PRODUCTION OF A B CELL GROWTH-PROMOTING ACTIVITY, (DL)BCGF, FROM A CLONED T CELL LINE AND ITS ASSAY ON THE BCL₁ B CELL TUMOR*

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It has long been clear that there are two components of the B cell response to antigen. There is an early phase of proliferation in which the responding B cell population is expanded many times, and a later phase of differentiation in which the already proliferating B cell moves on to the immunoglobulin-secretion stage. It has been suggested that separate signals are involved in each phase (1); it has also been realized that there must be an initial lag phase (2) before the onset of proliferation; more recent studies (3) have defined this activation step as a separate event.

It has been many years since the need for a helper T cell in the B cell response to antigen was defined (4, 5) and since the demonstration that the T cell could be replaced by a helper factor (6).

It is now known that a number of T cell- and macrophage-derived nonantigen-specific factors make up the activity previously defined as "helper factor." Current studies are underway to characterize each factor and to determine at which stage it acts and what is its exact role. Parker (7) has shown that B cell proliferation can be seen in cultures of positively selected B cells stimulated with anti-μ. He showed that anti-μ alone was not sufficient, but that interleukin 2- (IL-2) containing supernatants were also required. Immunoglobulin secretion required an additional factor (or factors) not provided in IL-2, but which were present in the supernatant of concanavalin A- (Con A) stimulated T cells. Howard et al. (8; and M. Howard, personal communication) also showed that a number of factors were required for B cell proliferation. In her analysis, the extent of proliferation to anti-Ig was proportional to the third power of the B cell concentration. The slope of this curve was reduced to two by the addition of an IL-2-containing supernatant from EL4 and to nearly one by the further addition of an 18-mol wt cut from the supernatant of P388D₁ (presumably, interleukin 1 [IL-1]). The B cell growth factor (BCGF) activity present in the EL4 supernatants could be separated from the IL2 activity by phenyl Sepharose.

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Abbreviations used in this paper: AOFS, AOFS.21.10.9 cell line; ATS, anti-thymocyte serum; BCGF, B cell growth factor; Con A, concanavalin A; DL, the Denner CC11.75 cell line; (DL)BCGF, factor(s) in DL supernatants responsible for promotion of cell growth; (DL)TRF, factor(s) in DL supernatants responsible for cell-replacing factor activity in antibody production; FCS, fetal calf serum; [³²P]UDR, [³²P]iododeoxyuridine; IL-1, interleukin 1; IL-2, interleukin 2; LPS, lipopolysaccharide; αmm, alpha-methyl mannoside; MoAb, monoclonal antibodies; PFC, plaque-forming cells; SI, stimulation index; SRBC, sheep erythrocytes; TRF, T cell-replacing factor.
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A similar activity has been isolated from the supernatants of phytohemagglutinin-stimulated human peripheral blood lymphocytes by Kishimoto et al. (10).

In the studies of Howard et al. (8; and M. Howard, personal communication) this combination of factors did not induce Ig secretion which was only seen when two additional factors were added: One was an IL-2-containing supernatant from EL4, and the other a T cell-replacing factor (TRF) containing supernatant from the T cell hybridoma described by Takatsu et al. (11).

Very recently, DeFranco et al. (3) have shown that low concentrations of anti-\( \mu \) (1 \( \mu g/ml \)) induce blast transformation, but higher concentrations and/or other factors are required for the cells to enter into the S phase. Other TRF activities and some B cell secretion factors have been described (12-17).

In our own studies (18-20) we have shown that antibody-forming cells were only obtained when both an IL-2-containing supernatant and a TRF-containing supernatant from the long-term alloreactive Dennert line C.C3.11.75 (DL) were present. In this system we measured the IgM response to sheep erythrocytes (SRBC) of anti-Thy-1 and anti-Lyt-2 plus complement-treated Sephadex G-10-passaged B cells from the spleens of anti-thymocyte serum- (ATS) injected mice. Similar observations have been reported by Leibson et al. (14) using a factor present in Con A-induced supernatants. We have called the activity revealed by the IL-2 synergy assay (DL)TRF.

A puzzling feature of all these studies is the apparent requirement for the T cell growth factor IL-2. We have found that although some IL-2-containing supernatants contained a factor that supported B cell proliferation, this activity did not parallel the activity of these supernatants. However, the TEF activity of these supernatants, as measured in the antibody response assay, did closely parallel the T cell growth factor activity over a 1000-fold range of activity (19, 20). For this and other reasons we have concluded that it is IL-2 itself rather than another factor present in the IL-2-containing supernatants that is required.

The DL culture supernatants also have proliferation-inducing activity when assayed in the single cell system described by Wetzel and Kettman (21) in which the frequency of B cell clonal expansion induced by mitogens and other agents can be examined. We found that the frequency of B cells induced to clonal expansion by lipopolysaccharide (LPS) and dextran sulfate under suboptimum conditions can be increased severalfold by the presence of DL culture supernatants (22). This observation and others raise the question of whether these supernatants contain a single activity, (DL)TRF, active in both assays, or whether a separate factor (or factors) is involved.

In the course of our studies, we found that most (DL)TRF-containing culture supernatants of the (DL) line contained a factor that stimulated B cell proliferation. We have called this factor (DL)BCGF. In this paper we will present the results on our studies of this B cell-growth-promoting activity. We describe here the conditions for the production of large amounts of this activity; its assay, both with normal B cells and the in vivo-passaged B cell tumor line BCL1; and demonstrate that it can be absorbed onto BCL1. This B cell-growth-promoting activity can be shown to be distinct from both IL-2 and (DL)TRF. The activity described here is compared with the activities present in a number of other sources of B cell-growth-promoting activities.
Materials and Methods

Animals. Mice of strains CBA/N, DBA/2J, C57BL/6J, BALB/c, and BALB/c ByJ were raised in our own colony from breeding pairs originally obtained from The Jackson Laboratory, Bar Harbor, ME. BALB/c Ke breeding pairs were obtained from the Salk Institute, La Jolla, CA. All F1 hybrid mice were bred in our own colony.

Monoclonal Antibodies (MoAb). The cell line making anti-Thy-1.2 F7D5 was a kind gift from Dr. Phil Lake (University College, London England). The line making anti-Thy-1.2 H0.13.4 was a kind gift of Dr. Ann Marshak-Rothstein (Massachusetts Institute of Technology, Cambridge, MA) and anti-Lyt-2.2 AD4.15 was a kind gift of Dr. Michael Bevan (Scripps Clinic and Research Foundation, La Folla, CA). All antibodies were from ascites taken from Pristane-injected mice inoculated with tumor cells.

Tumor Lines. The in vivo-passaged BCL1 tumor was a kind gift of Dr. Sam Strober, Stanford University School of Medicine, Stanford CA. (23). The tumor was maintained by intravenous injection of 10^6 cells from the spleen of tumor-bearing mice 6-10 wk after inoculation. BALB/c ByJ or BALB/c Ke were used interchangeably as tumor-bearing animals. Palpable tumors appeared in these mice 4 wk after injection. Tumor cells were used in proliferation experiments at anytime between 1 wk after the appearance of tumors (5 wk) and the death of the animals (8-10 wk). BCL1 cells were B cells expressing IgM (24); such cells have previously been shown to proliferate and secrete IgM in response to LPS (24) and in responses to anti-Ig and/or certain TRF (25) including (DL)TRF (16).

Factor-producing Lines. IL-1-containing supernatants were obtained by taking the 4- or 5-d culture supernatant of P388D1 cells (26) originally a gift of Dr. Steven Mizel. IL-2-containing supernatants were obtained from FS6.14.13 (27) or AOF5.21.10.9, gifts of Doctors John Kappler and Philippa Marrack (National Jewish Hospital, Denver, CO). The supernatants were induced with 2 μg/ml Con A in the absence of fetal calf serum (FCS). Alpha-methyl mannoside (α mm) was added to the resultant supernates at a final concentration of 10 mM. (DL)TRF and (DL)BCGF supernatants were prepared as described previously (18) by stimulating 10^5 DL cells/ml (28) with 2.5 × 10^6 stimulator cells from the spleen of mice bearing Ia^+ that were treated with anti-Thy-1.2 plus complement and mitomycin (25 μg/ml). Supernatants were collected after 18-24 h and stored frozen at -70°C. In some cases supernatants were made in the absence of stimulators. DL cells were cloned at 0.5 cells per well and several lines with desirable growth and factor production characteristics were chosen. Supernatants were obtained from these sublines by similar methods. Supernatant from the TRF-producing clone B151K12 (TAKB15) (11) was a kind gift of Dr. Kiyoshi Takatsu.

Assay for Normal B Cell Populations. (C57BL/6 × DBA/2)F1, (BDF1) mice (or, on occasion, other strains) were injected intraperitoneally at 3 and 1 d before killing with 0.06 ml/mouse of ATS (Microbiological Associates, Walkersville, MD). Spleens were removed and treated with optimum doses of two anti-Thy-1.2 MoAb (F7D5 and H0.13.4) and one anti-Lyt-2.2 MoAb (AD4.15) plus selected guinea pig complement. Cells depleted of T cells were then passaged through Sephadex G-10 as described previously (18). The resulting cells were cultured at 8 × 10^6 cells/ml in microcultures containing a total vol of 120 μl. Factors or other stimuli were added in 10-20-μl vol. Proliferation of B cells was determined by adding 0.1 μCi of [125I]-iododeoxy uridine ([125I]UdR) to cultures on day 3. 6 h later cultures were harvested and washed on a multiple automatic sample harvester and the incorporation of [125I]UdR determined with a Searle model 1197 gamma counter (Searle Instruments, Chicago, IL). Geometric means and SE of the counts per minute of triplicate cultures were determined. Machine backgrounds (30-60 cpm) have been subtracted from the counts per minute obtained. Stimulation indices (SI) were obtained by the ratio of test counts per minute divided by control counts per minute.

BCL1 Proliferation. Spleens were removed from mice bearing the BCL1 tumor (recoveries varied from 8 × 10^6-1.3 × 10^6 per mouse), treated in vitro with two Thy-1.2 MoAb and one anti-Lyt-2.2 MoAb as for normal B cells. In many cases cells were subsequently passaged on Sephadex G-10. The resulting cell population was resuspended at 5 × 10^5/ml and 5 × 10^4 cells were added to microtiter cultures (3596; Costar, Data Packaging, Cambridge, MA). Factors or other stimuli were added in 10- or 20-μl vol. Proliferation was determined as described above for normal B cells.
**TRF Activity.** The ability of supernatants to replace T cells in the antibody-forming response was determined as described previously (18). B cells prepared as above for normal B cell proliferation were cultured at $6 \times 10^5$ per well in the presence of 10–30% IL-2-containing supernatant (FS5.14.13 or AOFS 21.10.9) and 0.01% SRBC. After 4 d of culture, direct plaque-forming cells (PFC) to sheep erythrocytes (SRBC) were determined by the slide modification of the Jerne plaque assay. Geometric means and SE of triplicate cultures were determined.

**IL-2 Activity.** IL-2 activity was measured as reported previously (18) by incubating $5 \times 10^3$ cells of an IL-2-dependent NK cell line with several 1:2 dilutions of putative IL-2-containing supernatants. $[^{125}\text{I}]\text{UdR}$ was added at 24 h, and cultures were harvested and the incorporation of label was determined as for B cell proliferation. IL-1 activity was determined in a thymocyte costimulator assay, as reported previously (18).

**Results**

**Assay of B Cell-Growth-promoting Activity from the DL T Cell Line.** When cultures of the long-term alloreactive T cell line DL (28) or clones derived from this line (29) are stimulated with IAk-positive T cell-depleted spleen cells, the 24-h culture supernatants contain both an activity that we have called (DL)TRF, which is necessary for the B cell direct PFC response to SRBC, and a B cell-growth-promoting activity. Our studies with the (DL)TRF activity have been previously described (18). This study concerns the second activity, which promotes B cell proliferation.

We have assayed this (Fig. 1) activity in two separate assays by measuring the 6-h uptake of $[^{125}\text{I}]\text{UdR}$ by B cells after 3 d of culture in the presence of DL culture supernatants. In the first assay (Fig. 1 A) the B cells are obtained from the anti-Thy-1, anti-Lyt-2 + complement-treated Sephadex G-10 column-passaged spleen cells

![Figure 1](image-url)
obtained from mice injected 3 d and 1 d before killing with ATS as described in Materials and Methods. In this assay, in contrast to the assay described by Parker (7) and by Howard et al. (8), no anti-Ig is added. Significant stimulation is seen when as little as 0.1% DL culture supernatant is present, and the inhibition at higher concentrations if often seen.

In the second assay (Fig. 1 B), the B cells are from the B cell tumor line BCL₁. BCL₁-enriched populations were obtained from the anti-Thy-1 anti-Lyt-2 spleen cells from mice inoculated with BCL₁ 4–8 wk before killing. DL culture supernatants contained B cell-growth-promoting activity, as measured in both assays, and the degree of stimulation of [¹³¹I]UdR uptake was found to be concentration dependent (Fig. 1). Significant stimulation was seen when as little as 0.1% supernatant is present in the assay.

Culture supernatants from other sources are also active in the normal B cell proliferation assay (Fig. 1 A). Thus a modest stimulation is seen with a supernatant from the TRF-producing hybridoma TAKB₁5, the IL-1-containing supernatant of P₃₈₈D₁, and the IL-2-containing supernatant of AOFS.2₁.₁₀.₉ (hereafter referred to as AOFS).

In the BCL₁ proliferation assay (Fig. 1 B), only the supernatants from DL have appreciable activity, with a low level of activity present in the TAKB₁5 supernatant. P₃₈₈D₁ and AOFS supernatants are negative. The results from a number of experiments are summarized in Table I.

The activities in the AOFS and DL supernatants are additive or slightly synergistic in the normal B cell assay but do not contribute to BCL₁ proliferation (Table II, experiment 1). The normal B cell proliferation is enhanced by the presence of anti-μ serum both in the presence or absence of DL supernatants (Table II, experiment 2). The BCL₁ proliferation was mostly unaffected by the presence of anti-μ serum. The

| Table I |
|---|---|---|---|---|
| **Summary of Factor Activities in the Proliferation of Normal and BCL₁ B Cells** |
| Additions | Normal B cells | BCL₁ B cells |
| | SI ± SE | N | SI ± SE | N |
| IL-1 | | | | |
| P₃₈₈D₁ | 2.96 (2.17–4.04) | 6 | 0.83 (0.80–0.95) | 4 |
| IL-2 | | | | |
| FS6.14.13 | 4.16 (3.58–4.83) | 15 | 1.06 (0.89–1.26) | 3 |
| AOFS | 2.76 (2.48–3.07) | 15 | 0.96 (0.84–1.09) | 4 |
| TRF | | | | |
| DL | 2.75 (2.42–3.12) | 19 | 11.26 (9.69–13.08) | 13 |
| TAKB₁₅ | 3.77 (3.16–4.49) | 12 | 4.93 (2.81–8.62) | 3 |
| Other | | | | |
| LPS, 5 μg/ml | 14.38 (13.68–15.11) | 5 | 23.89 (18.48–30.87) | 3 |

Results have been pooled from a large number of experiments in which at least three different factors have been titrated and proliferation determined. Concentrations of factors from 10 or 15% to 0.01–0.05% have been tested in each case. The SI used was the highest one obtained in each titration and the geometric means of all the appropriate SI and their SEM are shown. N, number of separate determinations to calculate the mean SI. Normal B cells were from BDF₁, BALB/c, or DBA/2 mice.
Table II

| Experiment | Addition | B cell proliferation | BCL1 proliferation |
|------------|----------|----------------------|--------------------|
|            |          | cpm                 | SI                 | cpm                | SI                 |
| 1          | None     | 339 (316-362)       | 1.00               | 161 (113-224)      | 1.00               |
|            | DL, 0.5* | 2,760 (2,580-2,951) | 8.14               | 3,332 (3,25-3,752) | 21.94              |
|            | DL, 0.2  | 2,030 (1,950-2,111) | 5.99               | 2,902 (2,805-3,014)| 18.02              |
|            | DL, 0.02 | 1,608 (1,235-2,090) | 4.74               | 2,097 (1,832-2,374)| 13.02              |
|            | AOFs, 10 | 1,298 (1,217-1,385) | 3.83               | 152 (147-157)      | 0.94               |
|            | AOFs, 1C +DL, 0.5 | 4,248 (4,119-4,381) | 12.53              | 3,439 (3,141-3,809)| 21.48              |
|            | AOFs, 10 +DL, 0.1 | 3,661 (3,327-4,027) | 10.80              | 2,775 (2,489-3,093)| 17.24              |
|            | AOFs, 10 +DL, 0.02 | 2,252 (2,224-2,280) | 6.64               | 1,229 (1,072-1,408)| 7.63               |
| 2          | None     | 335 (316-356)       | 1.00               | 478 (458-499)      | 1.00               |
|            | DL, 1.0  | 814 (790-838)       | 2.43               | 5,993 (5,367-6,692)| 12.54              |
|            | DL, 0.2  | 780 (689-881)       | 2.33               | 3,739 (3,299-3,932)| 7.82               |
|            | DL, 0.04 | 613 (536-700)       | 1.83               | 2,316 (2,131-2,517)| 4.85               |
|            | Anti-μ    | 932 (925-938)       | 2.78               | 454 (453-466)      | 0.95               |
|            | Anti-μ + DL, 1.0 | 4,358 (4,286-4,431) | 13.01              | 2,600 (2,306-2,930)| 5.44               |
|            | Anti-μ + DL, 0.2 | 2,292 (2,076-2,531) | 6.84               | 2,024 (1,875-2,284)| 4.23               |
|            | Anti-μ + DL, 0.04 | 1,237 (1,106-1,384) | 3.69               | 1,418 (1,335-1,506)| 2.97               |

Results of two representative experiments in which DL supernatants mixed with other inducers were added to B cell proliferation assays are shown. Results are presented as both geometric mean counts per minute per culture (±SE) and as SI. A machine background of 50 cpm has been subtracted from all counts.

* Percentage (vol/vol).

The apparent depression seen when anti-μ was added to the highest concentration of DL supernatant was seen in some, but not all, experiments. Thus there are a number of differences in the characteristics of the two assays.

The amount of B cell-growth-promoting activity in a variety of DL supernatants is not proportional to the amount of (DL)TRF measured in the IL-2 synergy assay (S. L. Swain, unpublished results) and is therefore most likely the property of a different factor. More definitive evidence for this was provided by the absorption experiments shown below.

Absorption of B Cell-Growth-promoting Activity on BCL1 Cells. DL culture supernatants with high levels of B cell-growth-promoting activity were absorbed with in vivo passaged BCL1 as described in Materials and Methods. The resulting absorbed supernatants were assayed for residual growth-promoting activity in the two B cell-proliferation assays and for (DL)TRF activity in the PFC synergy assay. The results are shown in Fig. 2. It can be seen that the B cell-growth-promoting activity absorbed on BCL1, as measured in both the normal B (Fig. 2 A) and BCL1 (Fig. 2 B) assay, but there was only a slight reduction in the (DL)TRF activity as assayed in the synergy assay (Fig. 2 C). The presence of the absorbed supernatant did not inhibit the activity in the unabsorbed supernatant (legend to Fig. 2).
The BCL1-containing spleens were still able to absorb the activity when treated with anti-Thy-1.2 and complement or after glutaraldehyde fixation (Fig. 3). Comparable numbers of normal BALB/c spleen cells did not produce a measurable reduction in B cell-growth-promoting activity (Fig. 4). None of these absorption significantly affected the TRF activity of the supernatants (Table III).

**Production of B Cell-Growth-promoting Activity from T Cell Clones Obtained from the DL Line.** A number of clones or sublines have been obtained in our laboratory from DL cells. The clones were obtained by cloning at 0.5 cells per well. Some of these clones (Table IV, DL.A4 DL.B10) were able to produce large amounts of B cell-growth-promoting activity when cultured under the appropriate conditions. One of these clones DL.A4 (Table IV) produced appreciable levels of (DL)TRF and B cell-growth-promoting activity constitutively in the absence of stimulators of IL-2-containing supernatants. It should be noted that these clones are still antigen reactive and require
antigen stimulation for their propagation.

B Cells from X-linked Deficient (CBA/N × DBA/2)F1 Mice. B cells from female (normal) or male (deficient) F1 mice were cultured with a variety of culture supernatants containing B cell-growth-promoting activity (Table V). It can be seen that B cells from the deficient mice showed little or no response to these supernatants compared with those from the normal mice.

Discussion

B Cell Growth-promoting Activity and Its Assay. The results presented here show that there is a B cell-growth-promoting activity present in the culture supernatants of the DL cell line. This activity can be scored in two B cell assays. In one, the B cells are obtained from the spleens of normal mice; in the other, the B cells are obtained from mice carrying the in vivo B cell tumor line BCL1. The spleens from which the latter B cell preparation is made contain from 70 to 90% tumor cells. In both preparations, exhaustive efforts were made to remove non-B cells. The spleen cell suspensions were treated with anti-Thy-1 and anti-Lyt-2 MoAB and complement and were usually passaged through Sephadex G-10 columns. In the case of the normal B cell preparations the mice were injected with ATS before killing.

Our goal in developing a B cell-proliferation assay is to provide a system in which the responding B cell is acted on directly by only a single factor and responds by proliferation. There are a number of indications, however, that suggest that the
Fig. 4. Absorption of DL on normal and BCL1 cells. Aliquots of DL supernatant were absorbed with no cells (O), or with T cell-depleted BCL1 (●) or BALB/c (▲) spleen cells as described in the legend to Fig. 2. Supernatants were tested for their effect on proliferation on normal B cells and BCL1 cells. The TRF activity of supernatants absorbed in an identical manner is shown in Table III.

| Experiment | Cells for absorption          | PFC per culture ± SE |
|------------|------------------------------|----------------------|
|            | 5%*                         | 1%*                 | 0.2%*                |
| 1          | None                         | 305 (270–344)       | 127 (82–97)          | 51 (15–176)            |
|            | T-depleted BCL1              | 481 (361–641)       | 346 (302–396)        | 86 (73–101)            |
|            | Glutaraldehyde-fixed BCL1   | 209 (158–275)       | 151 (118–195)        | 101 (62–163)           |
| 2          | None                         | 132 (110–185)       | 109 (106–113)        | 82 (63–107)            |
|            | BCL1                         | 352 (154–804)       | 124 (104–148)        | 49 (34–72)             |
|            | BALB/c                      | 65 (44–96)          | 181 (151–210)        | 7 (5–12)               |

In experiment 1 the same supernatants shown in Fig. 3 were tested for TRF activity in synergy with IL2. In experiment 2 supernatants were prepared in an identical manner to those shown in Fig. 4 and were tested. PFC to SRBC were determined on day 4. The background PFC in cultures without TRF were 28 (24–32). The geometric means (±SE) of triplicate cultures is shown.

* Percent (vol/vol) of DL supernatants.

normal B cell assay and perhaps the BCL1 assay are still complex.

First, it is clear that the two assays do not have the same response characteristics. The BCL1 assay responds only to the DL line culture supernatants and those from the TRF-containing supernatants of the Takatsu hybridoma TAKB15. The normal B cell assay responds, in addition, to an IL-1-containing supernatant from cultures of
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Fig. 5. IL-2 induces B cell proliferation factor(s). Aliquots of a pool of DL cells at $10^5$ (in 1-ml Linbro cultures were incubated either with stimulators ($2.5 \times 10^6$ spleen cells from BALB.K, which had been treated with anti-Thy-1.2 MoAb and complement, and mitomycin) or with varying concentration of supernatants from AOFS (a source of IL-2). These supernatants were subsequently tested in the four assays shown.

### Table IV

| Cells          | Inducer    | BCL1 proliferation |
|----------------|------------|--------------------|
|                | cpm        | SI                 |
| None           | AOFS (IL2) | 119 (110–129)      | 1.0                 |
| DL-9           | None       | 495 (447–547)      | 4.2                 |
|                | AOFS (IL2) | 1,497 (1,487–1,507)| 12.6                |
| DL.A4          | None       | 1,032 (1,009–1,056)| 8.7                 |
|                | AOFS (IL2) | 1,812 (1,785–1,891)| 15.2                |
| DL.B10         | None       | 483 (411–545)      | 4.1                 |
|                | AOFS (IL2) | 1,433 (1,302–1,576)| 12.0                |

Cells of the original DL line (DL-9) and two subclones were incubated either alone or with 30% AOFS. Supernatants were collected after 24 h and tested for B cell growth-promotion activity.

P388D1 and to an IL-2-containing supernatant, AOFS.

The normal B cell assay shows additive effects when combinations of stimulators are present. The BCL1 assay responding only to the activity in the DL or TAKB15 supernatants is not affected by additional factors. We speculate that although the BCL1 B cell preparation contains predominantly the tumor BCL1 cell at a particular stage of differentiation, the normal B cell population may contain a variety of subsets or populations of B cells at different stages of differentiation. It is also possible that there are more contaminating, non-B cells present in the normal B cell population that can act as intermediaries in the response to a variety of factors.

The BCL1 assay is clearly less complex. It is of interest that, in contrast to other assays (3, 7, 8, 30) the BCL1 assay does not require the presence of the agent anti-$\mu$ to provide a polyclonal activator signal, nor does it require the presence of added IL-1.
TABLE V
X-linked Defect in B Cell Proliferation

| Experiment | Addition | (CBA/N × DBA/2)F1 ♀ | (CBA/N × DBA/2)F1 ♂ |
|------------|----------|----------------------|----------------------|
| 1          | None     | 250 (244-257)        | 1.00                 |
|            | AOF, 15% | 483 (425-526)        | 1.93                 |
|            | DL, 2%   | 529 (491-570)        | 2.12                 |
|            | LPS, 1 µg/ml | 3,198 (2,935-3,483) | 12.79               |
|            | Anti-Ig, 50 µg/ml | 9,124 (8,761-9,502) | 36.50               |
| 2          | None     | 294 (278-310)        | 1.00                 |
|            | FS6, 15.0% | 1,643 (1,476-1,826) | 5.59                 |
|            | FS6, 3.8% | 1,246 (1,186-1,310) | 4.24                 |
|            | FS6, 1.0% | 834 (819-848)        | 2.84                 |
|            | DL, 15.0% | 1,415 (1,344-1,490) | 4.81                 |
|            | DL, 3.0% | 1,129 (1,087-1,183) | 3.84                 |
|            | DL, 1.0% | 881 (835-926)        | 3.00                 |
|            | DL, 0.2% | 624 (593-659)        | 2.12                 |
|            | LPS, 10 µg/ml | 9,550 (9,111-10,010) | 32.48               |
|            | LPS, 2 µg/ml | 8,348 (8,054-8,651) | 28.39               |

Two representative experiments are shown in which the spleen cells of (CBA/N × DBA/2)F1 female and male littermates were compared for their proliferation in response to supernatants and other inducers. Results are presented both as geometric mean (counts per minute) per culture (±SE) and SI.

* Vol/vol.

or IL-2 (because these are absent from the DL supernatants).

Although the BCL1 assay may at first appear to fulfill the criteria of an ideal assay, as exemplified by the IL-2 assay that uses a T cell growth factor-dependent T cell line, we do not believe that the two assays are necessarily comparable. The BCL1 preparation is not a cloned line and experiments in which we have titrated the proliferation response against B cell number (now in progress) at least raise the possibility that other cells may still be involved. For this, and other reasons, we have referred to the activity as B cell growth-promoting factor rather than a growth factor per se. We have, however, used the abbreviation BCGF, because this is the abbreviation used by other groups (8, 10) to designate a similar activity. It should be emphasized, however, that the BCL1 assay in its present form does provide a very sensitive and selective assay for the B cell-growth-promoting activity and does not respond to IL-1, IL-2, or (DL)TRF (see below).

(DL)BCGF Can Be Distinguished from (DL)TRF, IL-2, and IL-1. The DL supernatants contain an activity, (DL)TRF, that we have measured in the IL2 synergy assay (18) in which the B cell response to SRBC is scored. Most preparations with high (DL)BCGF activity are devoid of measurable IL1 or IL2 activity. The (DL)BCGF activity can be reduced at least 100-fold by absorption to BCL1 tumor cells, whereas the (DL)TRF activity is reduced no more than the degree expected by the dilution inherent in the absorption procedure. The (DL)BCGF and (DL)TRF activities are clearly separated by this procedure. That the apparent absorption is not a result of the release of an inhibitor material is shown by the appropriate mix experiments (see...
legend to Fig. 2). Glutaraldehyde-fixed BCL1 preparations also offer compelling evidence that the effect is indeed a result of a passive process of absorption and does not appear to require any metabolic event. Anti-Thy-1 and complement-treated BCL1 preparations from ATS-pretreated mice are also effective, whereas comparable numbers of normal BALB/c T cell-depleted spleen cells show no measurable removal of activity. One might have imagined that normal spleen would contain some cells with receptors for the activity; the explanation for the striking difference between BCL1 and normal spleen is not immediately apparent.

The Production of (DL)BCGF. The DL line is maintained by weekly stimulation with IA^k-positive stimulator cells. In our previous studies (18) we have, in general, harvested culture supernatants 24 h after antigenic restimulation. Such culture supernatants contain somewhat variable amounts of (DL)TRF and, as described here, (DL)BCGF. We have adopted two strategies to increase the level of these activities, to produce supernatants with predominantly one activity or the other, and to demonstrate that the activities are the product of the T cell line and do not come from the stimulator cells.

In the first method we have shown that large amounts of (DL)BCGF activity can be obtained if DL is cultured in IL-2-containing supernatants instead of the IA^k-positive stimulator cells 1 wk after the last stimulation. This induction of increased factor production is reminiscent of the effect described by Raulet et al. (31) in which increased levels of TRF activity were obtained from cells cultured with IL-2.

In the second, we have derived clones or sublines of the original line. Several of these sublines, e.g., DL.B10, can be induced to produce high levels of (DL)BCGF activity on culture with IL-2-containing supernatants, whereas another, DL.A4, produces a substantial level of (DL)BCGF in the absence of IA^k or IL2 stimulation. These observations provide direct evidence that the (DL)BCGF activity is derived from the T cell line itself and provide an excellent source of material for purification and other studies.

Lack of Reactivity of B Cells from the Spleens of X-linked Deficient Mice. B cell preparations from the spleens of (DBA/2 x CBA/N)F1 male mice were prepared in the same way as described for the B cells for the normal B cell assay. These cells show little or no response to the (DL)BCGF preparations or other preparations that stimulated the B cells from normal (DBA/2 x CBA/N)F1 female littermates. This observation is compatible with the findings of a number of other investigators (32, 33).

One interpretation of this observation would be that the (DL)BCGF acts only on the subset of B cells that is missing in the X-linked deficient mice. The nature of the X-linked defect is still unclear, however, and other interpretations cannot be excluded yet. It is possible, for example, that in the deficient mouse B cells of both subsets fail to reach the differentiation state in which they can respond to the (DL)BCGF, whereas in normal mice both can respond.

Summary

Culture supernatants from a long-term alloreactive T cell line, the Dennert line C3.C3.11.75 (DL) contain a B cell-growth-promoting activity. This activity can be assayed on normal B cells or on the in vivo BCL1 tumor line. We have called this activity (DL)BCGF. This activity can be distinguished from the T cell-replacing factor activity we had earlier found in DL supernates [(DL)TRF], which is required
together with IL2 for the B cell plaque-forming cell response to erythrocyte antigens. The (DL)BCGF can be absorbed on untreated or glutaraldehyde-fixed BCL1. This absorption does not remove (DL)TRF activity. The production of (DL)BCGF is greatly enhanced when DL is cultured with IL2-containing supernatants. Sublines or clones of DL (DL.B10 and DL.A4) have been obtained that make large amounts of (DL)BCGF in the absence of any stimulator cells or IL2. B cells from the Xid-deficient male (DBA/2 × CBA/N)F1 mice do not respond to (DL)BCGF.

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