lower concentration of antigen if the IgG2a was specific for and reacted with molecules on the surface of APC. Alternatively, the principal APC in such cultures may be macrophages and other Ia-bearing cells, such as dendritic cells. Indeed, in most experiments of this type, responses are reported to be macrophage dependent. Under such circumstances, when low antigen concentrations are used, each T cell is stimulated relatively poorly (i.e., by APC bearing small amounts of antigen). Such T cells would be expected to produce small amounts of activating factor(s). Syngeneic B cells, specific for the antigen that stimulated the T cells, would be bound by the responding T cells because those T cells would recognize the antigen and the restriction elements on the B cells. This would lead to activation of antigen-specific syngeneic B cells. In contrast, neither allogeneic antigen-specific B cells nor syngeneic non–antigen-specific B cells would interact with the T cells. The former would fail to interact because, although they would have bound antigen to their receptors, they do not display a restriction element for which the T cells were co-specific. The latter cells would fail to interact with T cells because, although they express the proper restriction elements, they fail to capture sufficient antigen on their membrane when low concentrations are used since their receptors are not specific for that antigen. Of course, allogeneic B cells lacking receptors for the antigen in question would present neither the appropriate restriction element nor the proper antigen. This would lead to a situation in which antigen-specific B cells bearing class II molecules syngeneic to the T cells would be preferentially activated; that is, to an MHC-restricted T cell–B cell interaction. Furthermore, in hapten-carrier systems in which carrier-specific T cells are used and anti-hapten antibody responses are measured, cellular interactions could be achieved only if the hapten and carrier were linked to one another.

The results of Asano et al. (34) fit this scenario fairly well. They found that stimulation of B cells by cloned in vitro T cell lines was not MHC restricted at high antigen concentrations, but was restricted and required hapten-carrier linkage at low antigen concentrations. An apparent discrepancy between our results and theirs is that Asano et al. (34) found that xid B cells could not be stimulated to become hapten-specific antibody-producing cells in a non–MHC-restricted manner when high antigen concentrations were used. In our experiments, allogeneic xid B cells proliferated ~50% as well as did allogeneic B cells from normal mice in the presence of irradiated syngeneic B cells, 11.4 T cells, and antigen. A possible reason for this difference may be that antigen-specific responses in cultures receiving high antigen concentrations may require very substantial relative expansion of hapten-specific clones. Receptor cross-linkage by antigen could cause such expansion by making B cells sensitive to BSF-p1; xid B cells fail to proliferate in response to anti-μ together with BSF-p1, suggesting that they would not display such clonal expansion. This could lead to the striking difference reported in antigen-specific antibody responses of normal and xid B cells when high antigen doses are used. By contrast, we would predict that in

such systems measurement of total Ig production would reveal little or no difference between normal and xid B cells because receptor cross-linked stimulation of clonal expansion would play a lesser role in total Ig synthesis in such cultures.

Although nonspecific activating factors produced by stimulated T cells may explain many examples of MHC-restricted B cell stimulation, it does not rule out the possibility that activation mediated by truly MHC-restricted mechanisms does exist. Indeed, there are reports of MHC-restricted helper factors (45, 46) and allogeneic effect factor (AEF) has many of the properties one would expect of an MHC-restricted factor (47). Such factors are believed to act early in the B cell response, although that has not been demonstrated directly. Stimulation of B cells by some helper T cells may involve this putative MHC-restricted event, whereas other T cells, such as the in vitro lines and hybridomas used in this work, may be capable of releasing additional factors that bypass this requirement. The apparent MHC-restricted stimulation of B cells by 11.4 T cells responding to B10.RIII B cells could be due to secretion of an MHC-restricted factor, but as we have already pointed out, this phenomenon may be explained by the action of nonspecific factors.

Pathway of B Cell Responses. The results presented in this paper and the results of others suggest a more complicated view of B cell responses than is generally discussed. Each of the various events involved in causing a resting B cell to enter the cell cycle, to proliferate and to differentiate into an antibody-secreting cell, may be triggered by several different stimuli. For example, entry into G_1 phase is clearly stimulated by anti-μ, presumably by some antigens, and is also stimulated in an MHC-unrestricted manner by antigen-stimulated T cell lines as demonstrated here. This is also the step in the B cell response postulated to be controlled in an MHC-restricted manner, based upon experiments showing that large B cells from the spleen no longer need this signal (2). Since both anti-μ and antigen-stimulated T cells can each cause virtually all resting B cells to enlarge, these two signals would seem to represent parallel pathways of activation. If an MHC-restricted signal does exist, it may represent a third parallel pathway. Although B cells may be activated by each of these stimuli, it is not clear whether the consequences of the activation process is the same in each case. Detailed examination of the properties of G_1 phase B cells resulting from distinct stimuli will be required to determine the equivalence or lack of equivalence of the various stimuli.

The value of experiments such as the ones reported here is to point out the nature of the steps in the B cell response caused by stimulated helper T cells and thereby suggest the existence of presently unknown lymphokines with novel functions. Based on these experiments, we propose the existence of a B cell activating factor that will act on a resting B cell and cause it to enter G_1 phase in an MHC-unrestricted manner.

Summary

Resting B lymphocytes are activated, proliferate, and differentiate into antibody-secreting cells when cultured with long-term lines of major histocompatibility complex (MHC)-restricted, antigen-specific T cell in the presence of the

antigen for which the T cells are specific. Under optimal conditions, essentially all B cells are activated and ~35% enter S phase in the absence of antigens for which the B cells are specific. Activation and proliferation are observed in cells from both normal mice and mice with the *xid*-determined immune defect. Highly purified B cells bearing Ia molecules for which the T cells are "cospecific" can present antigen to T cells with the resulting T cell stimulation leading to the activation and proliferation of the antigen-presenting B cells. However, B cells that do not bear Ia molecules for which the T cells are cospecific are also activated and proliferate if antigen and a source of antigen-presenting B cells or macrophage-rich cells of proper histocompatibility type are present. Thus, resting B cells, both normal and "xid", can be activated by non-MHC restricted factors without receptor cross-linkage. Experiments are presented that support the concept that local production and action of such unrestricted activating factors may be responsible for the MHC-restriction of T cell–B cell interaction seen in many circumstances.

We wish to thank Dr. Carl Hansen for providing B10.*xid* mice, Dr. Kenji Nakanishi for advice and for reagents and Dr. Richard Asofsky, B. J. Fowlkes, and Dr. Stephen Hedrick for antibody reagents. Expert technical assistance for flow microfluorometry was provided by William Leiserson and Linette Edison.

Received for publication 31 October 1983.

References

1. Bretscher, P. 1975. The two signal model for B cell induction. *Transplant. Rev.* 23:37.
2. Andersson, J., M. H. Schreier, and F. Melchers. 1980. T-cell–dependent B cell stimulation is H-2 restricted and antigen dependent only at the resting B-cell level. *Proc. Natl. Acad. Sci. USA.* 77:1612.
3. Dutton, R. W. 1975. Separate signals for the initiation of proliferation and differentiation in the B cell response to antigen. *Transplant. Rev.* 23:66.
4. DeFranco, A. L., J. T. Kung, and W. E. Paul. 1982. Regulation of growth and proliferation in B cell subpopulations. *Immunol. Rev.* 64:161.
5. Nakanishi, K., M. Howard, A. Muraguchi, J. Farrar, K. Takatsu, T. Hamaoka, and W. E. Paul. 1983. Soluble factors involved in B cell differentiation: identification of two distinct T cell-replacing factors (TRF). *J. Immunol.* 130:2219.
6. Sell, S., and P.G. H. Gell. 1965. Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with an anti-allotype serum. *J. Exp. Med.* 122:423.
7. Parker, D. C. 1975. Stimulation of mouse lymphocytes by insoluble anti-mouse immunoglobulins. *Nature (Lond.).* 258:361.
8. Sieckmann, D. G., R. Asofsky, D. E. Mosier, I. M. Zitron, and W. E. Paul. 1978. Activation of mouse lymphocytes by anti-immunoglobulin. I. Parameters of the proliferative response. *J. Exp. Med.* 147:814.
9. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T-cell derived B-cell growth factor distinct from interleukin 2. *J. Exp. Med.* 155:914.
10. Kishimoto, T., and K. Ishizaka. 1975. Regulation of antibody response in vitro. IX. Induction of secondary anti-hapten IgG antibody response by anti-immunoglobulin and enhancing soluble factor. *J. Immunol.* 114:585.
11. Parker, D. C., J. J. Fothergill, and D. C. Wadsworth. 1979. B lymphocyte activation

by insoluble anti-immunoglobulin: induction of immunoglobulin secretion by a T cell-dependent soluble factor. *J. Immunol.* 123:931.
12. Tse, H. Y., J. J. Mond, and W. E. Paul. 1981. T lymphocyte-dependent B lymphocyte proliferative response to antigen. I. Genetic restriction of the stimulation of B lymphocyte proliferation. *J. Exp. Med.* 153:871.
13. Marrack, P., and J. W. Kappler. 1980. The role of H-2-linked genes in helper T cell function. VII. Expression of *I* region and immune response genes by B cells in bystander helper assays. *J. Exp. Med.* 152:1274.
14. Cammisuli, S., C. Henry, and L. Wofsy. 1978. Role of membrane receptors in the induction of in vitro secondary anti-hapten responses. I. Differentiation of B memory cells to plasma cells is independent of antigen-immunoglobulin receptor interaction. *Eur. J. Immunol.* 8:656.
15. Augustin, A., and A. Coutinho. 1980. Specific T helper cells that activate B cells polyclonally. *J. Exp. Med.* 151:587.
16. Ratcliffe, M. J. H., and M. H. Julius. 1982. H-2-restricted T-B cell interactions involved in polyspecific B cell responses mediated by soluble antigen. *Eur. J. Immunol.* 12:634.
17. Zubler, R. H., and O. Kanagawa. 1982. Requirement for three signals in B cell responses. II. Analysis of antigen- and Ia-restricted T helper cell-B cell interaction. *J. Exp. Med.* 156:415.
18. Brautigen, D. L., S. Ferguson-Miller, and E. Margoliash. 1978. Mitochondrial cytochrome c: preparation and activity of native and chemically modified cytochromes c. *Methods Enzymol.* 53:128.
19. Ashwell, J. A., A. L. DeFranco, W. E. Paul, and R. H. Schwartz. 1984. Antigen presentation by resting normal B cells: radiosensitivity of the antigen presentation function and two distinct pathways of T cell activation. *J. Exp. Med.* 159:881.
20. Mond, J. J., G. Norton, W. E. Paul, I. Scher, F. D. Finkelman, S. House, M. Schaefer, P. K. A. Mongini, C. Hansen, and C. Bona. 1983. Establishment of an inbred line of mice that express a synergistic immune defect precluding in vitro responses to type 1 and type 2 antigens, B cell mitogens, and a number of T cell-drived helper factors. *J. Exp. Med.* 158:1401.
21. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63.
22. Lanier, L. L., G. A. Gutman, D. E. Lewis, S. T. Griswold, and N. L. Warner. 1982. Monoclonal antibodies against rat immunoglobulin kappa chains. *Hybridoma.* 1:125.
23. DeFranco, A. L., E. S. Raveche, R. Asofsky, and W. E. Paul. 1982. Frequency of B lymphocytes responsive to anti-immunoglobulin. *J. Exp. Med.* 155:1523.
24. Ly, L., and R. Mishell. 1974. Separation of mouse spleen cells by passage through Sephadex G-10. *J. Immunol. Methods.* 5:239.
25. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture diseases coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* 15:47.
26. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844.
27. Kimoto, M., and C. G. Fathman. 1980. Antigen-reactive T cell clones. I. Transcomplementing hybrid I-A- region gene products function effectively in antigen presentation. *J. Exp. Med.* 152:759.
28. Sredni, B., and R. H. Schwartz. 1981. Antigen-specific, proliferating T lymphocyte clones. Methodology, specificity, MHC restriction and alloreactivity. *Immunol. Rev.* 54:187.

29. Heber-Katz, E., R. H. Schwartz, L. A. Matis, C. Hannum, T. Fairwell, E. Apella, and D. Hansburg. 1982. The contribution of antigen-presenting cell MHC-gene products to the specificity of antigen-induced T cell activation. *J. Exp. Med.* 155:1086.
30. Mosier, D. E. 1981. Primary *in vitro* antibody responses by purified murine B lymphocytes in serum-free defined medium. *J. Immunol.* 127:1490.
31. Jackson, S., J. A. Sogn, and T. J. Kindt. 1982. Microdetermination of rabbit immunoglobulin allotypes by ELISA using specific antibodies conjugated with peroxidase or with biotin. *J. Immunol. Methods.* 48:299.
32. Kung, J. T., S. Sharrow, D. G. Sieckmann, R. Lieberman, and W. E. Paul. 1981. A mouse IgM allotypic determinant (Igh-6.5) recognized by a monoclonal rat antibody. *J. Immunol.* 114:585.
33. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.
34. Asano, Y., M. Shigeta, C. G. Fathman, A. Singer, and R. J. Hodes. 1982. 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. *J. Exp. Med.* 156:350.
35. Leanderson, T., E. Lundgren, E. Ruuth, H. Borg, H. Persson, and A. Coutinho. 1982. B cell growth factor (BCGF): distinction from T cell growth factor and B cell maturation factor. *Proc. Natl. Acad. Sci. USA.* 79:7455.
36. Lernhardt, W., C. Corbel, R. Wall, and F. Melchers. 1982. T-cell hybridomas which produce B lymphocyte replication factors only. *Nature* (*Lond.*). 300:355.
37. Schimpl, A., and E. Wecker. 1972. Replacement of T cell function by a T cell product. *Nature New Biol.* (*Lond.*). 237:15.
38. Takatsu, K., K. Tanaka, A. Tominaga, Y. Kumahara, and T. Hamaoka. 1980. Antigen-induced T cell-replacing factor (TRF). III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.* 125:2646.
39. Swain, S., G. Dennert, J. Warner, and R. Dutton. 1981. Culture supernatant of a stimulated T cell line have helper activity that syngengizes with IL-2 in the response of B cells to antigen. *Proc. Natl. Acad. Sci. USA.* 78:2517.
40. Pure, E., P. C. Isakson, K. Takatsu, T. Hamada, S. L. Swain, R. W. Dutton, G. Dennert, J. W. Uhr, and E. S. Vitetta. 1981. Induction of B cell differentiation by T cell factors. Stimulation of IgM secretion by products of a T cell hybridomas and a T cell line. *J. Immunol.* 127:1953.
41. Katz, D. H., M. Graves, M. E. Dorf, H. Dimuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VII. Cooperative responses between lymphocytes. VII. Cooperative responses betweeen lymphocytes are controlled by genes in the I region of the H-2 complex. *J. Exp. Med.* 141:263.
42. Jones, B., and C. A. Janeway, Jr. 1981. Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted. *Nature* (*Lond.*). 292:547.
43. Keller, D. M., J. E. Swierkosz, P. Marrack, and J. W. Kappler. 1980. Two types of functionally distinct synergizing helper T cells. *J. Immunol.* 124:1350.
44. Hedrick, S. M., and R. H. Schwartz. 1983. The T cell response to allelic determinants on Igh-1-encoded IgG2a antibodies: dual recognition examined by using antigenic antibodies with binding affinity for Ia molecules. *J. Immunol.* 130:1958.
45. Lonai, P., J. Puri, and G. Hammerling. 1981. H2-restricted antigen binding by a hybridoma clone that produces antigen-specific helper factor. *Proc. Natl. Acad. Sci. USA.* 78:459.
46. Andersson, J., and F. Melchers. 1981. T cell-dependent activation of resting B cells:

requirement for both nonspecific unrestricted and antigen-specific Ia-restricted soluble factors. *Proc. Natl. Acad. Sci. USA.* 78:2497.

47. Delovitch, T. L., J. Watson, R. Battistella, J. F. Harris, J. Shaw, and V. Paetkau. 1981. In vitro analysis of allogeneic lymphocyte interaction. V. Identification and characterization of two components of allogeneic effect factor, one of which displays H-2-restricted helper activity and the other, T cell-growth factor activity. *J. Exp. Med.* 153:107.
48. Julius, M. H., H. von Boehmer, and C. L. Sidman. 1982. Dissociation of two signals required for activation of resting B cells. *Proc. Natl. Acad. Sci. USA.* 79:1989.