ANTIIMMUNOGLOBULIN-TREATED B CELLS RESPOND TO A B CELL DIFFERENTIATION FACTOR FOR IgG1

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The process by which B lymphocytes switch from synthesis of IgM to other isotypes has been characterized on both the cellular and molecular level (1, 2). However, the signals that induce isotype switching and the activation state of the responding B cell are not well understood. A T cell–derived factor (originally termed B cell differentiation factor γ [BCDFγ]) enhances IgG1 secretion from LPS-stimulated B cells (3, 4), and suppresses LPS-induced IgG3 production (5). Some sources of this lymphokine are capable of inducing IgG secretion in the absence of LPS (6), suggesting that B cells activated by stimuli other than LPS are sensitive to the actions of BCDFγ.

A T cell–derived lymphokine called B cell stimulatory factor (BSF-1) induces responsiveness to anti-Ig antibodies (7), and increases Ia expression on resting B cells (8, 9). Recent evidence (10) suggests that BSF-1 is identical to BCDFγ. BSF-1 appears to act at an early stage of anti-Ig stimulation, since cells treated with anti-Ig alone rapidly lose responsiveness to this lymphokine (11). This contrasts with studies on BCDFγ, which indicated that it was most effective on B cells previously stimulated by LPS (5). These data imply that anti-Ig, but not LPS, renders B cells insensitive to BSF-1. In experiments reported here, B cells cultured with anti-Ig (anti-Ig blasts) were found to secrete high levels of IgG1 in response to a supernatant containing a mixture of lymphokines; this response was blocked by monoclonal anti-BSF-1.

Materials and Methods

Mice. BALB/c and C57BL/6 mice were obtained from Cumberland Farms, Clinton TN, and used at 6–12 wk of age.

Reagents. Affinity-purified antibodies of RIA were obtained from Jackson Immunoresearch, Avondale, PA (goat anti–mouse IgM + IgG, and anti-μ chain and anti-γ chain), and from Southern Biotechnology Associates, Birmingham, AL (goat anti-IgG1, -IgG3, -IgG2a, -IgG2b, and goat anti-κ). Myeloma standards were obtained from Litton Bionetics, Charleston, SC (MOPC 141, MOPC 21, J606, and MOPC 195). Monoclonal rat anti-BSF-BSF1 (12) was generously provided by Dr. W. E. Paul (National Institutes of Health, Bethesda, MD), and a control rat monoclonal (anti–mouse λ chain) was provided by Dr. E. Vitetta. Radiochemicals were obtained from New England Nuclear, Boston, MA. *S. typhosa* LPS was purchased from Difco Laboratories, Detroit, MI.

Preparation of B Cell Blasts. Goat anti-mouse Ig or anti-μ chain antibodies were conjugated to cyanogen bromide–activated Sepharose (Sigma Chemical Co., St. Louis,

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MO) at a ratio of 1 mg protein per 1 ml swollen gel. B cells (40 ml at 10^6 cells/ml) were added to a 75 cm^2 flask (Falcon Labware, Oxnard, CA) with 80 μl of packed beads (2 μg protein per milliliter final concentration) and incubated for 24–48 h. Cells and beads were then removed and centrifuged; the pellet was resuspended in RPMI-1640, layered over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ), and centrifuged for 10 min at 400 *g*. The cells were collected from the interface and washed twice. In some experiments, anti-Ig blasts and/or B cells were fractionated on Percoll (Sigma Chemical Co.) density gradients. Anti-Ig blasts were essentially all found in the light-density fractions (<1.065 g/ml), while >75% of splenic B cells were of density >1.080 g/ml.

Blasts were also prepared by culture of B cells with either dextran sulfate (DxS) (50 μg/ml) or 8-mercaptoguanosine (8-MG) (0.5 mM). After 24 or 48 h, cells were washed and recultured in microtiter wells.

T Cell Supernatants. Lymphokine-containing supernatant (SN) was obtained from EL-4 cells as previously described, except serum-free conditions were used (6). This preparation contains substantial BCDFγ/BSF-1 activity, as assayed on LPS-treated B cells (3) and with anti-Ig–induced B cell proliferation (7). For the experiment shown in Table IV, EL-4 SN was passed over a Con A–agarose column. Material that induced proliferation of anti-Ig blasts was found in the flow-through, while BSF-1 was retained and eluted with 0.4 M α-methyl mannoside.

Cell Culture. Splenic B cells were prepared by treatment with monoclonal anti-Thy 1 (kindly provided by Dr. E. S. Vitetta) and baby rabbit complement (Pel-Freeze Biologicals, Rogers, AR). B cells and blasts were cultured in 96-well flat-bottomed plates (4×10^4 cells in 200 μl RPMI-1640 supplemented with 5% FCS [Gibco Laboratories, Grand Island, NY] and 50 μM 2-ME) for 5 d at 37°C in a humidified atmosphere with 10% CO_2.

Radioimmunoassay. Culture supernatant was assayed for Ig isotypes by a solid-phase RIA. Polyvinyl chloride plates (Falcon Labware) were coated with an antibody (goat anti–IgM + IgG), which recognized all tested isotypes. After incubation with sample, the plate was washed and incubated with ^{125}I-labeled isotype-specific antibody. The concentration of Ig was determined by comparison to standard curves of myeloma Ig with a microcomputer program (kindly provided by L. Simpson). The isotype-specific antibodies had negligible (<0.1%) crossreactivity with inappropriate heavy chain isotypes or with light chains.

Proliferation Assay. Anti-Ig blasts were cultured as above for 48 h, then pulsed with [^{3}H]thymidine (1 μCi/well). After 16 h, wells were harvested onto glass fiber filters and radioactivity was assessed by liquid scintillation counting.

Results

Lymphokine Stimulation of IgG Secretion from Anti-Ig Blasts. Splenic B cells were cultured for 48 h with anti-Ig–coated Sepharose beads; after removal of the beads, culture of these blasts with EL-4 SN resulted in a striking increase in the amount of IgG1 secreted into the medium. The pattern of IgG isotypes secreted by normal B cells and anti-Ig blasts stimulated with lymphokines in EL-4 SN alone is shown in Table I. EL-4 SN induced low levels of all IgG isotypes from normal B cells, whereas anti-Ig blasts cultured with EL-4 SN secreted predominantly IgG1 (Table I). Similar results were obtained with B cell blasts prepared with anti-Ig or anti-μ (Table I), and with anti-Ig blasts separated on Percoll density gradients (data not shown). The magnitude of the IgG1 response from anti-Ig blasts was markedly higher than that observed with B cells. In six separate experiments, anti-Ig blasts secreted an average of 3.9 μg/ml IgG1 in response to EL-4 SN, vs. 0.3 μg/ml from splenic B cells. The IgG isotype profile of B cells and anti-Ig blasts cultured with LPS is also shown in Table I. Both cell types yielded similar results: substantial IgG3 secretion, less IgG1, and still lower amounts of IgG2a and IgG2b (Table I).

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TABLE I
Profile of IgG Isotypes Produced by B Cells and B Cell Blasts

Exp.	Cell type	Addition	Isotype (ng/ml)			
			IgG3	IgG1	IgG2a	IgG2b
1	B Cells	None	3	1	2	0
		LPS	1,200	458	86	85
		EL-4 SN	22	105	78	11
1	Anti-μ blasts	None	2	6	2	0
		LPS	632	291	32	55
		EL-4 SN	37	3,883	11	4
2	B cells	None	0	0	6	1
		LPS	417	303	47	46
		EL-4 SN	73	494	48	57
3	Anti-Ig blasts	None	5	16	12	7
		LPS	93	314	34	16
		EL-4 SN	25	3,251	63	38

Blasts were prepared as described in Materials and Methods, and recultured at 2×10^5 cells/ml with LPS (25 μg/ml) or a maximally effective dilution of EL-4 SN; B cells were treated similarly without culture with Sepharose. Results presented are the mean of triplicate wells.

TABLE II
IgG Isotypes Produced by Other B Cell Blasts

Pre-incubated with:	Addition to secondary culture	Isotype (ng/ml)			
		IgG3	IgG1	IgG2a	IgG2b
No addition (24 h)	None	2	0	0	0
	LPS	1,180	280	24	14
	EL-4 SN	17	283	9	4
8-MG (1 mM) (24 h)	None	3	9	2	1
	LPS	360	116	37	16
	EL-4 SN	15	142	75	29
DxS (50 μg/ml) (24 h)	None	2	7	0	0
	LPS	875	430	12	11
	EL-4 SN	35	282	16	3
DxS (48 h)	None	0	0	0	0
	LPS	2,240	519	2	7
	EL-4 SN	59	615	2	0

B cells were cultured for 24 or 48 h at 10^6 cells/ml with the indicated additions, washed and cultured in microtiter plates at 2×10^5 cells/ml for 5 d.

IgG Response of Other B Cell Blasts. The heightened IgG1 response of anti-Ig blasts could be typical of B cell blasts produced in vitro. To examine this possibility, B cells were cultured for 1–2 d with DxS or 8-MG, two chemicals that induce B cell blasts (13, 14). The results of a representative experiment are shown in Table II. The viability of cells cultured for 48 h with 8-MG was poor, so only data for 24-h 8-MG blasts is shown. In contrast to results obtained with anti-Ig blasts, the isotype profile with both 8-MG blasts and DxS blasts were similar to that obtained with normal B cells, except that the DxS blasts secreted very little IgG2a or IgG2b in response to either stimulus.

Anti-BSF-1 Inhibits the IgG1 Response of Anti-Ig Blasts. An mAb against BSF-1 was previously shown (12) to inhibit BCDFγ activity. Preliminary experiments

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TABLE III

Effect of Monoclonal Anti-BSF-1 on Responses of Anti-Ig Blasts

Exp.	Addition	IgG1 (ng/ml)		IgM (ng/ml)	
		Control	Anti-BSF-1	Control	Anti-BSF-1
1	None	<10	<10	700	800
	EL-4 SN	794	16	7,700	6,600
2	None	<10	<10	100	300
	EL-4 SN	2,022	116	20,500	23,100

* B cells were incubated for two days with GAMIg-Sepharose (Exp. 1) or GAMu Sepharose (Exp. 2), then re-cultured for five days in the presence or absence of a suboptimal concentration of EL-4 SN. Either a control antibody or 11B11 anti-BSF-1 were added at a 1:4000 dilution.

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TABLE IV

Proliferative Responses of Anti-Ig Blasts

Exp.	Addition	[^{3}H]Thymidine incorporation (cpm × 10^{-3})	
		Control	Anti-BSF-1
1	None	2.6	2.9
	LPS	27.0	27.2
	EL-4 SN	76.7	67.4
2	None	2.9	1.3
	EL-4 SN	36.6	32.0
	EL-4 SN (Con A eluate)*	3.2	1.4

Anti-Ig blasts were cultured for 2 d, then assayed for incorporation of [^{3}H]thymidine.
* EL-4 SN was passed over a Con A-agarose column and eluted as described in Materials and Methods; a dilution giving half-maximal BSF-1 activity was added.

determined optimal concentrations of antibody required to neutralize BCDFγ activity on LPS-treated B cells (data not shown). When anti-BSF-1 was included in cultures of anti-Ig blasts, the IgG1 response to EL-4 SN was suppressed (Table III). The same wells were also assayed for polyclonal IgM secretion induced by EL-4 SN; anti-BSF-1 had little or no effect on this response, indicating that the inhibition of IgG1 secretion was isotype specific.

Proliferative Responses of Anti-Ig Blasts. It was possible that anti-Ig blasts prepared here proliferated in response to BSF-1, in contrast to previous results (11, 15). This was examined by determining the effect of anti-BSF-1 on proliferation in response to EL-4 SN, and by testing a partially purified preparation of BSF-1 on anti-Ig blasts (Table IV). Crude EL-4 SN caused marked proliferation of anti-Ig blasts, in agreement with the results of Muller et al. (15); addition of anti-BSF-1 had little effect on this response, suggesting that it was not dependent on BSF-1 in the EL-4 SN. The lymphokine that is mitogenic for anti-Ig blasts does not bind to Con A–agarose (L. Simpson and P. Isakson, unpublished data), whereas BSF-1 is bound. As shown in Table IV, the eluate from a Con A–agarose column had little mitogenic activity on anti-Ig blasts; this same fraction had substantial BSF-1 activity in a costimulator assay (7), but did not induce IgG secretion from anti-Ig blasts (data not shown).

Discussion

The T cell–derived lymphokine BSF-1 increases the number of resting B cells that enter S in response to anti-Ig (7, 16). Anti-Ig–pretreated cells are not sensitive to BSF-1 (17), suggesting that BSF-1 acts primarily during early stages of entry into cell cycle. In contrast, studies (5) with BCDFγ, which is apparently identical to BSF-1, showed that it was most effective with previously activated cells. Two important points can be made based on experiments reported here. First, B cells treated with anti-Ig secrete high levels of IgG1 in response to lymphokines present in EL-4 SN. Second, anti-Ig blasts appear to be capable of responding to a lymphokine with the antigenic properties of BSF-1. The latter conclusion is based on the ability of anti-BSF-1 to specifically inhibit IgG1 secretion, and provides strong evidence that anti-Ig blasts are responsive to BSF-1. Thus, treatment with anti-Ig may render B cells insensitive to one effect of BSF-1 (proliferation enhancement) but not another (isotype switching).

The experiment shown in Table IV suggests that BSF-1 alone is incapable of either maintaining proliferation of anti-Ig blasts or inducing IgG1 secretion. Thus, another lymphokine capable of maintaining B cell proliferation and inducing Ig secretion appears to act in concert with BSF-1 on anti-Ig blasts. It should be noted that complete inhibition of IgG1 secretion was not always obtained with anti-BSF-1 (cf. Exp. 2, Table III). A definitive explanation for this finding will require the use of purified lymphokines. The finding that B cell blasts produced by 8-MG or DxS do not show heightened IgG1 responses (Table II) could reflect low responsiveness to either BSF-1 or to another lymphokine.

In conclusion, anti-Ig blasts can be induced to switch isotypes by T cell–derived lymphokines alone. Since anti-Ig blasts do not divide or secrete Ig in the absence of further stimulation, they may provide a useful model for elucidating signals for growth and for stimulation of IgM vs. IgG secretion.

Summary

We have determined whether B cells previously activated by anti-Ig (anti-Ig blasts) are responsive to lymphokines that induce isotype switching. Culture of anti-Ig blasts with a mixture of lymphokines, including BSF-1, resulted in marked secretion of IgM and IgG1, but not other IgG isotypes. The IgG1 response of anti-Ig blasts to lymphokines was 13-fold greater than was observed with splenic B cells. B cell blasts induced by 8-mercaptoguanosine or dextran sulfate did not secrete high levels of any IgG isotype in response to lymphokines alone. An mAb against BSF-1 suppressed the IgG1 response of anti-Ig blasts, but not the IgM response to lymphokines. These data suggest that anti-Ig–treated B cells respond to at least one of the effects of BSF-1.

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References

1. Cebra, J. J., J. L. Komisar, and P. A. Schweitzer. 1984. C_H isotype switching during normal B-lymphocyte development. *Ann. Rev. Imm.* 2:493.
2. Honjo, T. 1983. Immunoglobulin genes. *Ann. Rev. Immunol.* 1:499.
3. Isakson, P. C., E. Pure, E. S. Vitetta, and P. Krammer. 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. *J. Exp. Med.* 155:734.
4. Pure, E., P. C. Isakson, J. W. Kappler, P. Marrack, P. H. Krammer, and E. S. Vitetta. T cell–derived B cell growth and differentiation factors. Dichotomy between the responsiveness of B cells from adult and neonatal mice. *J. Exp. Med.* 157:600.
5. Isakson, P. C., E. Pure, E. S. Vitetta, and P. H. Krammer. 1982. T Cell–derived B cell differentiation factors (BCDF): Definition of BCDFu and BCDFg. *In* B and T Cell Tumors, E. S. Vitetta, editor. Academic Press, New York. 391.
6. Isakson, P. C., and L. Simpson. 1984. Phorbol esters inhibit murine B cell differentiation to immunoglobulin secretion but not proliferation. *J. Immunol.* 133:1788.
7. 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.
8. Noelle, R., P. H. Krammer, J. Ohara, J. W. Uhr, and E. S. Vitetta. 1984. Increased expression of Ia antigens on resting B cells; an additional role for B-cell growth factor. *Proc. Natl. Acad. Sci. USA.* 81:6149.
9. Roehm, N. W., H. J. Leibson, A. Zlotnick, J. Kappler, P. Marrack, and J. C. Cambier. 1984. Interleukin-induced increase in Ia expression by normal mouse B cells. *J. Exp. Med.* 160:679.
10. Vitetta, E. S., J. Ohara, C. Myers, J. Layton, P. J. Krammer, and W. Paul. 1985. Serological, biochemical and functional identity of B cell stimulatory factor-1 and B cell differentiation factor for IgG1. *J. Exp. Med.* 162:1726.
11. Oliver, K., R. J. Noelle, J. W. Uhr, P. H. Krammer, and E. S. Vitetta. 1985. B-cell growth factor (B-cell growth factor 1 or B-cell-stimulating factor, provisional 1) is a differentiation factor for resting B cells and may not induce cell growth. *Proc. Natl. Acad. Sci. USA.* 82:2465.
12. Ohara, J., and W. E. Paul. 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor 1. *Nature (Lond.).* 315:333.
13. Goodman, M., and W. O. Weigle. 1982. Induction of immunoglobulin secretion by a simple nucleoside derivative. *J. Immunol.* 128:2399.
14. Wetzel, G. D., S. L. Swain, R. W. Dutton, and J. R. Kettman. 1984. Evidence for two distinct activation states available to B lymphocytes. *J. Immunol.* 133:2327.
15. Muller, W., R. Kuhn, W. Goldmann, H. Tesch, F. I. Smith, A. Radbruch, and K. Rajewsky. 1985. Signal requirements for growth and differentiation of activated murine B lymphocytes *J. Immunol.* 135:1213.
16. DeFranco, A. L., E. S. Raveche, and W. E. Paul. 1985. Separate control of B lymphocyte early activation and proliferation in response to anti-IgM antibodies. *J. Immunol.* 135:87.

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