

## T CELL-DERIVED B CELL GROWTH AND DIFFERENTIATION FACTORS

Dichotomy between the Responsiveness of B Cells  
from Adult and Neonatal Mice\*

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B cells are the precursors of antibody-secreting cells. Since the frequency of B cells with receptors for a particular antigen is low, polyclonal stimuli have been used to study B cell activation. These stimuli include mitogens, such as lipopolysaccharide (LPS)<sup>1</sup>, antibodies specific for surface immunoglobulin (sIg), and soluble factors from T cells. The results of studies with polyclonal activators suggest that activation of B cells from adult mice occurs in two stages: (a) the induction of proliferation by anti-Ig and T cell-derived B cell growth factor(s) (BCGF) (1–3), and (b) differentiation to Ig secretion by T cell-derived differentiation factors (BCDF) (4–8). Depending on the source of T cell-derived factors, the induction of IgM secretion may require concomitant cross-linking of Ig receptors by anti-Ig (7, 8) or can occur in the absence of anti-Ig (8–11). The lymphokine(s) in T cell supernatants (SN) that induces B cells to secrete IgM has been termed B cell differentiation factor(s) for IgM or BCDF<sub>μ</sub> (8, 12). In addition to BCDF<sub>μ</sub>, we have defined a second lymphokine (BCDF<sub>γ</sub>) that induces IgG<sub>1</sub> secretion by LPS-stimulated B cells (13).

In the present study we have distinguished BCDF<sub>μ</sub>, BCDF<sub>γ</sub>, and BCGF by their molecular weights, ability to activate neonatal B cells, and presence or absence in a panel of T cell SN. Furthermore, we have shown that BCDF<sub>μ</sub> and BCDF<sub>γ</sub> stimulate B cells from both neonatal and adult mice, whereas BCGF acts only on the latter. This finding might partially explain the relative immunoincompetence of immature B cells.

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<sup>1</sup> *Abbreviations used in this paper:* AEF, allogeneic effect factor; BCDF, T cell-derived B cell differentiation factor; BCDF<sub>μ</sub>, T cell-derived B cell differentiation factor for IgM; BCDF<sub>γ</sub>, T cell-derived B cell differentiation factor for IgG; BCGF, T cell-derived B cell growth factor; BCSF, B cell stimulating factor; BRMF, T cell-derived replication and maturation factor; C, complement; Con A, concanavalin A; FCS, fetal calf serum; [<sup>3</sup>H]TdR, tritiated thymidine; IL-2, interleukin 2; LPS, lipopolysaccharide; OVA, ovalbumin; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; RAMIg, rabbit anti-mouse Ig; Rαμ, rabbit anti-mouse μ chain; Rαγ, rabbit anti-mouse γ chain; RIA, radioimmunoassay; S-200, Sephacryl-200; sIg, surface immunoglobulin; SN, supernatant; TRF, T cell replacing factor.

### Materials and Methods

**Animals.** Adult BALB/c or (C57BL/6 × DBA/2) F<sub>1</sub> (BDF<sub>1</sub>) (Cumberland Farms, Clinton, TN) mice were used at 8–20 wk of age. Neonates were bred in our colony and were used at 5–17 d of age.

**BCL<sub>1</sub> Tumor.** The BCL<sub>1</sub> tumor was maintained by in vivo passage in BALB/c mice. Peripheral blood lymphocytes (PBL) were prepared from mice that carried the tumor for at least 8 wk. 90% or greater of PBL were tumor cells as determined by the expression of the BCL<sub>1</sub> idiotype (14).

**Antibodies.** Affinity-purified rabbit antibodies against mouse Ig (RAMIg),  $\mu$  (R $\alpha\mu$ ),  $\gamma$  (R $\alpha\gamma$ ), and  $\delta$  (R $\alpha\delta$ ) heavy chains have been described (14, 15). Goat anti- $\mu$  and goat anti- $\delta$  were prepared using the same immunogens. Sera were affinity purified, absorbed, and assayed as described (14). Goat anti-ovalbumin was prepared by immunizing a goat with 1 mg of crystallized ovalbumin (OVA) in complete Freund's adjuvant. The goat was boosted at intervals and bled, and the pooled serum was affinity purified on Sepharose-OVA. Two monoclonal anti-Thy-1 antibodies were used (14): the mouse IgM<sub>k</sub>-anti-Thy-1.2 product of the HO-13.4 hybridoma cells, obtained from the Cell Distribution Center of the Salk Institute, La Jolla, CA, and the rat IgG-anti-Thy-1 product of the HO-12.4 hybridoma cells, obtained from Dr. Noel Warner (Becton Dickinson & Co., Palo Alto, CA). The rat anti-Lyt-1 and anti-Lyt-2 hybridomas (16) were obtained from the Cell Distribution Center of the Salk Institute. The purification of the monoclonal anti-Lyt-1 and anti-Lyt-2 antibodies has been described previously (8). The rabbit antibodies were coupled to cyanogen-bromide-activated Sepharose-4B as previously described (17).

**Deletion of T Cells.** T cells were removed by treatment of normal spleen cells or BCL<sub>1</sub> PBL with monoclonal anti-Thy-1.2 (HO-13.4) and baby rabbit complement (C) (Pel-Freeze Biologicals, Inc., Rogers, AR). Alternatively, cells were treated with a cocktail of rat hybridoma antibodies directed against Thy-1 (HO-12.4), Lyt-1 (53.7.313), and Lyt-2 (52.6.72), followed by R $\alpha\gamma$  and C, as described (8).

**Culture Conditions.** Normal B cells or BCL<sub>1</sub> PBL were cultured in flat-bottomed microtiter plates (Linbro Scientific Co., Hamden, CT) at  $2-5 \times 10^5$  cells/ml in Hepes-buffered RPMI 1640 supplemented with penicillin, streptomycin, and gentamycin (10  $\mu$ g/ml), 50  $\mu$ M 2-mercaptoethanol, and 10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, NY). Cultures were incubated in an atmosphere of 83% N<sub>2</sub>, 10% CO<sub>2</sub>, 7% O<sub>2</sub> at 37°C.

**Radioimmunoassay (RIA) for Secreted Ig.** SN from 4–6-d cultures of stimulated cells were assayed for the presence of secreted IgM and IgG by a solid phase RIA as previously described (13, 18). Microtiter plates (Cooke Engineering, Alexandria, VA) were coated with affinity-purified RAMIg and 1% FCS. Secreted IgM was bound to the RAMIg-coated plates and detected with [<sup>125</sup>I]R $\alpha\mu$ . IgG was detected by using [<sup>125</sup>I]R $\alpha\gamma$ . These reagents had negligible cross-reactivity (<1%) with inappropriate heavy chains or light chains. For quantitation, standard curves using purified (BCL<sub>1</sub>) IgM ( $\mu\lambda$ ) (19), MOPC-104E ( $\mu\lambda$ ) (Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, MD), or mouse IgG ( $\gamma\kappa\lambda$ ) were included in each assay.

**BCDF <sub>$\mu$</sub>  Assay.** B cell differentiation to IgM secretion was induced by culturing BCL<sub>1</sub> PBL or normal B cells at  $5 \times 10^5$  cells/ml for 4 d, alone or in the presence of T cell SN (12). IgM secretion was then assessed by RIA.

**BCDF <sub>$\gamma$</sub>  Assay.** B cell differentiation to IgG secretion was induced as previously described (13). Briefly, normal B cells were cultured at  $2 \times 10^5$ /ml in microtiter plates with 20  $\mu$ g/ml LPS (*Salmonella typhosa*; Difco Laboratories, Detroit, MI). After 24 h the appropriate concentration of T cell SN was added. After 6 d IgG secretion was assessed by RIA.

**BCGF Assay.** B cell growth factor activity was detected by a modification of the method described by Howard et al. (1, 2). Briefly,  $2.5-5 \times 10^5$  B cells/ml were cultured in the presence of anti- $\mu$  or anti- $\delta$  bound to Sepharose. T cell SN was added either immediately or after 24 h. On day 3, cultured cells were pulsed for 16 h with 1  $\mu$ Ci/culture [<sup>3</sup>H]thymidine, harvested, and counted.

#### T Cell SN

**T CELL LINES PK 7.1.1.A AND PK 7.1.2.** Secondary alloreactive AKR anti-C57BL/6 (B6) cells ( $10^4$ /ml) were subcultured weekly in the presence of irradiated (3,300 rad) B6 stimulator cells

( $10^6$ /ml) in medium supplemented with rat spleen concanavalin A (Con A) SN as a source of interleukin 2 (IL-2) (20). These cells have been maintained in culture for >1.5 yr. The 7.1.2E8 and 7.1.2C7 cells are clones obtained by limiting dilution cloning of 7.1.2. Lymphokine release was induced (after washing the cells in IL-2-free medium containing 2% FCS) by pulsing the cells for 3 h with 10  $\mu$ g/ml Con A, washing, and culturing for 24 h in medium lacking both IL-2 and Con A. Cells were removed by centrifugation and the cell-free SN was filtered, aliquoted, and stored at  $-70^\circ\text{C}$ .

**EL-4 TUMOR.** EL-4 SN was prepared by stimulating  $10^6$  cells/ml of a cloned subline obtained from Dr. J. Farrar (National Institutes of Health) (21) with 20 nM phorbol myristate acetate (Sigma Chemical Co., St. Louis, MO). Cell-free SN were collected after 48 h. The SN was brought to 50% saturation with ammonium sulfate and the pellet was discarded. Lymphokines were then precipitated by bringing the SN to 85% saturation with  $(\text{NH}_4)_2\text{SO}_4$ . The precipitate was dissolved in phosphate-buffered saline (PBS) (10–20% of the original culture medium volume), dialyzed, and stored at  $-70^\circ\text{C}$ .

**B6.** The B6 cell line was obtained by fusion of the FS6.14.13.AG2.1 cell line with T cells from spleen and lymph nodes of C57BL/10 mice as previously described (22). Lymphokine-containing SN were obtained by culturing B6 cells at  $10^6$  cells/ml with 4  $\mu$ g/ml Con A for 24 h. SN containing  $\alpha$ -methylmannoside were tested along with control medium.

**GEL FILTRATION ANALYSIS.** Gel filtration analysis was performed on a  $1 \times 50$ -cm column of Sephacryl-200 (S-200) (Pharmacia Fine Chemicals, Piscataway, NJ). The sample of T cell SN was applied (maximum volume of 1 ml) and the material was eluted in PBS. 0.8–1-ml fractions were collected and the column fractions were brought to 5% (vol/vol) with FCS. Fractions were filter sterilized and tested for lymphokine activity.

## Results

*Definition of T Cell-derived B Cell Growth and Differentiation Factors.* BCGF is defined as a T cell-derived lymphokine that sustains anti-Ig-induced proliferation of B cells (1, 2). The proliferative response of B cells from adult mice to anti-Ig alone is optimal on day 1 and falls to background levels by days 3–4 of culture. In the presence of BCGF, proliferation is sustained. Thus, the response is optimal on days 3–4 and is the same or greater than the response to anti-Ig alone at day 1.

BCDF $_{\mu}$  is defined as a T cell-derived lymphokine that induces IgM secretion by either normal or neoplastic B cells (8). BCDF $_{\gamma}$  is defined as a lymphokine that induces IgG $_1$  secretion in LPS-stimulated B cells (13). SN were considered negative for each lymphokine if they exhibited <10% of the activity obtained with the reference SN 7.1.1a.

*Presence of BCGF, BCDF $_{\mu}$ , or BCDF $_{\gamma}$  in T Cell SN.* We first attempted to distinguish among BCGF, BCDF $_{\mu}$  and BCDF $_{\gamma}$  by analyzing the SN from different T cell lines and clones. Table I shows that SN from some T cell lines (e.g., SN from EL-4, 7.1.2E8, and 7.1.1a) contain all three lymphokines, whereas others (SN from B6 and 7.1.2C7) contain only one. In addition, there was no correlation between the activity of BCGF, BCDF $_{\mu}$ , and BCDF $_{\gamma}$  in different SN (data not shown). Taken together, these results suggest that the three lymphokine activities are carried by different molecules. Alternatively, SN lacking a particular lymphokine activity contain another lymphokine that suppresses the activity of the first lymphokine, so that the first lymphokine is not absent but rather suppressed. Distinguishing between these possibilities will only be possible when the lymphokines and/or suppressor factors have been purified.

*Gel Filtration Analysis of BCGF, BCDF $_{\mu}$ , and BCDF $_{\gamma}$ .* SN from 7.1.2 was analyzed by gel filtration on a S-200 column. 1 ml of SN was chromatographed on a  $1 \times 50$ -cm column. 0.8 ml-fractions were collected and immediately supplemented to 5% (vol/vol) with FCS. Each fraction was filter sterilized and assayed for lymphokines. As

TABLE I  
*Lymphokines Present in SN from Different T Cell Lines and Clones*

| T cell source | Reference  | Lymphokines* |                   |                   |
|---------------|------------|--------------|-------------------|-------------------|
|               |            | BCGF         | BCDF <sub>μ</sub> | BCDF <sub>γ</sub> |
| 7.1.2 E8      | 12, 20     | +            | +                 | +                 |
| EL-4          | 1, 2       | +            | +                 | +                 |
| 7.1.1a        | 12, 13, 20 | +            | +                 | +                 |
| 7.1.2 C7      |            | -            | -                 | +                 |
| B6            | 22         | -            | +                 | -                 |

\* SN were classified as negative if they contained <10% of the activity of a reference SN (7.1.1a).

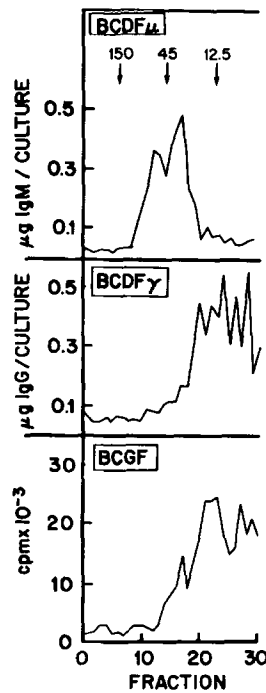


FIG. 1. Gel filtration separation of BCDF<sub>μ</sub>, BCDF<sub>γ</sub>, and BCGF. 1 ml of SN from Con A-activated 7.1.2 cells was chromatographed on a S-200 column (1 × 50 cm) at 4°C in PBS. 0.8-ml fractions were collected and immediately supplemented with FCS to a final concentration of 5% (vol/vol). Fractions were filter sterilized and assayed for BCDF<sub>μ</sub> on BCL<sub>1</sub> cells (top panel), BCDF<sub>γ</sub> on LPS-activated B cells (middle panel), or BCGF activity on anti-Ig-treated B cells (lower panel) as detailed in Materials and Methods. Each fraction was tested at a final concentration of 10% (vol/vol) in the BCDF<sub>μ</sub> and BCGF assays and at a final concentration of 20% (vol/vol) in the BCDF<sub>γ</sub> assay. Each assay was performed in duplicate and the experiments were repeated three times.

shown in Fig. 1, this method separated BCDF<sub>μ</sub> from BCGF and BCDF<sub>γ</sub>. BCDF<sub>μ</sub> consistently eluted as two broad peaks averaging 30,000 and 60,000 mol wt, respectively. In contrast, BCDF<sub>γ</sub> and BCGF could not be distinguished from each other since both eluted in broad peaks with apparent mol wt of <20,000. Similar results were obtained with SN from EL-4 and 7.1.1a (data not shown).

To confirm the results obtained above, the column fractions from four different

experiments were pooled and retested for  $BCDF_{\mu}$  and  $BCDF_{\gamma}$ . As shown in Fig. 2, the pool of the fractions eluting at 30–60,000 mol wt had excellent  $BCDF_{\mu}$  activity (A) and little or no  $BCDF_{\gamma}$  activity (B). In contrast, the pool of the fractions eluting at <20,000 mol wt had  $BCDF_{\gamma}$  activity (B) but no  $BCDF_{\mu}$  activity (A). Thus,  $BCDF_{\mu}$  is distinct from  $BCDF_{\gamma}$ .

*Effect of BCGF on Adult and Neonatal B Cells.* Adult B cells can be induced to

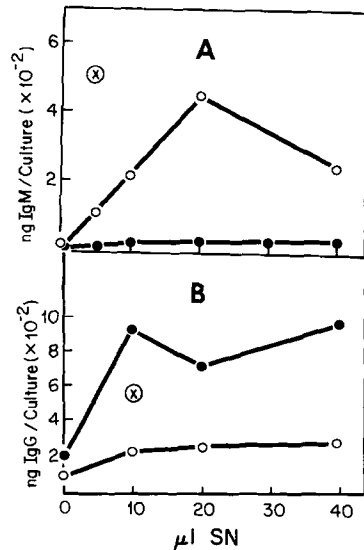


FIG. 2. Response of splenic B cells from adult mice to 7.1.2 SN separated by gel filtration on S-200. 7.1.2 SN was processed and tested as described in Fig. 1. Fractions eluting at 30–60,000 mol wt (○) and fractions eluting at <20,000 mol wt (●) were pooled separately. The indicated volumes of each pool were assayed for  $BCDF_{\mu}$  (A) or  $BCDF_{\gamma}$  (B). An optimal concentration of unfractionated 7.1 SN (2.5  $\mu$ l/culture) was included in each assay (⊗).

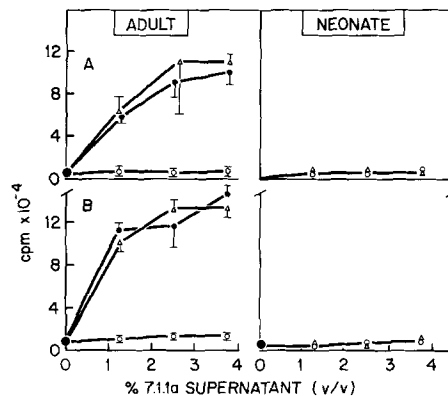


FIG. 3. Response of B cells from adult and neonatal mice to BCGF. Splenic B cells were prepared from either adult or 13-d-old BALB/c mice and cultured at (A)  $2.5 \times 10^5$ /ml or (B)  $5 \times 10^5$ /ml in the presence of control (○), anti- $\mu$  (●) (adult only), or anti- $\delta$  ( $\Delta$ ) antibodies coupled to Sepharose. SN from 7.1.1a was added at the indicated concentrations. After 3 d, cultures were pulsed with [ $^3$ H] thymidine ([ $^3$ H]TdR) and harvested 16 h later. Results are expressed as the mean  $\pm$  SD. These experiments were repeated eight times with neonatal mice ranging in age from 5 to 17 d and adult mice ranging from 8 to 12 wk of age.

proliferate in vitro by treatment with anti-Ig (17, 18, 23–27); proliferation can be sustained by the addition of BCGF (1, 2). Because B cells from neonatal mice (<21 d old) do not proliferate in the presence of anti-Ig alone (8, 26) but do respond to BCDF<sub>μ</sub> (8), we determined whether these cells could respond to BCGF. Fig. 3 shows a typical response of adult B cells to a BCGF-containing T cell SN. It is of interest to note that anti-μ and anti-δ antibodies were equally effective in rendering these B cells responsive to BCGF. In contrast, neither anti-μ (data not shown) nor anti-δ-treated B cells from neonatal mice proliferated in response to BCGF. Similar results were obtained by using B cells from 5–17-d-old mice, although B cells from mice that were 28 d old behaved like adult B cells. This suggests that either (a) anti-Ig-treated B cells from neonatal mice do not express receptors for BCGF; (b) neonatal B cells might express BCGF receptors and bind BCGF but fail to proliferate; (c) neonatal spleens contain cells that suppress the BCGF response; or (d) neonatal spleen cells lack accessory cells necessary for the BCGF response. Although it is not possible to distinguish between possibilities a and b, we have performed experiments that suggest that possibilities c and d are unlikely. Thus, mixtures of equal numbers of spleen cells from adult and neonatal mice give the same BCGF response as the adult spleen cells alone. This suggests that the neonatal B cells do not contain suppressor cells that suppress the BCGF response and that accessory cells that might be absent from the population of neonatal spleen cells (but present in the adult spleen) are not responsible for the failure of immature B cells to respond to BCGF.

Some proliferation was observed when B cells from either adult or neonatal mice were treated with T cell SN alone (in the absence of anti-Ig). This response was particularly pronounced when using spleen cells from neonatal mice. To determine if this mitogenic response to SN alone was due to B cells, Ig<sup>+</sup> cells were deleted with anti-Ig and C before culture. The removal of sIg<sup>+</sup> cells did not change the mitogenic effect of T cell SN alone (Table II). In contrast, the response to BCDF<sub>μ</sub> was reduced by at least 80% (data not shown). This indicates that B cells had been effectively deleted by anti-Ig and C. We therefore conclude that the proliferative response induced by SN alone is not due to B cells in the culture. Since the 7.1 and EL-4 SN

TABLE II  
*Mitogenic Response to 7.1.1a SN of Spleen Cells from Neonatal Mice Is Not Due to B Cells*

| Experiment number                  | Additions of 7.1.1a SN‡ | Treatment of cells* |             |
|------------------------------------|-------------------------|---------------------|-------------|
|                                    |                         | C                   | Anti-Ig + C |
| [ <sup>3</sup> H]TdR incorporation |                         |                     |             |
| <i>cpm</i>                         |                         |                     |             |
| 1                                  | –                       | 882                 | 492         |
|                                    | +                       | 9,627               | 12,195      |
| 2                                  | –                       | 656                 | 687         |
|                                    | +                       | 8,498               | 10,521      |

\* Cells were cultured at  $2.5 \times 10^5$ /ml ( $\pm$  7.1.1a SN) for 3 d, pulsed with [<sup>3</sup>H] TdR, and harvested 16 h later. Data are mean counts per minute of triplicate cultures of  $10^6$  input cells. IgM secretion in response to 7.1.1a SN was decreased by >80% following treatment with anti-Ig and C.

‡ 3.75% (vol/vol).

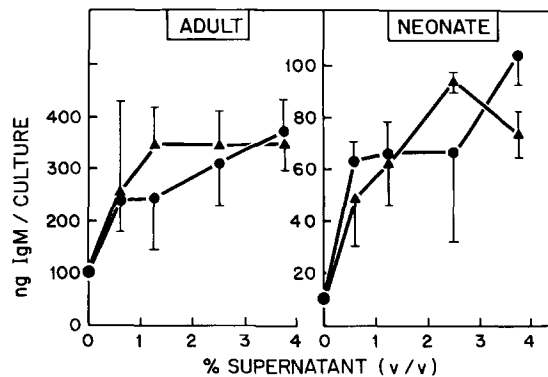


FIG. 4. Response of B cells from adult and neonatal mice to  $BCDF_{\mu}$ . Splenic B cells from adult and 13-d-old BALB/c mice were cultured at  $5 \times 10^5$ /ml with the indicated concentrations of 7.1.1a SN (▲) or 7.1.2 SN (●) for 4 d. IgM secretion was detected by RIA as described in detail in Materials and Methods. Experiments were repeated eight times.

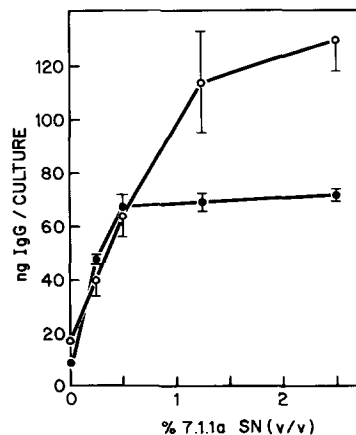


FIG. 5.  $BCDF_{\gamma}$ -mediated induction of IgG secretion by LPS-stimulated B cells from adult or neonatal mice. Splenic B cells were prepared from adult or 15-d-old BALB/c mice and cells were cultured at  $2.5 \times 10^5$ /ml with  $20 \mu\text{g}/\text{ml}$  LPS and the indicated concentration of 7.1.1a SN. IgG secretion was measured by RIA on day 6. Adult B cells (○); neonatal B cells (●). Experiments were repeated three times. Neonatal mice ranged in age from 10 to 21 d.

contain other lymphokines (such as CSF and MAF [20]), background proliferation may be due to the effect of these lymphokines on other cells, e.g., hematopoietic progenitor cells and monocytes.

*Response of B Cells from Adult and Neonatal Mice to  $BCDF_{\mu}$  and  $BCDF_{\gamma}$ .* In view of the failure of neonatal cells to respond to BCGF, it was of particular importance to evaluate their response to BCDF. The data in Fig. 4 confirm previous results (8) and show the effect of increasing amounts of 7.1.1a or 7.1.2 SN on IgM secretion by adult and neonatal B cells. Clearly, both  $BCDF_{\mu}$ -containing SN induce IgM secretion by B cells from both adult and neonatal mice. The response of the neonatal B cells is ~25% of that obtained with adult B cells, which is consistent with the number of immunocompetent B cells in spleens from neonatal mice.

We also compared the effect of  $BCDF_{\gamma}$  on adult and neonatal B cells. Cells were

cultured at low density in the presence of LPS and the effect of increasing concentrations of a BCDF $_{\gamma}$ -containing SN (7.1.1a) on IgG secretion was determined. As shown in Fig. 5, both adult and neonatal B cells secrete IgG in response to LPS and this response can be greatly enhanced by BCDF $_{\gamma}$ . Similar results were obtained in three separate experiments using cells from 10–17-d-old mice. Surprisingly, neonatal B cells secreted ~50% as much IgG as adult B cells even though there was no evidence for proliferation. These results suggest that, in the absence of growth, immature B cells are more responsive to BCDF $_{\gamma}$ , i.e., that either a higher percentage of the cells are induced to secrete IgG or the levels of IgG secreted per cell are higher.

These results demonstrate that B cells from both adult and neonatal mice respond to BCDF $_{\mu}$  and BCDF $_{\gamma}$ . Neonatal B cells do so in the absence of BCGF-mediated proliferation.

### Discussion

The data presented in this report suggest that BCGF, BCDF $_{\mu}$ , and BCDF $_{\gamma}$  are three distinct lymphokines. This conclusion is based on three lines of evidence: (a) Each of the three lymphokines is present in some T cell SN but not others; (b) gel filtration distinguishes BCDF $_{\mu}$  (30–60,000 mol wt) from BCGF/BCDF $_{\gamma}$  (<20,000 mol wt); and (c) BCGF and BCDF show different target cell activities. Thus, both immature and mature B cells respond to BCDF $_{\mu}$  and BCDF $_{\gamma}$ . In contrast, B cells from neonatal mice are unresponsive to anti-Ig and BCGF, whereas adult B cells are highly responsive. These observations support the notion that BCGF is distinct from both types of BCDF and that BCDF $_{\mu}$  and BCDF $_{\gamma}$  are different from each other. The biological and biochemical properties of the three lymphokines are summarized in Table III.

Several other T cell-derived lymphokines that induce polyclonal B cell growth and differentiation have been described (4, 5, 9–11, 27–37). This family of lymphokines has been referred to as B cell stimulating factors (BCSF) (34). BCSF include T cell replacing factor (TRF) (4, 5), allogeneic effect factor (AEF) (29, 30), BCGF (1, 2, 31, 33), BCDF (8, 13), and B cell replication and maturation factors (BRMF) (11). These factors are all defined by their ability to induce growth and/or differentiation of normal or neoplastic B cells. The relationship of these lymphokines to one another is unknown.

At least two lymphokines (AEF and TRF) that induce differentiation to IgM and IgG secretion may be different from BCDF. In contrast to BCDF $_{\gamma}$  (unpublished results), AEF exhibits H-2 restriction (35). However, since AEF has not been evaluated using LPS-stimulated cells, it is not possible to formally exclude the possibility that

TABLE III  
*Properties of BCGF, BCDF $_{\mu}$ , and BCDF $_{\gamma}$*

| Lymphokine       | Biological activity                               | Target B cells          | Apparent mol wt |
|------------------|---|-------------------------|-----------------|
| BCGF             | Sustains anti-Ig-induced proliferation            | Adult mice              | <20,000         |
| BCDF $_{\mu}$    | Induces IgM secretion                             | Adult and neonatal mice | 30–60,000       |
| BCDF $_{\gamma}$ | Induces IgG $_1$ secretion in LPS-treated B cells | Adult and neonatal mice | <20,000         |



BCDF $_{\gamma}$  and AEF are related. Since the 7.1 SN lacks conventional TRF (5, 20), BCGF, BCDF $_{\mu}$ , and BCDF $_{\gamma}$  must be different from TRF.

Lymphokines that induce B cell proliferation include BCGF and BRMF. BCGF is defined by its ability to sustain the proliferation of anti-Ig (1, 2) or mitogen-stimulated (33) B cells. Other lymphokines that sustain replication of slowly dividing B cells in the absence of other signals (e.g., anti-Ig) may exist and may be different from the BCGF as defined by others (1, 2, 31, 33) and ourselves in this study. BRMF (11) induces both growth and maturation of B cells in the presence of histoincompatible adherent cells and antigen. However, maturation occurs in both resting B cells and B cell blasts, whereas growth occurs only in B cell blasts (11). It is therefore possible that BRMF contains both BCGF and BCDF $_{\mu}$ .

Two lymphokines that induce the secretion of isotypes other than IgM have been described: (a) a lymphokine that specifically binds to IgE and induces IgE secretion by antigen-activated B cells (37), and (b) a lymphokine that induces IgG $_1$  secretion by sIgG-negative, LPS-stimulated B cells (BCDF $_{\gamma}$ ) (13). The existence of T cell subsets that induce the secretion of particular isotypes (38–40) raises the possibility of the existence of additional isotype-specific lymphokines for other classes and subclasses of Ig. Isotype switching in activated B cells may be directed by T cell-derived lymphokines. Analogous with the stimulation of T cell growth by IL-2 (41), such class and subclass-specific BCDF may bind to specific receptors on B cells.

Perhaps the most provocative finding in this paper is that anti-Ig alone as well as anti-Ig and BCGF induce proliferation of B cells from adult but not neonatal mice, whereas both mature and immature B cells can secrete IgM and IgG in response to BCDF $_{\mu}$  and BCDF $_{\gamma}$ , respectively. One interpretation of these data is that anti-Ig has two functions: (a) to induce a limited proliferative response in adult B cells, and (b) to induce the appearance of receptors for BCGF in adult but not neonatal B cells. Whether adult B cells responsive to BCDF require a signal from sIg receptors is presently unclear, since anti-Ig is not required for BCDF activity *in vitro*. It is possible, however, that BCDF $_{\mu}$  stimulates B cell blasts that have already been activated by antigen *in vivo*. This possibility is supported by data from Julius et al. (42), who have reported that T cells induce polyclonal differentiation only in large, and not in resting, B cells. Terminal differentiation of resting B cells requires cross-linking of sIg in addition to a T cell signal. We have also recently found that BCDF acts on large but not small B cells in the spleen (Layton et al., unpublished results).

The inability of neonatal B cells to proliferate in response to anti-Ig and BCGF is consistent with their inability to proliferate in response to LPS despite the fact that LPS induces immature B cells to secrete all isotypes of Ig (43). Neonatal B cells are relatively immunoincompetent to challenge with specific antigen. Our results might partially explain this incompetence by suggesting that neonatal B cells terminally differentiate in the absence of clonal expansion, and thus yield a poor antibody response. The implications of these findings for the ease of susceptibility to tolerance induction of immature as opposed to mature B cells (44, 45) is unclear. However, it is conceivable that terminal differentiation in the absence of clonal expansion is one mechanism for putative B cell tolerance as originally suggested by Sterzl and Trnka (46). If this were the case, one might postulate the existence of a suppressor mechanism in adult bone marrow or neonatal spleen that prevents the *in situ* terminal differentiation of clones of immature B cells reactive with foreign antigens. Alternatively,

central lymphoid tissues might lack BCDF-producing T cells. In this regard, a number of laboratories have described suppressor monocytes in the bone marrow (47, 48) and neonatal spleen (49) that prevent antibody responses of normal B cells *in vitro*. It is possible that these suppressor cells exert their effect via the suppression of BCDF and prevent immature B cells from terminally differentiating.

### Summary

In these studies we have determined the molecular weights of B cell growth factor (BCGF) (<20,000), and B cell differentiation factors (BCDF) that induce immunoglobulin M (IgM) secretion (BCDF<sub>μ</sub>) (30–60,000) and IgG secretion (BCDF<sub>γ</sub>) (<20,000). Thus, the molecular weight of BCDF<sub>μ</sub> is distinct from that of BCGF and BCDF<sub>γ</sub>; BCGF and BCDF<sub>γ</sub> cannot be distinguished. In addition, BCGF, BCDF<sub>μ</sub>, and BCDF<sub>γ</sub> are distinguishable by their presence or absence in different supernatants from a panel of mitogen-induced T cell clones. These results suggest that the three lymphokines are different. This conclusion is supported by their differential biological effect on B cells from adult and neonatal mice. Thus, treatment with anti-Ig induces B cells from adult mice to proliferate and this proliferation is sustained by BCGF. In contrast, even in the presence of BCGF, anti-Ig does not induce B cells from neonatal mice to proliferate. However, BCDF<sub>μ</sub> and BCDF<sub>γ</sub> induce IgM and IgG secretion in B cells, respectively, from both adult and neonatal mice. Thus, mature B cells can both clonally expand and differentiate in response to anti-Ig, BCGF, and BCDF, whereas immature B cells can only differentiate. The poor response of neonatal B cells to anti-Ig and BCGF may partially explain the relative immunoincompetence of immature B cells.

*Note added in proof:* W. Lernhardt et al. (*Nature [Lond.]*, 1982, **300**:355) and M. Okada et al. (*J. Exp. Med.*, 1983, **157**:583) have described hybridomas that make either BCGF or BCDF, further supporting the notion that these are two different lymphokines. Moreover, Mayer et al. (*J. Exp. Med.*, 1982, **156**:1860) have recently described a BCDF<sub>α</sub>.

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