

IDENTIFICATION OF A B CELL DIFFERENTIATION  
FACTOR(S) SPONTANEOUSLY PRODUCED  
BY PROLIFERATING T CELLS IN MURINE LUPUS  
STRAINS OF THE *lpr/lpr* GENOTYPE\*

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The MRL/Mp-*lpr/lpr* (MRL/l) mouse develops a T cell lymphoproliferative syndrome associated with a systemic lupus erythematosus (SLE)<sup>1</sup>-like disorder (1). The disease is marked by massive lymph node enlargement, hypergammaglobulinemia, and production of multiple autoantibodies that include anti-DNA, anti-gp70, and rheumatoid factors (2).

The proliferating cells of MRL/l mice are of the Thy-1<sup>+</sup>, Lyt-1<sup>+</sup> phenotype (2-4). The thymus dependency of these cells is substantiated by the finding that neonatal thymectomy abrogates both the lymphoproliferation and autoimmune disorder (5, 6). The disease complex of MRL/l mice can be attributed to a single autosomal recessive gene locus, named *lpr* (1). Thus, mice of the congenic strain MRL/Mp-+/+ (MRL/n), which lack the *lpr* gene but share >98% of their genome with MRL/l mice, do not develop lymphoproliferation and develop autoimmunity late in life (1, 2).

Previous studies revealed that MRL/l T cells provide increased antigen-nonspecific help to B cells (7) and promote anti-DNA antibody production (8). This increase in helper activity occurs despite MRL/l T cells' markedly lower production of interleukin 2 (IL-2) (9-11).

In this paper we document the production of a B cell differentiation factor(s) (BCDF) *in vitro* by MRL/l T cells in the absence of mitogenic stimulation. The factor is not produced by lymphoid cells from the non-*lpr*-bearing congenic MRL/n strain or from several immunologically normal murine strains. A BCDF is also spontaneously

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<sup>1</sup> *Abbreviations used in this paper:* BCGF, B cell growth factor; BSA, bovine serum albumin; C, complement; CAS, Con A supernatant; Con A, concanavalin A; FCS, fetal calf serum; HCD, high cell density; IgSC, immunoglobulin-secreting cell; IL-2, interleukin 2; L-BCDF, B cell differentiation factor produced by lymphoid cells of mice of *lpr/lpr* genotype; LCD, low cell density; LPS, lipopolysaccharide; MRL/l-SN, supernatants derived from MRL/l lymphoid cells; PBS, phosphate-buffered saline; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes; TRF, T cell replacing factor.

produced by T cells of the autoimmune C57BL/6J-*lpr/lpr* (B6/l) strain. Although T cells synthesize a variety of lymphokines in response to T cell mitogens or to T-dependent antigens, in mixed lymphocyte culture or when cloned (12), to our knowledge this represents the first example of a T helper factor produced spontaneously by unstimulated and uncloned T cells.

In view of its origin from mice of the *lpr/lpr* genotype, we term this B cell differentiation factor(s) L-BCDF. L-BCDF increases immunoglobulin (Ig) secretion in unstimulated B cells; however, it has a much greater effect on anti- $\mu$ - or lipopolysaccharide (LPS)-activated B cells. Our experiments do not establish whether only one factor is present that acts on both stimulated and unstimulated B cells. This question is difficult to resolve because even "unstimulated" B cells may contain a small percentage of B cells activated *in vivo* or in culture (e.g., by fetal calf serum [FCS]). L-BCDF induces both IgM and IgG production and, interestingly, acts in the absence of either IL-2 or B cell growth factor (BCGF). Moreover, L-BCDF has no conventional T cell replacing factor (TRF) activity. Our results point to an association of the *lpr* gene with T cell proliferation, production of a BCDF, and autoimmunity.

### Materials and Methods

*Mice.* C3H/St (H-2<sup>k</sup>), C57BL/6 (H-2<sup>b</sup>), and BALB/c (H-2<sup>d</sup>) are bred and maintained in our colony. MRL/l (H-2<sup>k</sup>) and MRL/n (H-2<sup>k</sup>) were developed by Dr. E. M. Murphy and originally obtained from The Jackson Laboratory, Bar Harbor, ME, but were bred and maintained thereafter in our colony. C57BL/6J-*lpr/lpr* (B6/l) mice were kindly given to us by Dr. D. Johnson (Scripps Clinic and Research Foundation, La Jolla, CA) and also originated from The Jackson Laboratory. Mice used in these experiments were 6- to 18-wk-old females, unless otherwise indicated.

*Preparation of T Cell- and Macrophage-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 complement (C). This treatment completely abrogated the response to concanavalin A (Con A). Such T cell-depleted populations contained between 5 and 10% macrophages as determined by nonspecific esterase staining (13) or by indirect immunofluorescence with a monoclonal rat anti-mouse macrophage antibody (M1/70HL, lot E22800; Hybritech Inc., La Jolla, CA) (14). In a few experiments (see Results), such contaminating macrophages were removed by passage of the T cell-depleted populations over a Sephadex G-10 column as described (15). After such passage, the effluent cells contained <1% positive cells by nonspecific esterase and <0.3% by the monoclonal anti-Mac antibody.

For assaying responses to supernatants from splenic or lymph node cell cultures, B cells ( $2 \times 10^5$ – $2 \times 10^6$  cells/ml) were incubated in 96-well, flat-bottomed microtiter plates (3072; Falcon Labware, Becton Dickinson & Co., Oxnard, CA). Medium consisted of RPMI 1640 supplemented with 25 mM Hepes (M. A. Bioproducts, Walkersville, MD), 50  $\mu$ M 2-mercaptoethanol, a mixture of penicillin (50 U/ml) and streptomycin (50  $\mu$ g/ml) (M. A. Bioproducts), and 10% FCS (lot 29101276; Flow Laboratories, Inc., Rockville, MD). Cells were cultured in the presence of 7% CO<sub>2</sub> at 37°C.

In some experiments, B cells were activated with LPS or with Sepharose-bound anti- $\mu$ . LPS R595 derived from *Salmonella minnesota* (Calbiochem-Behring Corp., San Diego, CA) was used at a concentration of 2.5  $\mu$ g/ml.

Anti-mouse- $\mu$  antibody was a goat antibody prepared by hyperimmunization with MOPC-104E ( $\mu$ ,  $\lambda$ ). This antiserum was affinity-purified on Sepharose 4B mouse TEPC-183 ( $\mu$ ,  $\kappa$ ) columns and rendered specific by absorption to mouse IgG-Sepharose 4B columns. This anti- $\mu$  antibody was then conjugated to cyanogen bromide-activated Sepharose 4B beads at a concentration of 1 mg/ml of Sepharose. Conjugated beads were stored at 4°C in phosphate-buffered saline (PBS) containing 0.02% sodium azide. Before use, the beads were washed five times with sterile PBS and once with medium. They were then added in culture at a concentration of 2.5  $\mu$ g of anti- $\mu$ /well.

Proliferative responses of B cells to anti- $\mu$  were assayed on day 3 of culture, after an 18-h incubation with 1  $\mu$ Ci [ $^3$ H]thymidine/well.

**Plaque-forming Cell Assay.** Ig-secreting cells (IgSC) were assayed with a reverse hemolytic plaque assay as previously described (7). In all cases plaques were assayed after culturing B cells for 4 d.

**Production of Supernatants.** All supernatants derived from spleen or lymph node cells were produced under the same conditions. Cells from four or five mice were pooled and cultured at a density of  $2 \times 10^6$  cells/cm<sup>2</sup> per 0.62 ml of medium for 24 h. The medium used was the same as described above for B cell assays. Supernatants recovered were spun at 15,000 rpm for 15 min, passed through a 0.45- $\mu$ m filter (Acrodisc; Gelman Sciences, Inc., Ann Arbor, MI), and stored at  $-70^\circ\text{C}$  for a maximum of 60 d before use.

Con A supernatant (CAS) was produced under identical culture conditions except that spleen cells were incubated for 2 h with 4  $\mu$ g/ml Con A (Miles Laboratories, Inc., Elkhart, IN) at  $37^\circ\text{C}$ , washed three times with minimal essential medium, and then cultured in medium for 24 h. All CAS used in this study were derived from C57BL/6 female mice (6–10 wk old).

In some experiments, MRL/l spleen cells were depleted of various cell populations before incubation to produce supernatants. In this case, MRL/l T cell-depleted spleen cells were prepared by treatment with a combination of monoclonal anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.1 (all from New England Nuclear) and guinea pig C. Selective ablations of Lyt-1<sup>+</sup> or Lyt-2<sup>+</sup> populations were performed by using anti-Lyt-1.2 and C, or anti-Lyt-2.1 and C, respectively. To deplete B cells and adherent cells, petri dishes were coated with F(ab')<sub>2</sub> fragments of rabbit anti-mouse Ig (N. L. Cappel Laboratories Inc., Cochranville, PA) according to the technique of Wysocki and Sato (16). Spleen cells were incubated on these plates at a concentration of  $10^7$  cells/plate for 90 min. Recovered unbound cells consisted of >95% T cells as determined by immunofluorescence. Dennert line TRF (DL-TRF) derived from line C.C3.11.75, was a kind gift from Dr. G. Dennert, The Salk Institute, La Jolla, CA.

**Radioimmunoassays.** Solid-phase radioimmunoassays were used to measure IgM, IgG1, IgG2a, IgG2b, and IgG3. The IgM assay has been described (17). Briefly, polystyrene plates were coated with goat anti-mouse  $\mu$  chain-specific antibody, after which various dilutions of each sample were added to the wells. Bound IgM was detected with  $^{125}\text{I}$ -goat anti-mouse  $\mu$  antibody.

The IgG isotype-specific radioimmunoassays were direct binding assays. Plates were incubated with 100% supernatant, followed by addition of one of the following isotype specific rabbit antibodies purchased from Litton Bionetics Inc., Kensington, MD (catalogue numbers are in parentheses): anti-mouse IgG1 (8403-03), anti-IgG2a (8403-04), anti-IgG2b (8403-06), or anti-IgG3 (8403-07). The assay was then developed with  $^{125}\text{I}$ -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, also purchased from Litton Bionetics Inc.

**IL-2 Assay and Absorption.** The IL-2 assay was performed as described by Gillis et al. (18), using the IL-2-dependent cell line CTLL-2-15-H, which was a gift from Dr. M. Bevan (Scripps Clinic and Research Foundation). IL-2 was absorbed with  $4 \times 10^6$  cells of line CTLL-2-15-H per milliliter of supernatant by incubation at  $37^\circ\text{C}$  for 24 h. This treatment completely removed IL-2 activity from CAS.

**TRF Assay.**  $5 \times 10^5$  splenic B cells (BALB/c mice) were cultured in 96-well microtiter plates with  $10^6$  sheep erythrocytes (SRBC) in medium containing 10% FCS (as described above) and in the presence or absence of MRL/l-SN or CAS. Direct anti-SRBC plaques were assayed 4 d later.

## Results

**Production of a BCDF by Unstimulated MRL/l Lymphoid Cells.** Cells obtained from the spleens and lymph nodes of 4-mo-old MRL/l mice were cultured without stimulation for 24 h. Supernatants obtained from these cultures stimulated Ig secretion by unmanipulated (no mitogens added) B cells of several mouse strains (Fig. 1). MRL/l-derived supernatants (MRL/l-SN) were equally effective on B cells from mice of the *H-2<sup>b</sup>*, *H-2<sup>d</sup>*, or *H-2<sup>k</sup>* haplotypes. However, control MRL/n-derived

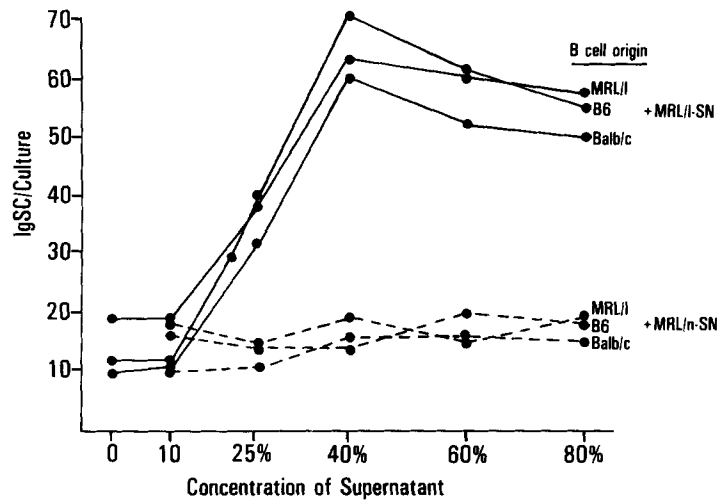


FIG. 1. Effect of MRL/l (—) or MRL/n (---) lymph node cell-derived supernatants on Ig secretion by splenic B cells of syngeneic and allogeneic mice. Increasing concentrations (vol/vol) of supernatants were added to splenic B cell cultures ( $5 \times 10^5$  cells/well). IgSC were determined at day 4 of culture. The results represent the mean of three experiments. 1 SD did not exceed 10% of the mean for any given point.

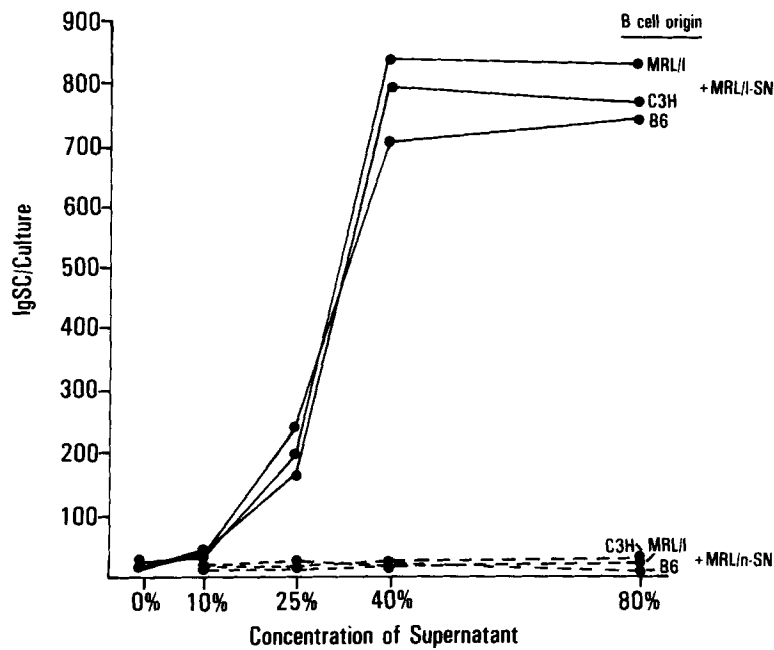


FIG. 2. Effect of MRL/l (—) and MRL/n (---) lymph node cell-derived supernatants on Ig secretion by splenic B cells of several strains activated with anti- $\mu$  antibodies.  $5 \times 10^5$  B cells were incubated with Sepharose-bound anti- $\mu$  ( $2.5 \mu\text{g/well}$ ) together with lymph node supernatants. IgSC were enumerated at day 4 of culture. The results represent the mean of three experiments. 1 SD did not exceed 5% of the mean for any given point.

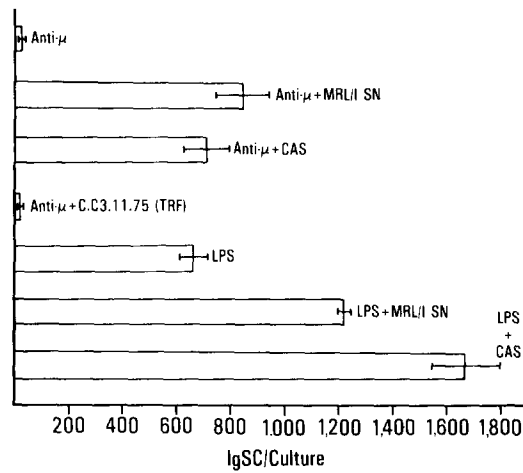


FIG. 3. Comparison of MRL/l-SN, CAS from normal mice (C57BL/6), and TRF on ability to enhance anti- $\mu$ - or LPS-induced Ig secretion.  $5 \times 10^5$  B cells with anti- $\mu$  (2.5  $\mu$ g/well) or LPS (2.5  $\mu$ g/ml) were cultured with optimal concentrations of MRL/l-SN, CAS, or supernatant from the TRF-producing line C.C3.11.75. IgSC were enumerated after 4 d of culture  $\pm$  SD. In the absence of LPS or anti- $\mu$  the following counts were obtained: untreated B cells,  $10 \pm 2$ ; MRL/l-SN,  $68 \pm 4$ ; CAS,  $35 \pm 3$ ; C.C3.11.75, not determined.

TABLE I

Comparison of Ig-inducing Ability by Lymphoid Cell-derived Supernatants from Various Mouse Strains

Cellular derivation of supernatant*	Strain of origin of supernatants					
	BALB/c	C57BL/6	C3H	MRL/n	MRL/l‡	C57BL/6-1‡
Spleen cells	<15§	<15	<15	<15	$683 \pm 45$	$727 \pm 14$
Lymph node cells	<15	<15	ND¶	<15	$823 \pm 106$	$324 \pm 85$

\* Supernatants were obtained after incubation of the respective cell populations for 24 h and added at various concentrations (20–80% vol/vol) to anti- $\mu$ -activated B cells ( $5 \times 10^5$  cells/well).

‡ These two strains are of the *lpr/lpr* genotype.

§ Numbers indicate IgSC per culture at optimal concentration of the respective supernatants. Background numbers of IgSC obtained with anti- $\mu$  alone have been subtracted.

¶ Not determined.

supernatants (see below) had no effect on Ig secretion. The response of MRL/l B cells to MRL/l-SN did not differ significantly from that of B cells from other strains.

This effect of MRL/l-SN was greatly enhanced by activating B cells from several strains with anti- $\mu$ -coated Sepharose beads (Fig. 2). Removal of macrophages from the T cell-depleted BALB/c target cell population had no significant effect on the MRL/l-SN activity ( $828 \pm 101$  IgSC/culture in T cell-depleted populations vs.  $873 \pm 79$  IgSC/culture in T cell- and macrophage-depleted populations). Therefore, no attempts were made to remove macrophages from the target population in subsequent experiments. As previously shown by others (19–21), anti- $\mu$  by itself has little effect on Ig secretion. However, when T cell-derived supernatants from the appropriate source, such as CAS, are added, then anti- $\mu$ -activated B cells are stimulated to secrete Ig. As can be seen from Fig. 3, both MRL/l-SN and CAS caused Ig secretion by anti- $\mu$ -treated B cells, whereas a supernatant from the TRF-producing line C.C3.11.75 had

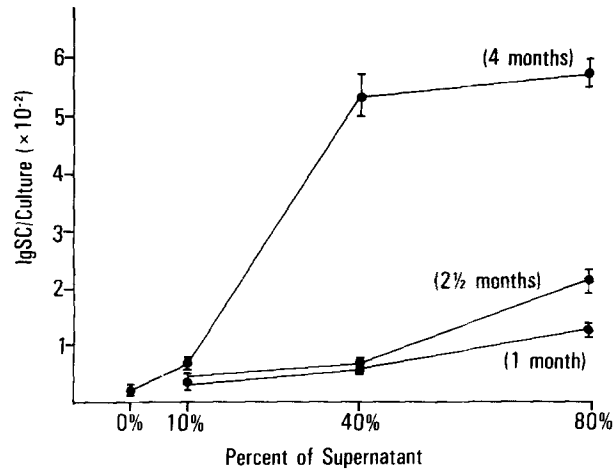


FIG. 4. Capacity of MRL/l-SN from mice of various ages to induce Ig secretion.  $10^5$  BALB/c B cells/culture were activated with anti- $\mu$ . Results represent IgSC/well  $\pm$  1 SD assayed after 4 d of culture. Similar results were obtained with supernatants from lymph nodes.

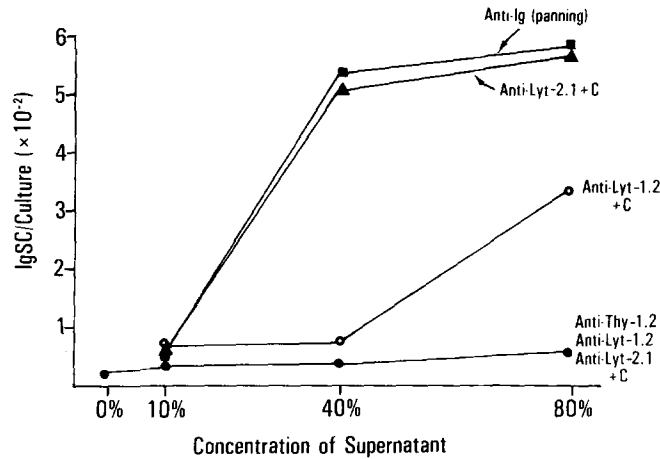


FIG. 5. MRL/l-derived lymphoid cell population responsible for the production of Ig secretion-inducing factor(s). Surface Ig<sup>+</sup> (B cells), T cells, or Lyt-defined T cell subsets were removed by appropriate treatments (see Materials and Methods) from spleen cells of 4-mo-old MRL/l mice. Supernatants were produced from the recovered cells and tested for their ability to induce Ig secretion in anti- $\mu$ -activated BALB/c B cells ( $10^5$  cells/well). The results represent the mean of three experiments. For each point, 1 SD represented  $<10\%$  of the mean.

no effect in this assay. In addition, both MRL/l-SN and CAS enhanced Ig secretion of LPS-activated B cells. Nevertheless, as shown later, there are significant differences between MRL/l-SN and CAS.

*Association of the *lpr/lpr* Genotype with Production of a BCDF.* Supernatants were prepared from the spleen and lymph nodes of adult ( $\geq 4$  mo old) MRL/Mp-+/-, BALB/c, C57BL/6 (B6), C3H, and C57BL/6J-*lpr/lpr* (B6/l) mice. Of these strains, only the supernatants derived from B6/l mice (spleens or lymph nodes) induced differentiation of anti- $\mu$ -activated B cells (Table I). The B6/l strain develops an autoimmune and T cell proliferative syndrome similar to that of the MRL/l mouse,

except that the disease appears in the former when they are several months older (11). On the other hand, as noted above, supernatants from the MRL/Mp-+/+ (MRL/n) substrain, which is congenic with MRL/l but lacks the *lpr* gene, were ineffective. We conclude that the *lpr* gene, regardless of the genetic background, is sufficient to induce the production of a BCDF.

*Age When MRL/l-derived L-BCDF Production Begins.* Analysis of MRL/l-SN from mice of different ages revealed that the production of L-BCDF began as early as 1 mo of age and therefore antedated morphologic evidence of lymphoproliferation (Fig. 4). Moreover, quantities of L-BCDF increased with age and roughly matched the degree of lymphoproliferation in older animals (2).

*Cell Where L-BCDF Originates.* Because the mesenteric lymph nodes of old MRL/L mice consist of >95% T cells (3), T cells were immediately suspected of producing the factor. Accordingly, depletion of adherent cells and B cells by panning with anti-Ig-coated plates had no effect on L-BCDF production by spleen cells (Fig. 5). Conversely, depletion of T cells from spleen cell populations by combined treatment with monoclonal anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.1 and C almost completely abrogated L-BCDF production. Treatment with anti-Lyt-1.2 and C only partially abrogated L-BCDF production, presumably because many MRL/l T cells express only a low density of Lyt-1.2 antigen (3, 4). In contrast, treatment with anti-Lyt-2.1 and C had no effect on L-BCDF production. Apparently, L-BCDF was produced by a Thy-1<sup>+</sup>, Lyt-1<sup>+</sup>2<sup>-</sup> cell, which is the dominant cell in MRL/l lymph nodes.

*MRL/l-SN Lacks BCGF-like Activity.* Although B cells proliferate in response to anti- $\mu$  in high cell density (HCD) cultures, they fail to respond to low cell density (LCD) cultures unless BCGF is added (22). We found that neither MRL/l-SN nor CAS induced proliferation of B cells in LCD or HCD cultures with anti- $\mu$  (Table II) or without anti- $\mu$  (data not shown). Nevertheless, MRL/l-SN induced Ig secretion very effectively in LCD conditions, despite little or no B cell proliferation. Interestingly, CAS could induce Ig secretion only in HCD cultures.

*Unlike CAS, MRL/l-SN Acts in the Absence of IL-2.* Using a standard assay we could not detect IL-2 in MRL/l-SN (Table III), although it was clearly present in CAS. To eliminate the possibility that low levels of IL-2 were present in MRL/l-SN and might have influenced this supernatant's activity, we absorbed supernatants with an IL-2-

TABLE II  
Effect of Cell Density\* on the Response of B Cells to MRL/l-SN vs. CAS from a Normal Mouse Strain

Treatment	Proliferation (SI)‡		Differentiation (SI)‡	
	LCD	HCD	LCD	HCD
Anti- $\mu$	0.9 ± 0.3	30.0 ± 4.7	1.9 ± 0.3	2.2 ± 1.5
Anti- $\mu$ + MRL/l-SN§	1.1 ± 0.3	28.6 ± 4.1	99.5 ± 19.0	84.0 ± 10.4
Anti- $\mu$ + CAS§	0.9 ± 0.4	29.4 ± 4.2	2.1 ± 0.3	70.5 ± 9.2

\* Cell cultures were set up at LCD ( $5 \times 10^4$  cells/250  $\mu$ l), or HCD ( $5 \times 10^5$  cells/250  $\mu$ l).

‡ Stimulation index (SI) = experimental/medium control. Proliferation is assayed by [<sup>3</sup>H]thymidine uptake, whereas differentiation is measured by reverse hemolytic plaque assay. Results represent the mean ± 1 SD of three experiments.

§ Results listed are at 40% (vol/vol) supernatant concentration. Varying the concentration of the supernatants (10–80%) had no significant effect on proliferation or on the differentiation response to CAS in LCD cultures.

TABLE III  
*IL-2 Dependency of CAS but Not MRL/l-SN for Induction of B Cell Differentiation*

Supernatant*	CTLL absorption‡	IL-2 activity (SI)§	Differentiation (IgSC/culture)
Medium	—	1.0	10
MRL/l-SN	—	1.2	820
CAS	—	24.8	728
MRL/l-SN	+	1.1	811
CAS	+	1.4	15

\* MRL/l-SN (80% vol/vol) and CAS (80% vol/vol) were added to anti- $\mu$ -activated B cells ( $5 \times 10^5$  cells/culture).

‡ 1 ml of supernatant was absorbed with  $4 \times 10^6$  cells of line CTLL-2-15-H for 24 h at 37°C.

§ [ $^3\text{H}$ ]thymidine uptake of the CTLL line; SI = counts per minute with supernatant divided by counts per minute with medium alone. The results of a representative experiment are shown.

TABLE IV  
*MRL/l-SN Enhances Both IgM and IgG Production in LPS-activated B Cells*

Strain of origin of B cells cultured with LPS*	Supernatant‡	IgM	Total IgG§	Isotypes secreted (percentage of total IgG)				IgG1, 2a, 2b/IgG3 ratio
				IgG3	IgG1	IgG2b	IgG2a	
		ng/ml	ng/ml					
BALB/c	—	230	33	50.1	6.1	25.0	18.8	1.0
	MRL/l-SN	603	227	6.6	18.5	49.3	25.6	14.1
MRL/l	—	613	55	16.4	7.2	30.9	45.4	5.1
	MRL/l-SN	1211	560	2.8	16.1	27.5	53.6	34.7

\* Anti-Thy-1.2 plus C-treated spleen cells were cultured at a concentration of  $5 \times 10^4$  cells/well for 6 d in the presence of 2.5  $\mu\text{g/ml}$  of LPS.

‡ MRL/l-SN was added at 40% (vol/vol).

§ Total IgG is the sum of the values obtained with the four IgG subclasses. IgM and IgG subclasses were determined by radioimmunoassay. The results shown represent the mean of three experiments. In all cases, SD was <10% of the mean.

TABLE V  
*Absence of Conventional TRF Activity in MRL/l-SN*

Supernatant*	PFC‡
Medium	$3 \pm 1$
CAS	$150 \pm 15$
MRL/l-SN	$2 \pm 1$

\* CAS and MRL/l-SN were added at a concentration of 40% vol/vol.

‡ Direct anti-SRBC PFC per  $10^6$  cultured cells  $\pm 1$  SD.

dependent cell line. This absorption had no effect on the ability of MRL/l-SN to induce differentiation (Table III). In marked contrast, IL-2 absorption completely removed the ability of CAS to induce B cell differentiation. We conclude that the differentiation factor present in MRL/l-SN is distinct from that in CAS.

*MRL/l-SN Enhances Both IgM and IgG Secretion in LPS-activated B Cells.* B cells were cultured at low cell density in the presence of LPS for 6 d. Although LPS by itself induced both IgM and IgG secretion, we found that both Ig classes were enhanced by the addition of MRL/l-SN (Table IV). Furthermore, IgG secretion was enhanced to a greater extent than IgM secretion. LPS by itself induced mostly IgG3 and IgG2b



secretion in BALB/c mice. The addition of MRL/l-SN with LPS had little effect on IgG3 secretion but considerably enhanced the production of IgG1, IgG2a, and IgG2b. This can be seen as a redistribution in the isotype percentages accounting for total IgG, and as a considerable increase in the IgG1, 2a, 2b/IgG3 ratio. It is noteworthy that MRL/l B cells produced more IgG2a in response to LPS alone than did those from BALB/c mice. This may reflect *in vivo* exposure of MRL/l B cells to L-BCDF, or may possibly be a primary property of MRL/l B cells.

*MRL/l-SN Lacks TRF Activity.* MRL/l-SN could not reconstitute the primary *in vitro* response of B cells to SRBC, whereas CAS gave a positive result (Table V). Thus, we could not demonstrate conventional TRF activity (23, 24) in MRL/l-SN. Furthermore, DL-TRF, the product of a cloned T cell line (25, 26), which can reconstitute primary *in vitro* responses to SRBC, could not induce Ig secretion by anti- $\mu$ -activated B cells (Fig. 3).

### Discussion

In this paper we document the *in vitro* production of an L-BCDF by unstimulated (i.e., in the absence of mitogens) lymph node and spleen cells of the MRL/l mouse. L-BCDF acts on unmanipulated B cells; however, its effect is much greater on anti- $\mu$ - or LPS-activated B cells. It should be noted in this respect that unmanipulated B cells may contain a small percentage of B cells that had been activated *in vivo* or by antigens and mitogens present in the culture media. Because the response of unmanipulated B cells was quite low, it may be that only activated B cells are responding to the L-BCDF. The factor appears to act directly on B cells and not indirectly via macrophage products. B cells from murine strains of three different H-2 haplotypes all responded to L-BCDF, indicating no apparent genetic restriction of this factor's activity. The animals' age when this factor appears precedes morphologic evidence of lymphoproliferation. However, the quantity of factor produced roughly matches the degree of lymphoproliferation in MRL/l mice >2 mo old. Unstimulated lymphocytes of the congenic strain MRL/n (which lacks the *lpr* gene) did not induce B cell differentiation; similarly, normal mouse strains did not produce a detectable BCDF. On the other hand, mice of the C57BL/6-*lpr/lpr* strain produced a similar factor, which suggests that the *lpr* gene is linked with both lymphoproliferation and L-BCDF production.

Cell depletion studies performed with monoclonal antibodies and C or by panning reveal that the cell producing L-BCDF is a T cell of Lyt-1<sup>+</sup>2<sup>-</sup> phenotype. Interestingly, this is the phenotype of the proliferating cell in MRL/l mice that accounts for >95% of all cells in MRL/l lymph nodes (4).

Early in the study of MRL/l-SN we found similarities between this supernatant and CAS derived from normal mouse strains. Thus, both MRL/l-SN and CAS could induce Ig secretion in anti- $\mu$ - or LPS-activated B cells. It soon became clear, however, that there were major differences between MRL/l-SN and CAS. First, we found that CAS could only induce Ig secretion in anti- $\mu$ -activated B cells in HCD cultures, whereas MRL/l-SN acted regardless of cell density. Second, the action of CAS was IL-2 dependent and could be completely abrogated by IL-2 absorption. On the other hand, MRL/l-SN did not contain detectable IL-2. The possibility that trace amounts of IL-2 were influencing the activity of MRL/l-SN was excluded by absorption with an IL-2-dependent line. Thus, L-BCDF (from MRL/l-SN) appears to induce Ig

secretion in the complete absence of IL-2. Recently, Parker (27) reported on the IL-2 dependency of CAS using a similar assay and suggested that in addition to IL-2, CAS contains a second factor (TRF) that induces B cell differentiation. Because our results demonstrate both an HCD requirement and an IL-2 requirement for CAS activity, we believe that this supernatant can only induce Ig secretion if sufficient residual T cells (there are fewer T cells per well in LCD cultures) are present in culture. This view is consistent with the results of T depletion studies performed by others (28) in the rat model. On the other hand, L-BCDF acts independently of cell density and appears to exert its effect directly on the B cell without the need for an intermediary helper cell.

MRL/l-SN had no effect on B cell proliferation in the presence or absence of anti- $\mu$ , regardless of cell density. Because BCGF has been shown to increase the proliferation of anti- $\mu$ -activated B cells in LCD cultures (22), we conclude that there is no BCGF-like activity in MRL/l-SN.

Although it is clear that L-BCDF is a factor distinct from IL-2 and BCGF, its relationship to TRF is more difficult to define. At the present time, it is not clear that TRF, as assayed by different groups, represents one factor (23, 24, 26, 29, 30). Indeed, there is no assay that detects TRF independent of any other factor. In the classic assay, TRF restores the primary in vitro response to SRBC (23, 24). In this respect, L-BCDF has no TRF activity. However, TRF activity has been shown to depend on the joint action of IL-2, BCGF, TRF, and possibly IL-1 (22, 26, 31). Some authors claim that TRF can only induce B cell differentiation in collaboration with IL-2 (26, 27). In fact, we found that DL-TRF, the product of a cloned T cell line (26), could not induce Ig secretion of anti- $\mu$ -activated B cells. Our results strongly suggest that L-BCDF is a factor distinct from TRF. Isakson et al. (32) have identified BCDF from cloned T cell lines that induce IgM secretion (BCDF $\mu$ ) or IgG secretion (BCDF $\gamma$ ) without having conventional TRF activity. On the basis of their data and our own, it would seem that there exists a family of BCDF that includes TRF and L-BCDF. The exact relationship of these BCDF evidently will require extensive studies and cannot be resolved at this time.

L-BCDF enhanced both IgM and IgG production by LPS-activated B cells. Interestingly, IgG production was more responsive to L-BCDF than IgM production. LPS by itself induced mostly IgG3 and IgG2b secretion in BALB/c mice, as previously reported (33, 34). The addition of L-BCDF to LPS-stimulated cultures had little additional effect on IgG3 secretion, but caused a marked increase in IgG1, IgG2a, and IgG2b secretion. We have recently found that surface Ig<sup>-</sup> B cells (pre-B cells) respond in a similar fashion (unpublished observation), which suggests that L-BCDF may induce switching from IgM to IgG production.

Analysis of serum immunoglobulins in SLE and especially in MRL/l mice reveals that with aging there is a switching from IgM to IgG production (7, 35). Furthermore, in the MRL/l mice the predominant isotypes produced are IgG1 and IgG2a.<sup>2</sup> We have also shown<sup>3</sup> that MRL/l mice produce a greater percentage of IgG2a and IgG2b in response to T-independent type 1 and T-dependent antigens when compared with

<sup>2</sup> Berden, J. H. M., L. M. Hang, P. J. McConahey, and F. J. Dixon. 1982. Analysis of vascular lesions in murine SLE. I. Association with serological abnormalities. *J. Immunol.* In press.

<sup>3</sup> Park, C. L., R. S. Balderas, T. Fieser, G. J. Prud'homme, F. J. Dixon, and A. N. Theofilopoulos. 1982. Isotypic profiles and other fine characteristics of immune responses of SLE mice to exogenous TD- and TI-antigens. *J. Immunol.* In press.

normal mice. Interestingly, MRL/l mice are highly prone to develop serum monoclonal gammopathies (36). The random analysis of 11 serum monoclonal peaks from MRL/l mice in our laboratory revealed that 5 were of the IgG2a subclass, 5 were of the IgG1 subclass, and 1 was of the IgG2b subclass, but none was of IgM or IgG3 isotype.<sup>4</sup> These results provide in vivo analogies for the in vitro effect of L-BCDF on B cells.

Recently,<sup>4</sup> we have investigated other SLE mouse strains (BXSB, NZB, NZB/NZW F<sub>1</sub>) for the production of lymphoid cell-derived factors that affect B cell function. Preliminary evidence suggests that such factors are present in other autoimmune strains at a time that antedates or coincides with the appearance of overt disease. Thus, we have evidence that autoimmune mice of genetic backgrounds different from MRL/l and lacking T cell proliferation also spontaneously produce factors that stimulate B cell function. Further studies will be necessary to determine whether these factors are related to L-BCDF. It is noteworthy that in all murine SLE strains we have studied the rise in production of B cell differentiation factor(s) is accompanied by a concomitant decrease in IL-2 production. This association may not be accidental. Indeed, it has been suggested (9-11) that IL-2 may act on regulatory T cells that prevent or delay the appearance of autoantibody production in murine SLE. Possibly, defective IL-2 production is a necessary prerequisite for increased BCDF production. This question is of more than theoretical interest because not only murine SLE, but also human SLE is characterized by a decrease in IL-2 production (37).

### Summary

Lymph node and spleen cells of the autoimmune MRL/Mp-*lpr/lpr* mouse strain spontaneously produce (in the absence of mitogenic stimulation) a factor(s) that induces B cell differentiation. This factor is not produced by the congenic MRL/n mouse strain that lacks the *lpr* gene or by normal mouse strains. However, lymphoid cells of the B6-*lpr/lpr* (B6/l) strain also produce a B cell differentiation factor. Although the factor acts on resting B cells, its effect is greatly magnified by activating the B cells with anti- $\mu$  or lipopolysaccharide. MRL/l mice begin producing the factor as early as 1 mo of age but levels increase with age and appearance of lymphoproliferation. Cell depletion studies reveal that this factor is produced by T cells of the Lyt-1<sup>+</sup>2<sup>-</sup> phenotype. Because of its association with the *lpr/lpr* genotype, we term this B cell differentiation factor L-BCDF. Functional analysis of L-BCDF reveals that it acts regardless of cell density in culture and in the absence of interleukin 2 (IL-2). In fact, the increase in the production of L-BCDF by MRL/l T cells with aging occurs concomitantly with a marked decrease in their ability to produce IL-2. No T cell replacing factor activity or B cell growth factor-like activity can be detected in MRL/l-derived supernatants. L-BCDF induces both IgM and IgG synthesis in lipopolysaccharide-activated B cells; however, it has a greater effect on IgG secretion. In particular, the production of IgG1, IgG2a, and IgG2b are markedly enhanced in the presence of L-BCDF. The spontaneous production of L-BCDF by T cells of SLE mice of *lpr/lpr* genotype suggests an association of this factor with autoimmunity.

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<sup>4</sup>Dong, Z. W., A. Bionda, L. M. Hang, F. J. Dixon, and A. N. Theofilopoulos. 1982. Analysis of spontaneous monoclonal IgG in MRL/l mice. Manuscript in preparation.

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