EVIDENCE FOR TWO DISTINCT CLASSES OF MURINE B CELL GROWTH FACTORS WITH ACTIVITIES IN DIFFERENT FUNCTIONAL ASSAYS

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B cell growth-promoting activities have recently been described by a number of investigators in both mouse and human models. These activities have been identified in mitogen-induced culture supernatants of normal cells (1, 2), cell lines (3–5), and hybridomas (6–13). These B cell growth factor (BCGF)[1] activities have been detected and measured in a number of different assays. These include the measurement of the proliferation of normal B cells after costimulation with anti-Ig (6, 7, 12, 13) or mitogens (2, 9, 10, 12, 14), or direct stimulation of B cells from B cell lymphomas (1–3, 11). Since the assay systems and the sources of the lymphokine activities have varied between individual investigators, it has not been possible to conclude whether one or more different factors were responsible for B cell proliferation.

Some of us recently described (3) a growth factor from the Dennert line C.C3.11.75, termed (DL)BCGF,[2] which maintained the growth of the in vivo BCL_1 tumor line and appeared to bind to receptors on the tumor cells (3). In our early attempts to characterize this material, it was clear that it was unusually

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[1] *Abbreviations used in this paper:* BCGF, B cell growth factor; DXS, dextran sulfate; Con A, concanavalin A; IFN, interferon; IL-2, interleukin 2; [^{125}I]UdR, [^{125}I]iododeoxy uridine; LPS, lipopolysaccharide; MAb, monoclonal antibody; pI, isoelectric point; PMA, phorbol myristate acetate; TRF, T cell-replacing factor.

[2] Factor preparation nomenclature: Since the possible relationships of different factors are unknown, we have designated factor preparations other than IL-1 and IL-2 by their activity (e.g., a preparation that has activity in a B cell growth assay is designated BCGF), preceded in parentheses by an indication of the cell line source of the relevant hybridoma (e.g., BCGF from the DL line is designated (DL)BCGF).

labile to extremes of heat and to low pH (S. Swain, J. Watson, R. Booth, and R. Dutton, unpublished results) and did not have the chromatographic properties reported for several other murine BCGF preparations (5, 6, 13).

In particular, the (EL_4)BCGF reported by Howard and colleagues (6), when assayed on anti-μ–induced B cells was more stable with a molecular weight of ~18,000 by gel filtration analysis, whereas (DL)BCGF gave low recoveries and activity in the 50,000–70,000 mol wt fractions (S. Swain, G. Wetzel, and R. Dutton, unpublished results). We therefore undertook a series of experiments to compare the activities of (DL)BCGF and two other BCGF preparations (EL_4 and FS6.14.13) using three assay systems. The results of these experiments reveal the existence of two distinct classes of BCGF that can be distinguished by the assays in which they can be measured. We have provisionally designated these two classes as BCGF I and BCGF II. The data suggest that BCGF I and BCGF II are active on B cells in different stages of differentiation and/or that they are specific for two subsets of B cells.

Materials and Methods

Animals

Mice of strains CBA/N, DBA/2J, C57BL/6J, BALB/c, and BALB/cByJ were raised in our own colony from breeding pairs originally obtained from The Jackson Laboratory, Bar Harbor, ME. BALB/cKe breeding pairs were obtained from the Salk Institute, La Jolla, CA. All F_1 hybrid mice were bred in our own colony.

Monoclonal Antibodies (MAb)

The cell line making anti-Thy-1.2, F7D5 was a kind gift from Dr. Phil Lake, then at 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 the line making anti-Lyt-2.2, AD4.15 was a kind gift of Dr. Michael Bevan, Research Institute of Scripps Clinic, La Jolla, CA. All antibodies were from ascites taken from Pristane-injected mice inoculated with tumor cells.

Tumor Lines

The in vivo-passed BCL_1 tumor was a kind gift of Dr. Sam Strober, Stanford University School of Medicine, Stanford, CA (15). The tumor was maintained by intravenous injection of 10^6 cells from the spleen of tumor-bearing mice 6–10 wk after inoculation. BALB/cByJ or BALB/cKe 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 any time between 1 wk after the appearance of tumors (5 wk post-injection) and the death of the animals (8–10 wk). BCL_1 cells were B cells that expressed IgM (16).

Factor Preparations

Sources of BCGF. (DL)BCGF was obtained as described previously (3, 17) from the supernatants of the C.C3.11.75 line either after stimulation with non-T cells, after no stimulation, or after stimulation with interleukin 2 (IL-2)-containing supernatants (3). The preparations used in these studies were all extensively characterized. They contained no IL-1 or IL-2 (17) and many of them did not contain interferon (IFN) (J. Klein, unpublished results). Most preparations did contain (DL) T cell-replacing factor (TRF), but their activity in the proliferation assays was not related to their content of TRF (3).

(EL_4)BCGF was prepared from phorbol myristate acetate (PMA)-stimulated EL_4 thymoma cells as detailed previously (6, 18). The BCGF was separated from IL-2 and TRF in these same supernatants by phenyl-Sepharose column chromatography as described

elsewhere (18). The final preparation contained no IL-1, IL-2, TRF, or IFN (18, 19; J. Klein, unpublished results).

(FS6)BCGF was an ammonium sulfate-concentrated preparation from concanavalin A (Con A)-stimulated FS6.14.13 hybridoma cells as described elsewhere (20). This supernatant was previously reported to have BCGF activity (21, 22). The supernatants used in these experiments contain large amounts of IL-2 but do not contain TRF or IFN.

(B151K12)BCGF was a kind gift of Dr. K. Takatsu, Osaka University, Japan. The preparation used in these experiments was constitutive supernatant from the B151K12 hybridoma prepared as described previously (23). This supernatant contains TRF activity in a TRF assay (K. Takatsu, unpublished results), in addition to its BCGF activity (3), but not IFN (J. Klein, unpublished results). Bulk EL_4 supernatant was the unseparated supernatant of PMA-stimulated cultures and is known to contain IL-2, TRF, and BCGF activities (6, 19).

BW.Mls supernatants were obtained by Con A stimulation of a hybridoma made by fusing BW5147 and short-term in vitro Mls-specific T cells (CBA/CaJ stimulated with CBA/J) (T. Beardsley and S. Swain, unpublished results). Supernatants made from subclones of the original are found that are respectively enriched for either IL-2 or BCGF (S. Swain, unpublished results). An ammonium sulfate concentrate rich in BCGF activity but with little IL-2 was used in the BCGF assays. No TRF or IFN is found in these supernatants.

Sources of IL2. $IL\text{-}2_{JW}$ was highly purified from Con A supernatant of normal spleen cells by chromatography and isoelectric focusing as described previously (24). $IL\text{-}2_{EL_4}$ was prepared from the same unseparated EL_4 supernatants that were the source of (EL_4)BCGF. The IL-2-containing fractions were taken after phenyl-Sepharose chromatography. They contained no BCGF or TRF (18, 19). $IL2_{BW.Mls}$ was obtained from supernatants of certain clones of BW.Mls, described above, that produced high amounts of IL-2 and very little BCGF.

Anti-Ig Sources

Anti-Ig was either selected goat anti-mouse μ-chain (whole affinity-purified Ig (6) or $F(ab')_2$ fragments prepared from rabbit antibodies to Ig (7, 21). Anti-μ or anti-Ig was generally used in concentrations of 2–10 μg in synergy with BCGF.

Mitogens

Lipopolysaccharide (LPS) was obtained from *Escherichia coli* 055:B5 by the Westphal technique and was purchased from Difco Laboratories, Detroit, MI. Dextran sulfate (DXS) was a sodium salt, 17% sulfur, 500,000 mol wt (Gibco Laboratories, Grand Island, NY).

Assays

Normal B Cells B cells were prepared as described previously (3, 6) by slight modification of the procedure described previously (20, 25). Mice were injected at days −3 and −1 or at day −2 with 0.06 ml antithymocyte serum (Mirobiological Associates, Walkersville, MD). Spleens of such mice were removed and treated with a cocktail of anti-T cell MAb including two anti-Thy-1.2, anti-Lyt-1.2, and/or anti-Lyt-2.2, and in some cases MK2.2 (20). T-depleted spleens were passaged through Sephadex G-10. Cells were cultured in triplicate at concentrations ranging from 2×10^4 to 1×10^5 per well with serial dilutions of factors or other stimuli. [^{125}I]iododeoxy uridine ([^{125}I]UdR) or [^{3}H]-thymidine was added for the last 6 h of a 72 h culture and the degree of proliferation was assessed by determining incorporation of the radiolabel. Geometric means and SE of triplicate cultures were determined and stimulation indices were obtained by the ratio of test counts per minute divided by control counts per minute. For anti-Ig–costimulated B cell proliferation, anti-Ig at 2–10 μg/ml was added to normal B cells. For DXS-costimulated proliferation, DXS at 50 μg/ml was added to B cells.

BCL_1 Proliferation. Spleens were removed from mice bearing the BCL_1 tumor (recoveries varied from 8×10^8 to 1.3×10^9 per mouse), treated in vitro with two Thy-1.2 MAb

and anti-Lyt-2.2 MAb plus complement 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 after 72 h as described above for normal B cells.

Determination of Cell Cycle State. Two-color flow cytofluorometry was used to simultaneously determine DNA content and RNA content of individual cells. A minimum of 10,000 cells were stained with acridine orange as described in detail elsewhere.[3] The fluorescence signals of each cell were optically separated into two wavelength bands: green (510–550 nm) corresponding to DNA content, and red (>630 nm) corresponding to RNA content. This was possible since AO interacts monomerically with DNA, yielding a characteristic fluorescence maximum at 530 nm, but interacts multimerically with single-stranded RNA and shows a fluorescence maximum at 640 nm. This technique of cell cycle analysis has the advantage that resting G_0 cells can be distinguished from activated G_1 phase cells.

Results

(DL)BCGF Does Not Synergize with Anti-μ for the Proliferation of Normal or BCL_1 Tumor B Cells. The proliferation of highly T-depleted B cells to (EL_4)BCGF is dependent, at low cell numbers per culture, on the presence of anti-Ig of either goat origin (6) or $F(ab')_2$ fragments from rabbits (22). (DL)BCGF was tested in four separate laboratories for its ability to synergize with anti-Ig preparations in this type of assay. The results of two representative experiments are illustrated in Fig. 1.

Fig. 1*A* shows that although phenyl Sepharose-purified (non–IL-2-containing) (EL_4)BCGF gives very good proliferation of normal B cells in the presence of anti-μ, the addition of anti-μ did not enhance (DL)BCGF-dependent proliferation. Fig. 1*B* shows a similar experiment comparing (DL)BCGF with factors present in concentrated supernatants of FS6.14.13. This second experiment was run in the presence of saturating levels of IL-1 from $P388D_1$ (25) as well as anti-Ig, thus ensuring that IL-1 limitation was not contributing to the failure of DL supernatants to support proliferation. The third panel (Fig. 1*C*) shows that the same (DL)BCGF preparation very actively supports the proliferation of the BCL_1 tumor line and that the addition of anti-μ to this assay does not enhance the proliferation induced by (DL)BCGF.

(EL_4) BCGF and (FS6)BCGF Do Not Support Proliferation of BCL_1 Cells. (EL_4) and (FS6)BCGF were next tested for their abilities to support the proliferation of the BCL_1 tumor. As shown in Fig. 2, neither of these factors supports BCL_1 proliferation, in marked contrast to (DL)BCGF.

(DL)BCGF Causes the Proliferation of Normal B Cells Costimulated With DXS. In an earlier study (3), we found that (DL)BCGF had a direct effect on normal B cells. The amount of proliferation seen in these normal B cells was small, however, and we suggested that it might be acting on a small subpopulation of already activated normal B cells that might be in a similar stage of development as the BCL_1 tumor cells (3). We have recently found that up to 90% of BCL_1 tumor cells isolated from BALB/c mice are cycling cells with a large proportion in G_1, whereas normal B cells are >95% resting cells in G_0 (Table I). Thus we felt it

[3] Wetzel, G. D., and J. R. Kettman. Activation of murine B lymphocytes. Different states of activation available to B cells during mitogenesis. Manuscript submitted for publication.

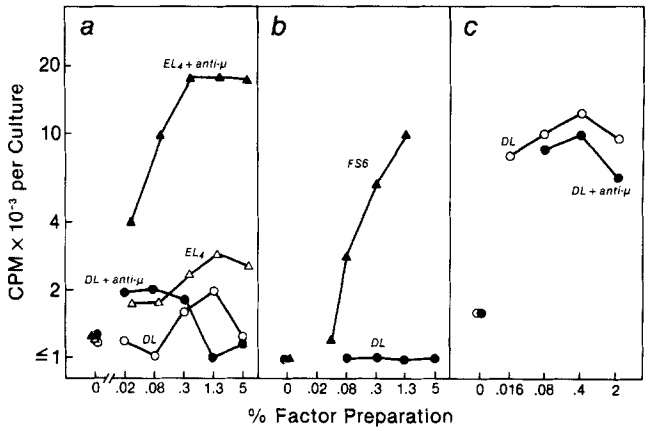

FIGURE 1. Activities of BCGF preparations costimulated with anti-Ig. (DL)BCGF was compared with two BCGF previously found to costimulate proliferation of B cells induced with anti-Ig. (*A*) 5×10^4 purified B cells were incubated alone (open symbols) or in the presence of 5 μg/ml purified goat anti-mouse Ig (closed symbols); with (DL)BCGF (○, ●) or phenyl-Sepharose purified (EL_4)BCGF (△, ▲) titrated into these cultures. [^{3}H]TdR was added for the last 6 h of a 72-h culture period. (*B*) 5×10^4 purified B cells were mixed with 10% constitutive supernatant of $P388D_1$ cells containing IL-1 and incubated with 5 μg/ml $F(ab')_2$ fragments of purified rabbit anti-mouse Ig sera. (●) (DL)BCGF; concentrated supernatants of FS6.14.13 (▲) were titrated into these cultures. Incorporation was determined as in *A*. (*C*) 5×10^4 purified BCL_1 B cells were incubated alone (○) or with 5 μg/ml purified $F(ab')_2$ fragments of rabbit anti-mouse μ-chain sera (●); (DL)BCGF was titrated into these cultures. [^{125}I]UdR was added for the last 6 h of a 72-h culture.

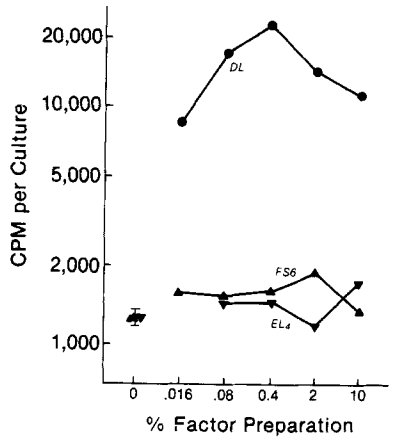

FIGURE 2. Activities of BCGF preparations in BCL_1 B cell proliferation. (DL)BCGF (●), (FS6)BCGF (▲), and (EL_4)BCGF (▼) were titrated into cultures of 5×10^4 BCL_1 B cells. [^{125}I]UdR was added for the last 6 h of a 72-h culture.

might be possible to induce a much larger response of normal B cells to (DL)BCGF in the presence of a costimulating agent that could stimulate a G_0 to G_1 transition of some of the normal B cells. The data in Tables II and III show our success in developing a costimulator assay with normal B cells and DXS. DXS stimulates a portion of B cells to go from G_0 to G_1 states (Table I), but by itself is quite ineffective in stimulating normal B cell proliferation (Table II).

TABLE I
Cycle Analysis of Stimulated and Unstimulated B Cells

	Percent cells		
	G_0	G_1	S,G_2,M
Normal B*	96.5	2.1	1.4
DXS-stimulated B‡	87.7	7.6	2.4
BCL_1 B Cells*	8.9	88.1	3.0

This table is one of three similar experiments.
* Normal B and BCL_1 B were obtained as indicated in Materials and Methods.
‡ Bulk cultures of 2×10^5/ml B cells were stimulated with 50 μg/ml of DXS for 40 h.

TABLE II
Activity of (DL)BCGF on Normal B Cells in Costimulation With DXS

Additions		Normal B cells		BCL_1 cells	
		cpm/culture	SI	cpm/culture	SI
None		181	1.00	163	1.00
DL supernatant	2%	376	2.08	1,372	8.42
	0.4%	258	1.43	935	5.74
	0.08%	213	1.18	504	3.09
DXS	50 μg/ml	499	2.76	278	1.71
DXS + DL supernatant	2%	3,368	18.61	934	5.73
	0.4%	2,109	11.65	475	2.91
	0.08%	1,164	6.43	262	1.61
LPS	50 μg/ml	4,004	22.12		

Purified normal B or BCL_1 cells at 5×10^4/well were incubated with or without 50 μg/ml of DXS and DL supernatant containing (DL)BCGF was added at doses ranging from 2 to 0.08%. Incorporation was determined by adding [^{125}I]UdR during the last 6 h of a 72-h culture. SI, stimulation indice. SI equal cpm in stimulated cultures divided by CPM in unstimulated cultures.

The addition of (DL)BCGF to DXS-costimulated B cells causes a very impressive synergy in B cell proliferation (Table II and Fig. 3). As might be predicted, DXS has no effect on the already high proliferation of BCL_1 B cells (Table II). It seems then that DXS has an effect on all or a subset of normal B cells which causes them to become responsive to (DL)BCGF.

(EL_4)BCGF Does Not Have Activity in the DXS-B Cell Costimulator Assay. Next we tested whether (EL_4)BCGF, which had activity in the anti-μ costimulator assay, would also synergize with DXS in normal B cell proliferation. (EL_4)BCGF did not synergize with DXS for the proliferative response of either unstimulated or DXS-activated normal B cells (Fig. 3). A further comparison of the effects of different sources of growth factors, including the unfractionated PMA-induced supernatant from the EL_4 line, Con A-induced FS6.14.13 supernatants, the B151K12 line (23), and our BW.MIs hybridoma line, are shown in Table III.

A clear pattern indicating two different BCGF activities emerges from these

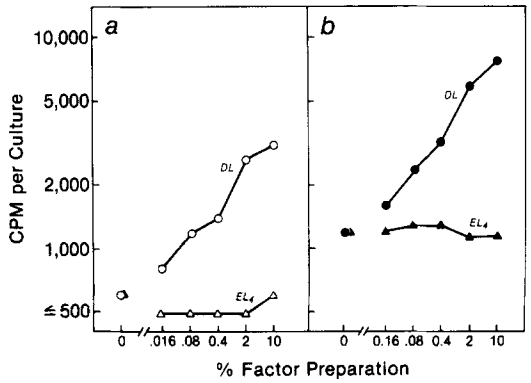

FIGURE 3. Activities of BCGF preparations on DXS-costimulated normal B cells. Purified B cells were cultured at 5×10^4/well alone (*A*, open symbols) or in the presence of 50 μg/ml DXS (*B*, closed symbols). The (DL)BCGF (○, ●) and (EL_4)BCGF (△, ▲) preparations tested in Figs. 1 and 2 were titrated into such cultures. [^{125}I]UdR was added for the last 6 h of a 72-h culture.

experiments. We will designate those BCGF activities that score in the anti-Ig costimulator assays and whose prototype is (EL_4)BCGF as tentatively belonging to the BCGF I class, and those which score in the BCL_1 proliferation assay and costimulate with DXS in normal B cell proliferation, whose prototype is (DL)BCGF, as belonging to the BCGF II class. A summary of the activities of a variety of different factor preparations in these assays is contained in Table IV. In addition to (EL_4)BCGF and FS6, BCGF I activity is found in unseparated EL_4 supernatants. BCGF II activity is found in supernatants we have tested from the BW.Mls line, B151K12 hybridoma, and bulk EL_4 supernatants.

Discussion

The testing of a variety of sources of BCGF activities in a panel of different B cell proliferation assays has allowed us to describe two distinct classes of BCGF whose prototypes are (EL_4)BCGF and (DL)BCGF. We have provisionally named these two classes BCGF I and BCGF II, respectively. BCGF I causes the proliferation of normal B cells that have been costimulated with anti-Ig but does not costimulate proliferation with DXS or stimulate proliferation of the BCL_1 tumor line. In a reciprocal fashion, BCGF II has no enhancing effect on the proliferation of normal B cells plus anti-Ig, but is very effective at costimulating in the presence of DXS and at stimulating the proliferation of the BCL_1 tumor line. Supernatants of various cell lines and hybridomas have been tested for these activities and many have one or both of them.

Two possible interpretations of the significance of these results for normal B cell responses can be considered. It is possible either that these two growth factors have effects on different stages of the differentiation of one subset of B cells or that they act on two separate lineages of B cells. This issue cannot be resolved from the data available, but it is worthwhile considering what we know about the individual assays and reagents for a background to approaching this question.

TABLE III
Comparisons of BCGF in Different Proliferation Assays

Assay	Expt.	Supernatant source	cpm/culture	Stimulation index
Anti-μ Induced Normal B	1	None	230	1.00
		FS6.14.13*	10,000	43.48
		DL‡	110	0.48
	2	None	1,320	1.00
		EL_4§	22,319	16.91
		DL‡	2,000	1.52
DXS-Induced Normal B	1	None	393	1.00
		EL_4§	524	1.33
		DL‡	5,143	13.09
	2	None	1,443	1.00
		EL_4§	2,692	1.87
		DL‡	9,333	6.47
		FS6.14.13*	3,786	2.61
		B151‖	16,395	11.36
	3	None	1,545	1.00
		EL_4 unseparated¶	6,518	4.22
		DL‡	6,004	3.89
		BW.Mls**	8,763	5.67
BCL_1	1	None	263	1.00
		DL‡	4,665	17.74
		El_4§	402	1.53
	2	None	364	1.00
		DL‡	6,569	18.05
		El_4§	863	2.37
		FS6.14.13*	647	1.77
		B151	3,760	10.33
	3	None	1,237	1.00
		EL_4 unseparated¶	6,828	5.52
		DL‡	7,977	6.45
		BW.Mls**	6,074	4.91

BCGF preparations were screened in three proliferation assays. Factor preparations were added over a range of 10–0.016% per culture. Peak proliferation is shown and was usually found at the 10 or 2% factor addition. Factor preparations listed below have all been tested in at least three such assays with similar results.

* FS6.14.13 was a 50× ammonium sulfate concentrate of a Con A-induced supernatant.

‡ DL supernatant from one batch of DL. Other batches gave similar results.

§ EL_4 was purified BCGF separated from EL_4 supernatant, as described in Materials and Methods.

‖ B151 was a 3× concentrated supernatant from the B151K12 line, a kind gift of Dr. K. Takatsu.

¶ "EL_4 unseparated" was unseparated PMA-induced supernatant of the EL_4 line.

** BW.Mls was the concentrated supernatant of the Con A-induced BW.Mls line as described in Materials and Methods.

The anti-Ig synergy assay and the BCGF I used in this assay have been extensively characterized (6, 18). It appears that anti-Ig by itself causes a size increase in the vast majority of B cells but that only about half of these B cells activated with anti-μ go on to proliferate in response to BCGF I (26). Preliminary data show that anti-Ig and BCGF I must be present very early in the culture, from 0 to 12 h (27). It has been suggested that anti-Ig is responsible for the

TABLE IV

Summary of Activities of Factor Preparations in B Cell Proliferation Assays

Factor preparation	BCL_1 proliferation	DXS-induced normal B proliferation	Anti-μ induced normal B proliferation	BCGF type
(EL_4)BCGF	–	–	++++	I
(FS6)BCGF	–	–	++++	I
(DL)BCGF	++++	++++	–	II
(B151)BCGF	+++	+++	–	II
(BW.Mls)BCGF	++	++	–	II
EL_4 unseparated	+++	+++	+++	I and II
Con A supernatant	–	–	–	None
IL2 purified (multiple)	–	–	–	None

BCGF activities were purified or obtained as described in Materials and Methods. "EL_4 unseparated" was unseparated PMA-induced supernatant of EL_4 line. Con A supernatants were obtained by 24-h stimulation of whole spleen. Multiple purified IL-2 were obtained from Con A supernatant and from EL_4 supernatant from the human Jurkat line; unpurified IL-2 from BW.Mls supernatants.

transition of resting B cells from G_0 to G_1 and that BCGF I may have an effect early in G_1 phase. Completion of the anti-Ig-driven cell cycle requires yet a third stimulus, provided by the monokine IL-1, which appears to act at a later point in G_1, driving the cells to enter S phase (27, 28). The resting B cells that respond to the later parts of this sequence are absent from the spleens of mice with the X-linked CBA/N defect (xid).[4] BCGF I from EL_4 has been partially characterized biochemically. It has an apparent molecular weight of 18,000 by gel filtration (6), 11,000 and 14,000 by sodium dodecyl sulfate-polyacrylamide electrophoresis (18), and isoelectric points (pI) of 6.4–6.7 and 7.4–7.6 (18). A factor that has activity on human B cells in costimulation with anti-μ and with chromatographic properties similar to those of murine (EL_4)BCGF has been recently described by Okada et al. (11). When the requirements to drive anti-Ig–induced B cells through both proliferation and differentiation to antibody formation are studied, at least two factors, in addition to (EL_4)BCGF and IL-1, are required that can be provided by the B15K12 hybridoma supernatants and by a pI 4.5 component of EL_4 supernatant (19). Since the B15K12 supernatants contain BCGF II activity (this paper), it is possible that one role of this supernatant is to provide a late-acting growth factor. Consistent with this notion, it has previously been observed that B15K12 supernatants enhance anti-Ig–induced BCGF I-dependent proliferation and increase cell recoveries (19).

In contrast to the BCGF I assay, the costimulation of DXS and BCGF II described here has not yet been extensively studied. DXS was originally described as a polyclonal B cell activator, thought to be particularly active on immature B cells (29). When the frequency of B cells able to initiate clonal growth was studied

[4] Howard, M., T. Malek, J. Ansel, W. Kell, D. Cohen, K. Nakanishi, B. Johnson, and W. E. Paul. Production of B cell growth factor by antigen-stimulated Lyt-1$^+$ 2$^-$ T lymphocytes. Manuscript in preparation.

by Wetzel and Kettman (30) in limiting dilution, there was a synergy between LPS and DXS such that together they could induce virtually 100% of all B cells to form clones, whereas LPS alone induced 1.5% and DXS alone induced 0.1–1% (30). In recent experience, DXS induces 5–40% of cells in bulk cultures of normal splenic B cells to enter G_1 phase during the first 48 h of culture (this paper), but only a small amount of division occurs without added factors, a situation similar to that discussed above for anti-μ stimulation. The number of G_1 phase cells found depends largely on the cell density at which the purified B cells are stimulated: at high cell densities (5×10^5/ml), large number of cells enter G_1 (up to 40%), while at lower cell densities ($1–2 \times 10^5$), many fewer cells are driven from G_0 to G_1 (5–20%) (G. D. Wetzel and S. Swain, unpublished results). This may reflect a requirement for accessary cells for this transition, similar to results found when the effects of LPS stimulation on the B cell cycle are analyzed (31).

The BCL_1 proliferation assay that we first used to identify BCGF II from DL supernatants uses the in vivo BCL_1 tumor line originally characterized by Slavin and Strober (15). The immunologically relevant biological properties of this line have been extensively studied by Knapp et al. (16) and by Isakson et al. (32). They have found that the BCL_1 cells from BALB/c tumor-bearing animals are B cells expressing IgM and IgD. These cells can be induced to differentiate to Ig-secretion with either LPS, anti-Ig plus Con A-induced spleen cell supernatant, or various T cell supernatants containing TRF or B cell differentiation factor, including supernatants from the C.C3.11.75 line, which contains (DL)TRF and (DL)BCGF (33). The low ratio of surface IgM/IgD and the functional similarity of BCL_1 B cells to B cells from young (2-wk old) mice have prompted the proposal that the BCL_1 B cells have the characteristics of immature B cells (32, 33). The results in this report are consistent with the view that BCL_1 B cells are reflective of a subset of normal B lymphocytes with a unique response pattern. Whether such a subset is "immature" is unclear since the correlation between surface phenotype of BCL_1 and B cells from young mice is purely circumstantial evidence for immaturity and since different lineages of B cells could well become immunocompetent at different stages of B cell ontogeny. The BCL_1 cells we harvest from the animal are found to be mostly in G_1 phase, with few resting G_0 cells (Table I).

The possibility that BCGF I and II act on different cell subsets is easily accommodated by the extensive documentation of B cell subpopulations. In particular, mouse splenic B cells are known to contain two major populations of approximately equal numerical size, defined by expression of B cell surface markers Lyb-3, -5, and -7. B cells either express all three (most often termed Lyb-5^+) or fail to express any of them (Lyb-5^-) (for a recent review see reference 34). These two subsets are properly distinguished with the appropriate alloantisera. These antisera are in extremely limited supply, so most of what is known about Lyb-5^+ and Lyb-5^- B cell subsets has been inferred from experiments using mice with an X-linked defect found in CBA/N mice, which contain only Lyb5^- B cells, compared with normal mice which have both Lyb-5^+ and Lyb-5^-

cells. It should be remembered that the Lyb-5$^-$ B cells present in the CBA/N mice as well as other cells in their spleens may not be equivalent to those in normal mice (35, 36). In particular, since they may be more immature, the failure of B cells from xid mice to respond in a situation should not be interpreted as definitively showing that only Lyb-5$^+$ cells are able to respond. The experimental observation is that most lymphokine-drive proliferation and differentiation in in vitro responses are not seen with B cells from xid mice, except for antigen-specific responses using in vivo-primed cells (reviewed in reference 37). In the latter case, a strict requirement for direct T-B interaction for linked recognition by T cells of Ag and B cell I region products has been seen (38). Recent experiments of Takatsu (personal communication) suggest that primed B cells from xid mice can indeed respond to TRF. B cells from xid mice do not proliferate in vitro to anti-μ plus BCGF I or to BCGF II in the absence of costimulation (3).[4] However, our recent experiments (U. Staerz, S. Swain, and R. Dutton, unpublished results) suggest that B cells from mature xid mice can proliferate moderately in response to DXS plus BCGF II and that the addition of DXS, IL-2, and supernatants containing (DL)TRF will produce sheep erythrocyte-specific plaque-forming cells. Thus it is likely that the Lyb-5$^-$ cells in xid mice can become responsive to at least some lymphokines after appropriate stimulation (polyclonal mitogens or in vivo priming). It will be necessary to isolate Lyb-5$^-$ cells from normal mice to determine if this response profile is also accurate for such cells and to gather further evidence to define whether the two subsets of B cells that respond, respectively, to anti-μ plus BCGF I and DXS and BCGF II, correlate with Lyb-5$^+$ and Lyb-5$^-$ subsets.

Recent studies (11, 39) have described two classes of BCGF activity in the human system that are made by T cell clones which appear to synergize in the proliferative response of some B cells. One of these, with an apparent molecular weight of ~17,000 synergizes with anti-Ig and appears to correlate with the BCGF I in the murine system. The second factor has a 50,000 mol wt and was active in proliferation of phytohemagglutinin-induced colonies of B cells (11) and of antiidiotype–stimulated BCLL cells (39). Further biochemical and biological experiments will be needed to determine if the murine BCGF II described herein is equivalent to the second human BCGF, but the circumstantial evidence suggests that this is a serious possibility. If this is so it might be expected that murine BCGF I and II would both contribute to the proliferation of anti-Ig–induced B cells and that there is some suggestion that this prediction is born out as discussed above. The facts that the presence of B15K12 supernatants, added late (48 h) in the response of anti-μ– and BCGF I-stimulated cells, increases the cell recovery at day 5 (19), and that B15K12 contains BCGF II (3 and this report) are consistent with such a model. Taken together these data suggest a model in which BCGF I and II are growth factors of subsets of one lineage of B cells in different states of differentiation. However, they in no way rule out the possibility that a separate lineage of B cells with a different response pattern might also exist and contribute to proliferation in some experimental situations.

Summary

Several previously described B cell growth factor (BCGF) activities from a number of mouse monoclonal T cell sources were compared in different functional assays. The results indicate that there are two distinct classes of BCGF defined by functional activity and source. BCGF I, whose prototype is (EL_4)BCGF, synergized with anti-Ig in the proliferation of normal splenic B cells but had no activity when dextran sulfate (DXS), rather than anti-Ig, was used to costimulate the same source of B cells. BCGF I also failed to directly stimulate BCL_1 tumor B cells. In contrast, BCGF II, whose prototype is (DL)BCGF, showed a reciprocal pattern of activity. BCGF II failed to synergize with anti-Ig–costimulated normal B cells to give good proliferative responses. Sources of BCGF II also directly stimulated (no anti-Ig or DXS added) B cells of the BCL_1 tumor-carrying mice. These results suggest that the two BCGF may have activity on two subsets of B cells that respond differentially to induction with the two polyclonal B cell activators, anti-Ig and DXS. The possibilities that these different patterns of response occur in separate lineages of B cells and/or in B cells in different states of differentiation is discussed.

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