B CELL DEPENDENCE ON AND RESPONSE TO ACCESSORY SIGNALS IN MURINE LUPUS STRAINS*

By GÉRALD J. PRUD'HOMME‡, ROBERT S. BALDERAS, FRANK J. DIXON, AND ARGYRIOS N. THEOFILOPOULOS

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

The study of murine systemic lupus erythematosus (SLE)[1] has revealed that several abnormalities controlled by multiple genes play a role in this autoimmune disease (1–4). Both B and T cell abnormalities have been reported, and the relative importance of a given defect varies from strain to strain (1, 2, 5, 6). For example, T helper cell hyperfunction plays a dominant role in the disease of MRL/l mice (6–10), whereas primary B cell abnormalities have been identified in BXSB, NZB, and (NZB × NZW)F_1 (NZB/W) mice (1, 2, 6, 11–14). In fact, these latter mice, unlike MRL/l mice (7, 9), develop SLE despite neonatal thymectomy (1, 2, 7, 15). Additionally, male BXSB early-life SLE can be transferred with anti-Thy-1.2 + complement (C) treated male bone marrow cells given to thymectomized, lethally irradiated female BXSB recipients.[2]

It is now clear that normal B cells require several signals to become activated, to proliferate, and to undergo differentiation to antibody-producing cells (16, 21). In this study we have used in vitro assays for which two or more identifiable signals are necessary to obtain B cell responses and compared lupus mice versus normal control mice in regard to: (*a*) the number of signals required for activation and differentiation and (*b*) the magnitude of responsiveness to these signals. Our hypothesis was that hyperactive B cells from SLE mice might bypass certain of the signals required by normal B cells, i.e., they might need fewer stimuli to differentiate or they might respond abnormally to the stimuli.

We have found that B cells from young BXSB and NZB/W mice give heightened responses to almost all stimuli tested. In particular, defects were noted in the regulation of IgM to IgG switching. Nevertheless, we found no evidence that the B cells of SLE mice could proliferate or secrete antibody in the absence of appropriate T cell- and/or macrophage-derived signals. These findings indicate that primary B cell defects

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‡ Supported by a Medical Research Council of Canada Research Fellowship. Present address: Gérald J. Prud'homme, M.D., McGill Cancer Center, McGill University, Montreal, Canada H3G 1Y6.

[1] *Abbreviations used in this paper:* B6, C57BL/6 mice; BCGF, B cell growth factor; C, complement; CAS, concanavalin A-derived spleen cell supernatants; Con A, concanavalin A; FCS, fetal calf serum; IgSC, Ig-secreting cells; L-BCDF, MRL/l lymph node-derived supernatants containing B cell differentiation factor(s); LPS, lipopolysaccharide, MLC-SN, supernatant from mixed lymphocyte culture; TI-1, thymus independent class 1 antigen; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes; TRF, T cell replacing factor.

[2] Hang, L. M., S. Izui, A. N. Theofilopoulos, and F. J. Dixon. Transfer of BXSB male SLE by T-depleted male bone marrow cells. Manuscript in preparation.

found in some, but not all, SLE strains of mice depend on accessory cell-derived signals for expression.

Materials and Methods

Mice. C57BL/6 ($H\text{-}2^b$), BALB/c ($H\text{-}2^d$), C3H/St ($H\text{-}2^K$), DBA/2 ($H\text{-}2^d$), and NZB/W ($H\text{-}2^{d/z}$) mice are bred and maintained in the Scripps Clinic animal colony. BXSB ($H\text{-}2^b$) and MRL/Mp-*lpr/lpr* (MRL/l, $H\text{-}2^k$) mice, which were developed by Murphy and Roths (3) and originally obtained from The Jackson Laboratory, Bar Harbor, Maine, are bred and maintained in the Scripps Clinic animal colony. All mice used in this study were 20–25-d old females, except the BXSB strain from which early SLE developing male mice of the same age were used. The mortality rates, serologic, histologic, and cellular characteristics of the SLE strains have been reviewed elsewhere (1).

Preparation of T Cell-depleted Populations and Culture Conditions. To deplete T cells, spleen cell suspensions were treated with monoclonal anti-Thy-1.2 (NEI-001, New England Nuclear, Boston, MA) and guinea pig C. This treatment completely abrogated the response to concanavalin A (Con A). In some experiments spleen cells were treated with a combination of monoclonal anti-Thy-1.2, anti-Lyt-1, and anti-Lyt-2 (all from New England Nuclear) and guinea pig C. For assaying responses to various stimuli, B cells (10^4 to 5×10^5 cells/250 μl medium) were incubated in 96 well, flat-bottomed microtiter plates (Falcon 3072, Becton Dickinson, Oxnard, CA). Medium consisted of RPMI 1640 supplemented with 25 mM Hepes (M. A. Bioproducts, Walkersville, MD) 50 μM 2-mercaptoethanol, penicillin (50 units/ml)-streptomycin (50 μg/ml) mixture (M. A. Bioproducts, Walkersville, MD), and 10% fetal calf serum (FCS) (lot 29101276, Flow Laboratories). Cells were cultured in the presence of 7% CO_2 at 37°C.

In some experiments B cells were activated with bacterial lipopolysaccharide (LPS) or with Sepharose-bound anti-μ. LPS R595 derived from *Salmonella minnesota* (Calbiochem-Behring Corp., La Jolla, CA) was used at a concentration of 2.5 μg/ml. The preparation of Sepharose-bound affinity purified anti-μ has been described (10). Anti-μ was added to cultures at a concentration of 2.5 μg per well.

Proliferative responses to LPS or anti-μ were assayed on day 3 of culture, after an 18-h incubation with 1 μCi [^{3}H]thymidine per well.

Plaque-forming Cell Assay. Ig secreting cells (IgSC) were quantitated with a reverse hemolytic plaque assay as previously described (6). IgSC were counted after 4 d of culture.

Production of Supernatants. Con A supernatant (CAS) and a B cell differentiation factor secreted spontaneously by cultured lymph node T cells of older MRL/l mice (L-BCDF) were produced as previously described (10). Briefly, CAS was derived from C57BL/6 (B6) spleen cells (6–10-wk old mice) incubated for 2 h with 4 μg/ml of Con A (Miles Laboratories, Elkhart, IN), washed, and re-cultured for 24 h in the absence of Con A. L-BCDF was produced by incubating MRL/l lymph node cells from 4-mo old mice for 24 h in medium without mitogens. CAS and L-BCDF were added at a concentration of 40% vol/vol to cultures, since previous titration studies (10) have shown at this concentration optimum responses of activated B cells from several normal and autoimmune strains.

Radioimmunoassays. The radioimmunoassays used to measure IgM, IgG1, IgG2a, IgG2b, and IgG3 in culture supernatants have been described (10, 22). Briefly, for the IgM assay polystyrene plates were coated with goat anti-mouse μ-chain specific antibody, after which various dilutions of each sample were added to the wells. Bound IgM was detected with ^{125}I-goat anti-mouse μ antibody.

The IgG isotype-specific radioimmunoassays were direct binding assays. Plates were incubated with 100% supernatant, followed by addition of one of the following rabbit anti-mouse antibodies: Anti-IgG1, anti-IgG2a, anti-IgG2b, or anti-IgG3, all purchased from Litton Bionetics (Kensington, MD). The assay was developed with ^{125}I-labeled goat anti-rabbit IgG. Specific absorptions were performed to reduce the cross-reactivity of the antibodies, and standard curves were established for each assay by coating the plates with graded amounts of mouse myeloma proteins of each isotype and subclass, also purchased from Litton Bionetics.

In Vitro Responses to Sheep Erythrocytes (SRBC). This assay was performed at a B cell density of 2×10^5 cells/ml as described by Zubler and Glasebrook (21). Cells were cultured in medium as

mentioned above (10% FCS) in 24 well, 3524 Costar plates (Costar, Cambridge, MA) in the presence or absence of SRBC, LPS, CAS, or L-BCDF. SRBC were added at a concentration of 2×10^6/ml; LPS was added at a concentration of 2.5 μg/ml; CAS and L-BCDF were added at 40% vol/vol. After 5 d of culture at 37°C with 7% CO_2, without rocking, indirect anti-SRBC plaque were assayed.

Results

LPS- and Anti-μ-mediated Proliferation in Low and Medium Density Cultures. In a previous study, we reported on the proliferative responses of splenocytes from various murine strains to LPS and anti-μ in high cell density cultures (5×10^5/250 μl) (12). At that density there was no consistent difference in proliferative responses of young autoimmune and young normal mice. At the high cell density, cellular confluence or rapid exhaustion of the media may have prevented a maximal proliferative response. In contrast, splenocytes of 1-mo old NZB and NZB/W mice cultured at a medium cell density (10^5 cells/250 μl) in the presence of LPS had 5- to 10-fold higher frequencies of IgSC than spleen cells from the other autoimmune strains or several normal strains (6). In the present study, at medium (10^5 cells/250 μl) and low cell density (5×10^4 cells/250 μl), both BXSB and NZB/W anti-Thy-1.2 + C treated spleen cells showed considerably higher responses to LPS than MRL/l cells or cells from immunologically normal strains (Fig. 1). Nevertheless, at 10^4 cells/250 μl proliferation was undetectable or slightly lower than that of control cells cultured in medium alone in all strains tested, unless CAS was added (Table I). BXSB and NZB/W were more responsive to CAS than other strains under these conditions. The MRL/l Thy-1.2^+, Lyt-1.2^+ proliferating cell product L-BCDF had no effect in this assay.

The proliferative response to anti-μ was even more sensitive to changes in cell density than with LPS. As reported by others (19), we found that normal B cells in low density cultures no longer proliferated in response to anti-μ (Fig. 2). In fact, at 5×10^4 cells/well anti-μ had a slight suppressive effect, giving counts lower than cells in medium alone. B cells of BXSB and NZB/W, but not MRL/l, mice gave higher responses than those of the other strains tested and were the only ones to show a positive response at a density of 5×10^4 cells/well. However, at 10^4 cells/well no proliferation could be detected in any strain tested. This response was not restored by CAS or L-BCDF (data not shown). Proliferation in response to anti-μ is thought to depend on the presence of B cell growth factor (BCGF) (19), presumably produced by residual T cells in high density cultures. BXSB and NZB/W B cells may thus be more sensitive to low levels of BCGF than B cells of other strains.

Effect of CAS and L-BCDF on LPS-induced IgM Secretion in Low Density Cultures. At cell densities as low as 5×10^4 cells/250 μl, in the presence of LPS BXSB and NZB/W B cells produced considerably more IgM than MRL/l, BALB/c, and B6-derived B cells (Table II). This response was enhanced by the addition of either CAS or L-BCDF. At a cell density of 10^4 cells/250 μl there was an abrupt decline in production of IgM in all strains, disproportionately to the decrease in cell numbers. However, even at this low density, IgM production could be enhanced by CAS or L-BCDF, and both BXSB and NZB/W were more responsive to these stimuli than the other strains.

Effect of L-BCDF and CAS on Anti-μ-treated B Cells. Anti-μ alone induced little Ig secretion in any strain tested, regardless of cell density (Fig. 3*A*). When L-BCDF was added, a marked increase in the number of IgSC was noted. BXSB and NZB/W B cells did not differ from normal strains in their requirement for L-BCDF in this assay,

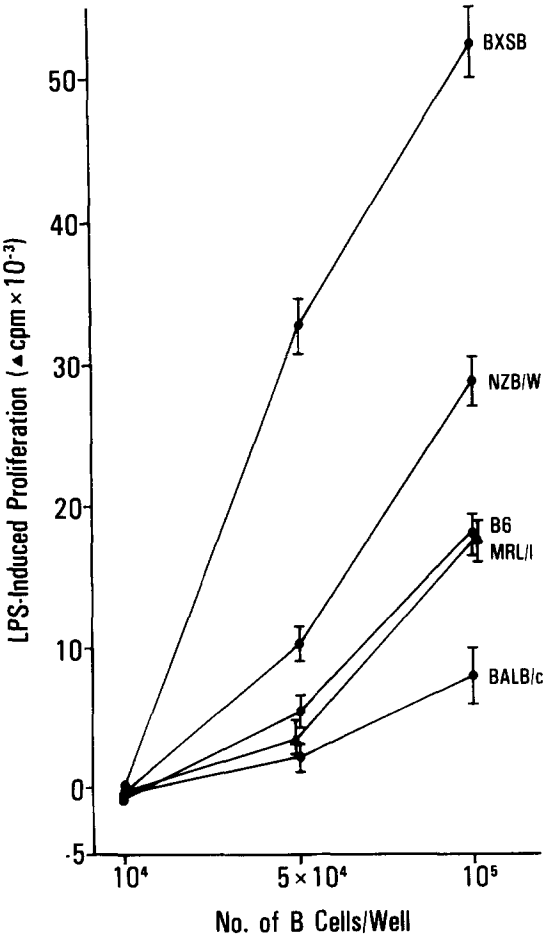

FIG. 1. LPS-induced proliferation in low to medium cell density cultures. Splenic B cells from 20- to 25-d-old mice were cultured at cell densities ranging from 10^4 to 10^5 cells/250 μl for 3 d. Δ cpm ± 1 SD = [^{3}H]thymidine uptake with LPS (2.5 μg/ml) − [^{3}H]thymidine uptake with medium alone. The responses of C3H/St and DBA/2 mice not depicted were similar to the response of B6 and MRL/l mice.

but gave higher responses than other strains. This finding held for concentrations of L-BCDF ranging from 20 to 80% vol/vol (data not shown). The results with anti-μ plus CAS differed in that this response was dependent on cell density (Fig. 3 *B*). Thus, at a cell density of 5 × 10^4 cells/250 μl, none of the strains responded, including BXSB and NZB/W. We have described this cell density effect before (10) and attribute it to a need for residual interleukin-2 dependent T cells in this assay. Thus, at a low cell density, too few residual T cells (and ? macrophages) seemed to be present for a detectable CAS-mediated response. Yet at higher cell densities (1–5 × 10^5 cells/250 μl), activated B cells of BXSB and NZB/W mice showed higher responses to CAS than MRL/l or normal mice.

CAS and L-BCDF Induced Ig Secretion in Nonmitogen-Treated B Cells. In this assay both CAS and L-BCDF increased the number of IgSC. The responses of B cells from BXSB and NZB/W mice were several-fold higher than those of control mice (Table III). It

TABLE I
LPS-Induced Proliferation in Low Density Cultures

B cell origin	[^{3}H]Thymidine uptake (Δ cpm)*					
	10^4 cells/well			5×10^4 cells/well		
	LPS‡	LPS + CAS	LPS + L-BCDF	LPS	LPS + CAS	LPS + L-BCDF
BALB/c	−176	926	−283	2,387	5,254	2,108
B6	−665	647	−701	5,391	10,782	4,900
BXSB	8	3,072	−55	32,877	48,021	33,720
NZB/W	−42	1,845	83	10,410	26,042	11,215
MRL/l	41	983	−19	3,690	10,490	2,711

* Assayed at day 3 of culture.

‡ LPS was added at a concentration of 2.5 μg/ml. CAS or L-BCDF was added at a concentration of 40% vol/vol. Varying the concentration of L-BCDF from 10 to 80% vol/vol did not significantly alter the results. Values listed represent the mean of three experiments and were derived by substraction of proliferative responses in cultures of cells with medium alone. Negative values indicate lower responses than those obtained with cells in medium alone.

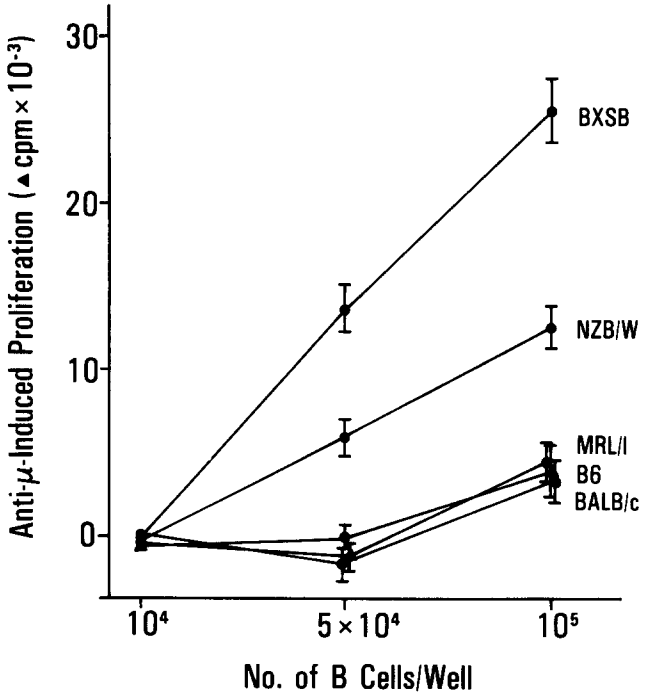

FIG. 2. Anti-μ induced proliferation in low to medium cell density cultures. See the legend to Fig. 1 for experimental conditions. Δ cpm ± 1 SD = [^{3}H]thymidine uptake with Sepharose-bound anti-μ (2.5 μg anti-μ/well) − [^{3}H]thymidine uptake with medium. The response of other normal strains tested not depicted (C3H/St, DBA/2) were similar to the response of BALB/c, B6, and MRL/l mice.

has been suggested that only activated B cells respond to antigen nonspecific helper factors (21). If this is the case, then BXSB and NZB/W mice may have an increased number of spontaneously activated B cells as others have indicated (13). On the other

TABLE II
LPS-Induced IgM Secretion in Low Density Culture

Strain of origin of B cells	IgM (ng/ml)*							
	10^4 cells/well				5×10^4 cells/well			
	Medium	LPS	LPS + CAS	LPS + L-BCDF	Medium	LPS	LPS + CAS	LPS + L-BCDF
BALB/c	<10	12	50	52	51	230	1,023	603
B6	<10	19	86	61	50	674	1,648	1,362
BXSB	<10	28	192	171	45	1,368	2,044	1,860
NZB/W	<10	30	180	153	66	1,056	2,072	1,632
MRL/l	<10	18	82	67	47	613	1,432	1,211

* IgM was measured by radioimmunoassay after 6 d of culture. See Table I for culture conditions. The results represent the mean of three experiments. One SD did not exceed 12% of the mean for values above 10 ng/ml. BXSB and NZB/W gave higher responses than other strains at all concentrations of CAS and L-BCDF tested (10–80%).

hand, activation may occur in vitro in this assay due to the possible presence of mitogens, or antigens (e.g., FCS) in the culture medium.

Response to SRBC in Low Density Cultures. Zubler and Glasebrook (21) have demonstrated that in low cell density cultures (2×10^5 B cells/ml) the addition of CAS, mixed lymphocyte culture supernatant (MLC-SN), or the products of cloned T cell lines was insufficient to reconstitute the primary in vitro response to SRBC. Even in the presence of both LPS and SRBC, no response was observed. However, with three signals present, i.e., antigen (SRBC), LPS, and CAS or MLC-SN, a response was elicited. We have obtained similar results using B cells from several strains and we believe that, in this assay, LPS replaces a signal normally mediated by T cells or macrophages but absent from CAS.

As can be seen from Table IV, all strains tested required the three signals (SRBC, CAS, and LPS) to give a high response. However, BXSB and NZB/W B cells differed from those of the other mice by generating responses of disproportionately greater magnitude. This finding is consistent with the higher sensitivity of the latter B cells to both LPS and CAS. However, we cannot exclude that other genetically determined factors may contribute to this hyperresponsiveness. Interestingly, L-BCDF could not replace CAS in this assay.

IgG Isotypes Produced Following LPS Stimulation. We previously reported that LPS-activated B cells produce increased amounts of IgG1, IgG2a, and IgG2b when L-BCDF is added to the cultures (10). We also reported and confirm in the present study (Table V) that MRL/l B cells produced increased amounts of IgG2a in response to LPS, possibly due to their in vivo exposure to L-BCDF. BXSB and NZB/W B cells produced much more IgG than normal strains or MRL/l B cells in response to LPS (Table V). This result paralleled their high proliferative response and high IgM production stimulated by LPS. In addition, LPS activated BXSB and NZB/W B cells produced higher percentages of IgG2a and IgG2b, but less IgG3, than control B cells.

These results resemble those we obtained following in vivo thymus independent-1 (TI-1) antigen injections (23). This high level of IgG2 production could reflect an increased rate of switching secondary to the magnified proliferative response to LPS

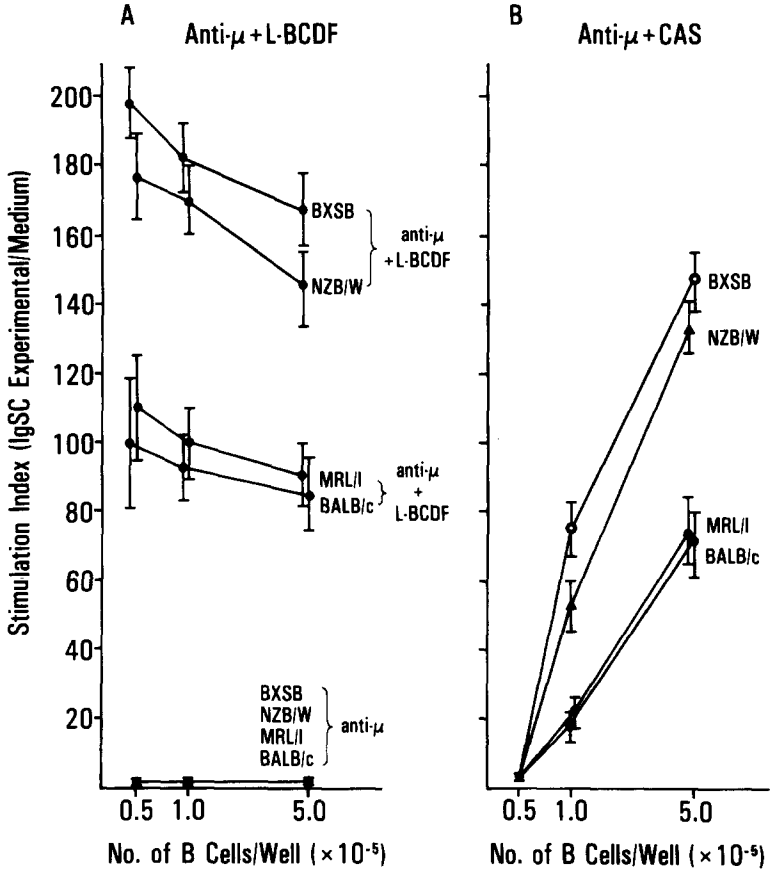

FIG. 3. Anti-μ induced differentiation. (*A*) Splenic B cells from various strains were cultured for 4 d in the presence of Sepharose-bound anti-μ (2.5 μg/well) at cell densities ranging from 5×10^4 cells/250 μl to 5×10^5 cells/250 μl, in the absence or presence of 40% vol/vol L-BCDF. Stimulation index ± 1 SD = Experimental/medium control, as assessed by number of IgSC per well. With anti-μ alone, the response of all strains was low and closely similar. (*B*) Response at various cell densities to anti-μ + 40% vol/vol CAS. Other normal strains tested (B6, C3H/St) did not differ significantly from BALB/c and MRL/l.

TABLE III

CAS and L-BCDF-Induced Ig Secretion in Nonmitogen-treated B Cells

			IgSC per culture*			
Treatment	C3H/St	B6	BALB/c	MRL/l	BXSB	NZB/W
Medium	1 ± 2	8 ± 2	6 ± 3	9 ± 3	6 ± 3	6 ± 3
CAS	14 ± 4	19 ± 5	15 ± 3	18 ± 4	195 ± 9	89 ± 8
L-BCDF	71 ± 11	92 ± 12	65 ± 7	87 ± 10	348 ± 21	306 ± 8

* 10^5 B cells per well were cultured for 4 d after which IgSC were counted using a reverse hemolytic plaque assay. Results represent the mean ± 1 SD of three experiments.

in these mice. Nevertheless, when L-BCDF was added, B cells of BXSB and NZB/W mice considerably increased their production of total IgG as did B cells of MRL/l and of normal mice. Addition of L-BCDF to normal B cells resulted in a proportional

TABLE IV

Response to SRBC in Low Density Cultures

Treatment*	Indirect anti-SRBC PFC/culture‡				
	B6	BALB/c	MRL/1	NZB/W	BXSB
Medium	14 ± 13	10 ± 2	12 ± 2	12 ± 2	13 ± 2
SRBC + CAS	80 ± 7	72 ± 5	75 ± 5	82 ± 6	84 ± 8
SRBC + LPS	75 ± 5	68 ± 6	62 ± 5	81 ± 5	94 ± 7
CAS + LPS	95 ± 8	80 ± 5	82 ± 6	114 ± 6	118 ± 7
SRBC + CAS + LPS	830 ± 60	792 ± 72	802 ± 58	1,784 ± 80	1,860 ± 60
SRBC + L-BCDF + LPS	62 ± 6	54 ± 4	44 ± 5	77 ± 4	79 ± 6

* 2×10^5 B cells/ml from each of the listed strains were cultured for 5 d. SRBC were added at a concentration of 2×10^6/ml. LPS was added at a concentration of 2.5 μg/ml, and CAS or L-BCDF were added at a concentration of 40% vol/vol. Varying the concentration of L-BCDF from 10 to 80% had no additional effect.

‡ Less than 50 plaque-forming cells (PFC) per culture were generated when only one of SRBC, CAS, LPS, or L-BCDF was added. Results represent the mean ± 1 SD of three experiments.

TABLE V

IgG Subclasses Produced Following LPS Stimulation with or without L-BCDF

Strain of origin of B cells cultured with LPS*	Supernatant‡	Total IgG§ (ng/ml)	Subclasses secreted (percentage of total IgG)			
			IgG3	IgG1	IgG2b	IgG2a
BALB/c‖	—	33	50.1	6.1	25.0	18.8
	L-BCDF	227	6.6	18.5	49.3	25.6
BXSB	—	797	4.2	10.5	61.7	23.6
	L-BCDF	1,000	3.0	26.7	46.9	23.4
NZB/W	—	341	8.2	13.8	46.3	31.7
	L-BCDF	723	6.4	29.0	41.5	23.1
MRL/l	—	55	16.4	7.3	30.9	45.4
	L-BCDF	560	2.8	16.1	27.5	53.6

* Splenic B cells were cultured at a density of 5×10^4 cells/well for 6 d in the presence of 2.5 μg/ml of LPS.

‡ L-BCDF was added at a concentration of 40% vol/vol.

§ Total IgG is the sum of the values obtained for the 4 IgG subclasses. IgG subclasses were determined by radioimmunoassay. The results represent the mean of three experiments.

‖ The response of B6, C3H/St, and DBA/2 B cells not depicted were similar to that by BALB/c cells.

decrease of IgG3 and increased IgG1, IgG2b, and IgG2a. In SLE mice a proportional increase in IgG1 was most apparent. This demonstrates that Ig switching mechanisms in autoimmune mice can still respond to T cell-derived signals.

Discussion

B cell hyperactivity is a feature common to all murine SLE strains. In this study we compared the responses of B cells from MRL/l, NZB/W, and BXSB autoimmune mice in a number of in vitro assays. We chose assays in which two or more identifiable stimuli or signals were necessary to obtain a response. Our basic hypothesis was that hyperreactive B cells might be capable of responding in the absence of one or more signals that are normally required for activation, proliferation, or differentiation of B cells. We made use of the fact that in very low density cultures (10^4 B cells/250 μl), normal B cells virtually do not proliferate or secrete Ig after LPS stimulation (24), unless accessory signals found in CAS are added. Similarly, B cells do not proliferate

in response to anti-μ in low cell density cultures, and do not secrete Ig at any cell density, unless T cell-derived factors are provided (19, 25). Presumably, LPS and anti-μ prime the B cells by inducing them to express receptors for antigen nonspecific factors that mediate proliferation (e.g., BCGF) or differentiation (e.g., T cell replacing factor [TRF], BCDFs) (18, 19, 21, 25–27). CAS is known to contain several lymphokines, which include interleukins (IL-1, IL-2), BCGF, and conventional TRF. Because of this complexity in several assays, we have also tested the response to L-BCDF, a factor we recently identified (10). This factor is produced by the proliferating T cells of autoimmune MRL/l mice and induces differentiation of anti-μ- or LPS-activated B cells, in the apparent absence of macrophages or T cells, while having little or no effect on B cell proliferation. This L-BCDF lacks IL-2, BCGF, or conventional TRF activity (10) as well as macrophage-activating factor, interferon, IL-1, and macrophage Ia recruiting factor (unpublished results). Some authors have described production of BCGF (19), BCDFs (26), or TRF (28) by cloned T cell lines. Unfortunately, such T cell lines may produce several factors (19, 29, 30), and purified products are not yet available for study. For this reason, we have not at the present extended our study to analyze lymphokines derived from T cell lines.

We have also looked at the in vitro anti-SRBC response of autoimmune strains since B cells are known to require several signals to respond to this antigen. In fact, it has been shown that in low density cultures, even in the presence of LPS, three signals are required to induce an anti-SRBC PFC response (21).

The mice used to study B cell function in this study were all 20–25-d old. This age range was chosen because none of the strains tested express significant disease at this early age. Thus, B cell abnormalities found at this age are unlikely to be secondary to the presence of circulating immune complexes, to the presence of antilymphocytic antibodies, or to other factors associated with autoimmunity.

We found that BXSB and NZB/W, but not MRL/l, B cells have higher proliferative responses to anti-μ or LPS than normal mice. However, at a cell density of 10^4 cells/250 μl proliferation was undetectable in any of the strains tested. Nevertheless, even at this low cell density, CAS induced a small degree of proliferation in LPS-activated B cells. Thus, such proliferation in BXSB and NZB/W mice, although apparently abnormally regulated, is not independent of accessory signals found in CAS.

The same situation holds for Ig secretion. BXSB and NZB/W secreted abnormally large amounts of IgM and IgG in response to LPS. However, at 10^4 cells/250 μl Ig secretion decreased sharply, disproportionately to the decreases in cell numbers, unless CAS or the T cell-derived factor L-BCDF was added. The results were similar following incubation with anti-μ. Here the situation was more clear-cut since anti-μ induced little Ig secretion in any strain tested, regardless of cell density, unless CAS or L-BCDF was added. These results suggest that the B cells of NZB/W and BXSB mice cannot bypass differentiation signals, presumably of T cell origin in this case.

Nonmitogen-stimulated B cells of BXSB and NZB/W mice also responded much better in terms of IgSC, to CAS and L-BCDF stimulation than MRL/l and normal mice. This may reflect a higher percentage of spontaneously activated in vivo B cells in NZB/W and BXSB as others have proposed (13). On the other hand, this result may indicate that antigens (e.g., FCS) and possible mitogens (e.g., 2-ME) present in the culture medium have a limited ability to activate B cells.

The pattern of IgG subclass expression in response to LPS was clearly abnormal in

both BXSB and NZB/W mice. Thus, both strains produced higher levels of IgG2a and IgG2b, but lower levels of IgG3, than normal mice. As we previously reported (10), MRL/l mice also secreted an unusually large amount of IgG2a in this assay. The reason for these abnormal responses in autoimmune mice is not clear but does not necessarily represent a primary defect in switching mechanisms as such. For example, in vivo exposure to soluble mediators could influence in vitro responses of B cells. On the other hand, the high rate of proliferation by BXSB and NZB/W B cells in the presence of LPS may give rise to a higher number of switching events resulting in more IgG2 secretion. In any case, the addition of L-BCDF increased IgG1 secretion considerably in all strains tested. Thus, switching from IgM to IgG1 synthesis appears to remain T dependent in autoimmune mice, as in normal mice (31, 32). IgG2 production paralleled the degree of proliferation, but IgG1 production did not, suggesting that the signals required for IgM → IgG2a, 2b switching are different than those required for IgM → IgG1 switching. The results we have obtained after LPS stimulation in vitro closely simulate the in vivo response to TI-1 antigens, which we previously reported (23). The large content of IgG subclasses 2a and 2b may be explained partially by the high rate of cell division in those mice following an LPS-like stimulus. Nevertheless, IgG1 synthesis remains highly T dependent.

In the LPS-SRBC assay, in accordance with the studies of Zubler and Glasebrook (21), we found that all strains tested, including autoimmune strains, required LPS, SRBC, and CAS to respond. It is noteworthy that, unlike standard TRF assays, this assay was performed at low cell density (2×10^5 cells/ml). Interestingly, L-BCDF could not replace CAS in this assay. This shows that an additional signal(s) not present in L-BCDF, either of T cell or macrophage origin, is necessary to induce a response in this assay. Candidates as the source of this signal include IL-1, IL-2, BCGF, or the recently described IL-X (20). In any case, BXSB, NZB/W and MRL/l B cells apparently required the same signals as normal B cells in order to respond.

The findings described above lead to a number of conclusions: (*a*) B cell proliferation and differentiation in lupus mice remain dependent on accessory signals of either macrophage or T cell origin. (*b*) BXSB, NZB/W, and MRL/l B cells appear to require the same number of signals as normal B cells to undergo polyclonal or antigen-directed responses. BXSB and NZB/W, but not MRL/l, B cells differ from normal B cells only by the higher sensitivity (or degree of response) to the signals they receive. (*c*) The regulation of IgM to IgG switching in response to LPS in vitro is disordered in NZB/W and BXSB mice, but IgG1 production remains largely T dependent in all SLE strains as in normal mice.

The reason for this higher sensitivity of BXSB and NZB/W B cells to the immunogenic signals they receive have not been established in the present study. It could be caused by better activation by the mitogens or a better response to T cell-derived factors. The first possibility is less likely since our studies (12) and those of Raveche et al. (13) indicate that B cells of 1-mo-old BXSB and NZB/W mice do not show different patterns of LPS- or anti-μ-induced activation, i.e., cells in the $S+G_2+M$ phase of the cell cycle, than normal B cells. Furthermore, Lyb-5^+ B cells, which are thought to respond to anti-μ are present in normal or near normal numbers in SLE mice (12). Thus, it appears that the increased response of BXSB and NZB/W B cells is not due to recruitment of more B cells by mitogens, but rather to a true hyperre-

sponse of their activated B cells to accessory T cell-derived signals. We are presently investigating the attractive possibility that these B cells may express an unusually high number of membrane receptors, or have receptors with a higher affinity for antigen nonspecific factors than normal B cells. However, the abnormality may also reside at the level of regulatory genes that control cell responsiveness after receipt of a signal. Another unanswered question is the role of T and other accessory cell defects in this genetic disease of BXSB and NZB/W mice. One could speculate that these animals' abnormal B cells are hyperresponding to normal levels of accessory cell signals, but overproduction of accessory signals may also occur that could play a role in magnifying Ig secretion and inducing IgM to IgG switching.

To summarize, it appears that B cell hyperactivity in murine strains with SLE-like disease is not caused by bypassing certain accessory signals necessary for B cell proliferation and differentiation. Our present and published studies suggest that in BXSB and New Zealand strains of SLE mice there is an inherent hyperresponsiveness of B cells to external stimuli. In contrast, in MRL/l mice the B cells do not hyperrespond to accessory signals but their proliferating Thy-1.2$^+$, Lyt-1$^+$, -2$^-$ cells hyperproduce B cell differentiation factors. Such abnormal responses to accessory signals or overproduction of the signals ultimately lead to polyclonal and autoantigen specific B cell expansion and Ig gene rearrangement that result in the production of pathogenic IgG type autoantibodies and disease.

Summary

B cell hyperactivity, a feature common to all lupus-prone murine strains, may be caused by hyperresponsiveness to, overproduction of, or bypassing of certain signals required for B cell activation, proliferation, and differentiation. In this study, we have compared the responses of B cells from three lupus-prone strains of mice (BXSB males, MRL and NZB/W females) and normal strains in a number of assays for which two or more signals are required to obtain a response. In medium to low density cultures of B cells from BXSB and NZB/W but not MRL/l lupus mice, the cells' proliferation induced by bacterial lipopolysaccharide (LPS) or anti-μ antibody was much higher than that of B cells from normal controls. At low B cell density, polyclonal activation by these substances and subsequent Ig secretion were dependent on accessory signals present in supernatants of concanavalin A-treated normal lymphocytes (CAS) or on the MRL/l proliferating T cell-derived B cell differentiation factor (L-BCDF) in both lupus-prone and immunologically normal mice. However, the responses of B cells from BXSB and NZB/W, but not MRL/l, mice to these accessory signals were higher than those of normal mice. Ig synthesis by fresh B cells of BXSB and NZB/W mice cultured in the absence of mitogens but in the presence of CAS or L-BCDF was higher than by similar cells from other strains, suggesting an increased frequency of B cells activated in vivo in these two autoimmune strains of mice. The patterns of IgG subclass secretion in response to LPS (without added CAS or L-BCDF) were abnormal in all lupus strains, with a predominance of IgG2b and/or IgG2a and low levels of IgG3, contrary to normal B cells for which IgG3 synthesis predominated. However, IgG1 synthesis in vitro by autoimmune and normal B cells alike was highly dependent on T cell-derived soluble mediators. Antigen-specific responses to SRBC in vitro of B cells from all lupus strains, like those of B cells from normal strains, required a minimum of three signals (antigen, LPS, T cell-derived

antigen nonspecific helper factors). Yet, once triggered, B cells of BXSB and NZB/W mice gave higher responses than those of the other strains.

We conclude that B cells of lupus mice have signal requirements similar to those of normal mice. Nevertheless, B cells of BXSB and NZB/W, but not MRL/l, lupus mice hyperrespond or process some accessory signals abnormally.

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