Long-term growth of human B cells and their use in a microassay for B-cell growth factor

(lymphokine/proliferation/anti-immunoglobulin)

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Communicated by Peter C. Nowell, May 5, 1983

ABSTRACT Normal human B lymphocytes, prepared from peripheral venous blood, have been stimulated with intact anti-IgM (μ chain specific) bound to an insoluble matrix. The activation event, in a subfraction of human B cells, was associated with subsequent receptivity to the mitogenic effects of exogenously added B-cell growth factor. The ability of the cell population to specifically absorb the B-cell growth factor was dependent upon the time of stimulation with the anti-IgM. Continuous replenishment of the growth factor resulted in the ability to maintain long-term growth-factor-dependent human B-cell populations. These cultured B lymphocytes were shown to specifically absorb the B-cell growth factor, suggesting the presence of membrane receptors for it. The cultured B lymphocytes were routinely maintained in logarithmic-phase growth, in the presence of growth factor, with a population doubling time of 36 hr. These cultured B cells have been utilized in a microassay for the assessment of B-cell growth factor activity that is accurate, sensitive, and precise.

Experimentation has demonstrated that an activated human B lymphocyte may be stimulated to enter the S phase of the cell cycle by factor(s) present in media conditioned by lectin-stimulated peripheral blood lymphocytes (1-4). One factor capable of stimulating human B cell proliferation, presently known as B-cell growth factor (BCGF), is a trypsin-sensitive protein with a molecular weight of 12,000-13,000 (5, 6). The protein has a mildly acidic isoelectric point (pH 6.3-6.6), is relatively unstable at 56°C (6, 7), is unaffected by low concentrations of reducing agents, and is stable between pH 3 and pH 8 (7). The human BCGF possesses functional specificity in that its action is strictly proliferative. The factor also has target cell specificity in that its scope is limited to B lymphocytes (3, 5).

Further experimentation on many aspects of the biological and biochemical characterization of the growth factor would be enhanced by the establishment of long-term cultures of normal human B cells dependent upon BCGF. In this report we describe a procedure for the polyclonal activation of human B lymphocytes by using anti-IgM (μ chain specific) bound to an insoluble matrix. Anti-immunoglobulin antibodies have previously been utilized in several experimental systems because of their efficacy in human lymphocyte activation; such studies have suggested an important role for the immunoglobulin receptor in B-lymphocyte regulation (8-11). This activation procedure is shown in the present paper to render a subset of cells subsequently responsive to the effects of BCGF and to provide conditions supporting the long-term growth of these B lymphocytes. The long-term BCGF-dependent populations provide an effective system for the sensitive, accurate assessment

of BCGF activity and provide evidence for the presence of growth factor receptors on these cells.

MATERIALS AND METHODS

Preparation of Growth Factors. BCGF-containing growth factor preparations were prepared essentially as described (5). Briefly, peripheral blood lymphocytes, pooled from multiple donors, were cultured for 72 hr at 1×10^6 cells per ml in RPMI 1640 medium supplemented with 0.75% phytohemagglutinin (PHA-M, Gibco) and 1% pooled human type AB serum or 0.25% bovine serum albumin (fraction V). Conditioned media were either prepared in our laboratory or kindly provided by Cellular Products (Buffalo, New York). The conditioned media were clarified by centrifugation, concentrated, and filtered with a Pelicon Diaflo concentrator and applied to a DEAE-Sepharose column. The applied material was eluted in stepwise fashion with 10 mM Tris-HCl/0.2 mM phenylmethylsulfonyl fluoride/2 mM 2-mercaptoethanol/0.12 M NaCl, pH 7.5. The material eluting from the DEAE column containing BCGF activity was utilized in the majority of the experiments in this report. For several experiments (see table legends), including those involved with cell-mediated absorption of the growth factor, BCGF of higher specific activity was prepared by further fractionation of the DEAE-purified material on successive Bio-Gel P-30 and P-100 gel filtration columns as described (5). Human interleukin 1 (IL-1) was prepared by ion-exchange chromatography and gel filtration as described (5).

Cell Purification. Cell preparations enriched for B lymphocytes were purified by negative selection as described (1, 5). Briefly, peripheral blood B lymphocytes were purified from the nonadherent fraction of cells that did not form rosettes with neuraminidase-treated sheep erythrocytes (E_n -rosettes). The E_n -rosette depletion procedure was routinely performed two times. Cells prepared in this manner were >88% surface Ig-positive, <1% E_n -rosette positive, and <4% nonspecific esterase positive.

Nucleotide Incorporation Assays. Routine assays for cell proliferation were done in 96-well microtiter plates. Cells were cultured in a final volume of 200 μ l in RPMI 1640 medium supplemented with 2.5-5% fetal calf serum. For those experiments using long-term cultured B cells, the fetal calf serum was heat inactivated for 60 min at 56°C. Cells were routinely cultured for 96 hr in those assays using freshly prepared B lymphocytes and for 40 hr in those assays using long-term cultures of B lymphocytes dependent upon BCGF. Sixteen hours prior to harvest, the cells were labeled with 1 μ Ci of [methyl- 3 H]thymidine (6 Ci/mmol; 1 Ci = 3.7×10^{10} Bq). For those studies

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Abbreviations: IL-1, interleukin-1; TCGF, T-cell growth factor; BCGF, B-cell growth factor; E_n -rosette, neuraminidase-treated sheep erythrocyte rosette.

using freshly prepared B cells from peripheral venous blood, 2×10^5 cells were cultured with and without insolubilized anti- μ (Bio-Rad), with and without multiple dilutions of growth factor, in round-bottom microtiter plates. The intact purified rabbit IgG against human μ chains (anti- μ) was bound to polyacrylamide beads. The effective antibody concentration in the cultures is indicated in the table legends. For those studies using long-term cultured B cells, 3×10^4 cells were routinely cultured with and without multiple dilutions of growth factor in flat-bottom microtiter plates.

Phenotypic Characterization. Membrane phenotyping was performed routinely on the freshly prepared B-cell-enriched populations and the long-term cultures. Determinations were performed on cytocentrifuge preparations by using a Leitz Orthoplan fluorescence microscope. Monoclonal antibodies against human IgM, IgG, and the monoclonal antibody B1 were kindly provided by Lee Nadler of the Sidney Farber Cancer Institute (Boston). Antiserum against Epstein-Barr virus nuclear antigen was kindly provided by Cellular Products (Buffalo, NY). Monoclonal antibodies OKT4, OKT8, and OKT11 were purchased from Ortho Pharmaceuticals (Raritan, NJ). Monoclonal antibodies against HLA-Dr and heteroantisera against polyvalent surface immunoglobulins were purchased from Cappel Laboratories (West Chester, PA.).

Cytofluorography. Cellular fluorescence measurements were performed on propidium iodide-stained cells by using the 50-H cytofluorographic system of Ortho Diagnostics (Ortho Diagnostic Instruments, Westwood, MA) equipped with helium-neon and 5-W argon lasers. The propidium iodide-stained cells were analyzed for red fluorescence. Simultaneous measurement of fluorescence signal peak values and signal areas permitted accurate discrimination of doublets. Cell cycle analysis was performed with the Ortho 2150 computer (Ortho Diagnostic Instruments); an iterative algorithm was implemented in which a constant or a polynomial function was used to model the S-phase compartment (12).

RESULTS

Experiments were first conducted to examine the effect of stimulation with insolubilized anti-IgM (μ chain specific) on the polyclonal induction of S-phase entry. Both the specificity of the antibody and the use of a support matrix were chosen because of previous data indicating the efficacy of these procedures in B-cell activation (9–11). Human peripheral venous blood B lymphocytes were exposed to various concentrations of whole IgG anti- μ antibodies bound to an insoluble matrix. The relative degree of S-phase entry was measured by thymidine incorporation both in the presence and in the absence of a growth factor preparation containing BCGF. As may be seen in Table 1, optimal proliferation at a fixed concentration of cells occurred at an effective antibody concentration of 15 $\mu\text{g}/\text{ml}$. Under the culture conditions described, one may note that a subfraction of the human B-cell population apparently entered the S phase of the cell cycle under the influence of anti- μ stimulation alone. Yet, it is also apparent that another subfraction was present that required not only the activating effect of anti- μ but also the T-cell product BCGF (13). It should be emphasized that control experiments using the insoluble matrix support alone or the matrix support with an irrelevant antibody failed to induce S-phase entry and failed to activate cells for the subsequent stimulatory effects of BCGF. To ensure that the B-cell proliferative response to combined anti- μ and growth factor stimulation was related to cell number, thymidine incorporation was measured at several different cell concentrations. The data from these experiments, presented in Table 2, dem-

Table 1. Effect of anti- μ and BCGF on human B cell proliferation

Anti- μ , $\mu\text{g}/\text{ml}$ *	[^3H]Thymidine incorporation, [†] cpm per 2×10^5 cells			
	10% BCGF	5% BCGF	1% BCGF	0% BCGF
30	12,808	10,447	10,122	5,218
15	34,980	16,448	10,526	6,430
5	20,222	15,116	8,186	6,222
1	9,016	7,295	4,248	4,466
0	7,252	6,488	2,176	848

*The concentrations listed refer to the actual amount of specific anti- μ antibody bound to the insoluble matrix support bead per ml of culture volume. The stock preparation of anti- μ (Bio-Rad, lot 23493) contained effective anti- μ antibody at 366 $\mu\text{g}/\text{ml}$ after reconstitution. The results presented above are from a single preparation using a single source of cells. Replicate factor preparations and replicate cell populations indicated the same finding, with anti- μ and BCGF proliferative synergy occurring at the same dilutions as tabulated.

[†]B-cell-enriched mononuclear cells, prepared directly from peripheral venous blood, were cultured in round-bottom microtiter plates at 2×10^5 cells per well. Thymidine incorporation was measured during the last 16 hr of a 96-hr total culture period. The BCGF percentages indicate the final amount of factor added to the cultures on a vol/vol basis. The BCGF was purified by ammonium sulfate precipitation, ion-exchange chromatography, and Bio-Gel P-30 and P-100 chromatography. The BCGF-enriched fractions used for these experiments contained only minimal contaminating T-cell growth factor (TCGF) (5).

onstrated a relatively linear relationship, at optimal antibody and growth factor stimulation, between thymidine incorporation and cell number, at a fixed culture volume. Also of interest was the observation that the ability to demonstrate a direct mitogenic effect of anti- μ was apparently dependent on cell concentration.

Confirmation for the role of BCGF in the induction of S-phase entry in a subpopulation of B lymphocytes was next examined by using techniques to assess the specific absorption of the growth factor. Previous experiments demonstrated that activated human T-cell-mediated absorption of TCGF from a growth factor preparation capable of stimulating B-cell proliferation failed to remove that activity associated with the B-cell proliferative response and demonstrated that activated B cells were incapable of absorbing TCGF (1, 3–5). Yet, to more fully establish the

Table 2. Effect of cell concentration on the B-cell proliferative response

Culture conditions*	[^3H]Thymidine incorporation, [†] cpm		
	5×10^4 cells	1×10^5 cells	2×10^5 cells
B cells	500	737	1,814
B cells + BCGF	3,215	6,445	12,840
B cells + anti- μ	356	1,314	3,851
B cells + anti- μ + BCGF	8,766	21,518	36,706

*BCGF utilized for these experiments was prepared through DEAE-Sephacrose ion-exchange chromatography. The activity of the BCGF on the B cells alone, in the absence of anti- μ , has been previously reported (5). Anti- μ was used at a final concentration of 15 $\mu\text{g}/\text{ml}$ and BCGF was used at a final concentration of 10% (vol/vol).

[†]B-cell-enriched populations were prepared directly from peripheral venous blood. The cells were grown, at the numbers indicated, for 96 hr. Thymidine incorporation was measured during the last 16 hr of the 96-hr culture period. The results presented are the mean values derived from three separate experiments. The standard errors of the mean for triplicates within an individual experiment were approximately 5%. The standard errors of the mean for replicate experiments were approximately 15%.

Table 3. Effect of time of anti- μ prestimulation on the BCGF-dependent proliferative response

Time of anti- μ prestimulation,* hr	$[^3\text{H}]$ Thymidine incorporation,† cpm		Stimulation index‡
	Anti- μ without BCGF	Anti- μ with BCGF	
0	6,284	46,421	7.4
24	3,219	41,144	12.8
48	1,595	33,212	20.8
72	1,148	27,098	23.6

* The group indicated as "0" refers to those B-cell-enriched populations to which both anti- μ and growth factor (or control media) were added simultaneously. The groups referred to as 24, 48, and 72 were first exposed to anti- μ for 24, 48, or 72 hr, respectively, prior to addition of either factor or control media.

† B-cell-enriched populations prepared directly from peripheral venous blood were cultured and labeled as described in the legend of Table 2. The results presented are the mean values from replicate experiments with standard errors of the same magnitude as indicated in the legend of Table 2. B cells were exposed to anti- μ for the time periods indicated. After these time intervals the cells were exposed to either control medium (group indicated as without BCGF) or a growth factor preparation containing BCGF (group indicated as with BCGF). The cells were grown for an additional 96 hr, with thymidine incorporation being determined during the last 16 hr of culture. Prior to addition of the control media, or the growth factor preparation, all samples were adjusted to 2×10^5 viable cells per well. The BCGF samples used for these experiments were fractionated by ammonium sulfate precipitation and DEAE chromatography. The anti- μ was used at 15 $\mu\text{g}/\text{ml}$ and the growth factor preparation was used at 10% (vol/vol).

‡ Stimulation index was determined by dividing the cpm incorporated by cells stimulated by anti- μ plus BCGF by the cpm incorporated by cells stimulated by anti- μ alone.

role of BCGF in normal B-cell proliferation a direct assessment of its absorption by activated normal B cells seemed obligatory. For these experiments human B lymphocytes were stimulated with anti- μ alone for periods of time between 0 and 120 hr. The cell populations were subsequently treated in two ways. One set of anti- μ -stimulated cells was utilized for the assessment of thymidine incorporation both in the presence and in the absence of growth factor. The second set of stimulated cells was incubated at 4°C, for 2 hr, with a growth factor preparation containing BCGF. After this incubation, the growth factor preparation was clarified and tested for the presence of residual B-cell proliferation-stimulating activity. As may be seen in Table 3, the inherent proliferative response to anti- μ alone diminished with time of incubation, whereas the relative effect of added growth factor, as measured by a stimulation index, increased with time. This change in proliferative requirements was also reflected by the increasing ability, with time, of the anti- μ -stimulated population to absorb BCGF (Table 4). To more effectively quantitate the subfraction of the cell population actually responsive to the growth factor stimulus, human B cells were stimulated with anti- μ for 48 hr, exposed to growth factor for the next 72 hr, and examined cytofluorographically for the number of cells in the S phase of the cycle. Table 5 presents data for the population percentages in the various stages of the cell cycle, as determined on alcohol-fixed, propidium iodide-stained preparations. There is apparently a subfraction of human B cells capable of entering the S phase in response to stimulation by anti- μ , yet there is a larger group of lymphocytes that require multiple signals for an effective proliferative response.

Given the data indicating that the fraction of cells apparently responsive to anti- μ alone diminished with time of stimulation, while the BCGF-dependent population apparently formed a

Table 4. Absorption of BCGF by anti- μ -stimulated B cells

Time of anti- μ prestimulation,* hr	$\Delta[^3\text{H}]$ Thymidine incorporation,† cpm		Activity absorbed,‡ %
	Pre- absorption	Post- absorption	
0	15,524	15,248	1.8
24	15,524	10,907	29.7
72	15,156	6,827	55.0
96	19,572	9,887	49.5
120	11,724	0	>95.0

* Time of anti- μ prestimulation refers to that time at which the B-cell-enriched population was exposed to anti- μ prior to being used for absorption of the BCGF preparation.

† $\Delta[^3\text{H}]$ Thymidine incorporation refers to those values seen with BCGF (both before and after absorption) tested on freshly prepared anti- μ -stimulated cells minus the values for incorporation seen with anti- μ alone. The values seen with anti- μ alone were similar to those in Tables 1 and 2. The B-cell-enriched populations were exposed to anti- μ for the indicated time periods. After this interval, 2.5×10^7 viable cells were incubated for 2 hr at 4°C with a preparation of BCGF. After this incubation, this preparation was tested for residual activity. Preabsorption values are those seen with the factor before exposure to the anti- μ -activated cells, and postabsorption refers to those values seen after exposure to the activated cells. The variation in thymidine incorporation seen with different preabsorption factor preparations was due to the inherent variability of the different target anti- μ -stimulated assay cells. The BCGF used for absorption was prepared by multiple steps as indicated in the legend of Table 1. As previously reported (1, 5) activated T blasts, used as a control, failed to absorb the BCGF activity.

‡ Percent activity absorbed is determined by simply dividing postabsorption by preabsorption cpm and subtracting the percent value from 100.0.

greater fraction of the proliferating pool, we next attempted to grow human B cells after a relatively long initial anti- μ activation period. Human B cells, exposed to insolubilized anti- μ for periods of time >144 hr, were thereafter exposed to a growth factor preparation containing BCGF. The growth-factor-containing medium (RPMI 1640 supplemented with 1–5% heat-inactivated fetal calf serum) was replenished every 48–72 hr and the membrane phenotypes of the proliferating cells were determined at frequent intervals. The membrane phenotyping included T-cell markers to assess any cellular expansion of those T cells (<1%, see *Materials and Methods*) known to be present in the starting peripheral venous blood B-cell-enriched preparation. These T cells, when present, were removed by suc-

Table 5. Cytofluorographic evaluation of cell cycle states

Exp.	Culture conditions	$[^3\text{H}]$ Thymidine incorporation,* cpm	% of cells in state†		
			G ₁	G ₂ /M	S
1	B cells + anti- μ (15 $\mu\text{g}/\text{ml}$)	1,612	83.9	3.5	12.6
	B cells + anti- μ + BCGF (10%)	32,025	58.6	3.7	37.6
2	B cells + anti- μ	2,020	86.0	0.8	13.2
	B cells + anti- μ + BCGF	36,045	53.8	8.2	38.0

* These values refer to the cpm seen in those cells, used for cytofluorographic evaluation. The B-cell-enriched population was first exposed to anti- μ for 48 hr. The viable cell number was adjusted to 2×10^5 cells per well, growth factor was added, and the cells were grown for the next 96 hr. Sixteen hours prior to harvest, the cells were labeled with $[^3\text{H}]$ thymidine and cpm incorporation was determined.

† The cells were first exposed to anti- μ for 48 hr and BCGF was added for the next 72 hr. The cells were then fixed, stained, and cytofluorographically evaluated.

Table 6. Surface phenotype of long-term-cultured B cells.

Marker antiserum	% positive*
Anti-polyvalent surface Ig	86
Anti-OKT11	0
Anti-IgM	66
Anti-IgG	7
Anti-B1	>95
Anti-HLA-Dr	85
Anti-EBNA	0

EBNA, Epstein-Barr nuclear antigen.

*The results presented represent the percent of cells positively reactive, as determined by fluorescence, for the markers tested. The long-term culture tested was factor-dependent line JM-7 that had been in culture for approximately 110 days prior to marker determinations. Replicate determinations on another long-term line (DVB-1) were essentially the same.

cessive E_n -rosette depletion procedures. Table 6 presents the membrane marker profile for a representative B cell population maintained with growth factor in logarithmic phase growth for a period of approximately 90 days. The B cell population possessed a large percentage of IgM-bearing cells, consistent with the anti- μ stimulation used at the beginning of culture. The high percentage expression of the B1 phenotype and the lack of T-cell membrane markers are indicative of the cell type uniformly present in those long-term populations after methodical E_n -rosette depletion during the early course of growth. Furthermore, these long-term-cultured B lymphocytes, to date, have been negative for Epstein-Barr nuclear antigen. Fig. 1 presents typical growth kinetics, over a span of 12 days, for a representative B-cell population cultured in the presence of BCGF. One may note that removal of factor led to a relatively rapid loss of viable cells with eventual loss of the cultured population. The rate of loss of viability, upon factor removal, was demonstrated to be related to cell concentration. Cells seeded at 2×10^5 per ml were shown to lose viability more rapidly than those started at higher concentrations when the factor was withdrawn. The calculated population doubling time of the cells shown in Fig. 1 was 36.1 ± 2.2 hr (mean $\pm$ SEM), dependent upon initial starting cell concentration. The human B cells were most readily maintained in logarithmic phase growth when grown at starting concentrations of 2×10^5 cells per ml, reaching a saturation concentration of approximately $1-1.2 \times 10^6$ cells per

FIG. 1. Growth characteristics of long-term-cultured B-cell populations. Human B cells were cultured in the presence of BCGF for 70 days. At this time the cells were seeded at 2×10^5 cells per ml in medium containing BCGF (—), or in medium without growth factor (---). The cells were cultured thereafter for either 48 hr or 96 hr prior to splitting to the original concentration and refeeding. The BCGF was used at 10% (vol/vol) of the culture medium, supplemented with heat-inactivated fetal calf serum.

Table 7. BCGF microassay with long-term-cultured B cells

BCGF, %	[³ H]Thymidine incorporation,* cpm per 3×10^4 cells
0.0	161 $\pm$ 35
0.6	1,734 $\pm$ 74
1.2	4,216 $\pm$ 794
2.5	9,087 $\pm$ 420
5.0	15,101 $\pm$ 679
10	18,763 $\pm$ 130

*The long-term-cultured B cells were removed from flasks, washed three times, and placed in flat-bottom microtiter plates in the presence of RPMI 1640 medium supplemented with 2.5% heat-inactivated fetal calf serum. The cells were grown, with and without growth factor, for 24 hr. At this time the cells were exposed to $1 \mu\text{Ci}$ of [³H]thymidine for the next 16 hr, after which they were harvested. The growth factor used was prepared through ion-exchange chromatography. The number of cells utilized in this assay was shown to be optimal for the parameters of efficiency of cell utilization, minimal background, and effective range of cpm incorporated. Assay for thymidine incorporation, in the presence of a fixed amount of growth factor and various cell numbers, revealed an essentially linear relationship between 1.5 and 3.5×10^4 cells. Results are mean $\pm$ SEM.

ml (data not shown). To this date, the human B cells have been in culture for periods longer than 8 months.

The long-term factor-dependent cells were next tested for their utility in a microassay to quantitate BCGF. Table 7 presents a representative dilutional experiment in which cells at a fixed concentration (3×10^4 per well) were exposed to increasing concentrations of a standard BCGF-containing growth factor preparation. Saturation of the proliferative response, as measured by incorporation of radio-labeled nucleotide, occurred at a factor concentration of 5% (vol/vol). The sensitivity of the assay was underscored by the observations that one may dilute a preparation of BCGF to a significantly greater extent than is possible in the standard anti- μ -based assay (refer to Table 1) and that the cell number used for the microassay may be reduced by a factor of 10. Furthermore, the precision of the assay was more than adequate in that the standard errors of the means of replicate determinations, at a fixed dilution, were invariably small (refer to Table 7).

Experiments using the long-term cultures next focused on the question of whether these B lymphocytes could effectively absorb BCGF. B lymphocytes (2.5×10^7) were incubated for 2 hr at 4°C with a growth factor preparation known to contain both TCGF and BCGF. After the incubation, the preparation was clarified and residual proliferation-stimulating activity was determined. Cells from the long-term B-cell cultures were able to effectively absorb BCGF from the preparations yet were unable to absorb TCGF in the sample. The degree of absorption of the BCGF by the long-term population (at a fixed number of cells) was equivalent to that seen with freshly prepared B lymphocytes after 72–96 hr of anti- μ stimulation. This specificity of absorption, and therefore the accuracy of the microassay procedure, was further confirmed by experiments examining the effect of partially purified IL-1 on the B-cell proliferative response. Long-term-cultured human B lymphocytes were used in a microassay to test the proliferative effectiveness of IL-1 prepared by ion-exchange chromatography and gel filtration on a Bio-Gel P-30 column (5). Human IL-1 prepared in this manner would not alone support the proliferative response of the long-term-cultured B lymphocytes.

DISCUSSION

The results presented herein provide further evidence for the specific role of human BCGF in the proliferative control of a

subfraction of normal peripheral venous blood-derived B lymphocytes. Utilizing anti-IgM (μ -chain specific), one may dissect out populations of cells that are differentially responsive to this initial activating event. One subset of cells, in agreement with several previous studies, appears to be directly stimulated to enter the S phase of the cell cycle, at specific cell concentrations, by exposure to the insolubilized antibody (9, 10). This result may truly reflect the fact that a small subpopulation of human B cells may enter the S phase of the cell cycle independently of BCGF. Yet, the B-cell-enriched populations used in the cytofluorographic studies were known to contain a small percentage of T cells (<1%) and monocytes (<4%) that, at present, cannot be ruled out as possible accessory cells (or sources of soluble factors) in the apparent growth-factor-independent response (14, 15). Another population of human B cells can be readily demonstrated to require the proliferation-stimulating activity of the T-cell-derived product BCGF in order to enter the S phase of the cell cycle (12). This subfraction of human B cells acquires receptivity to the growth factor after initial exposure to the activating signal, as previously demonstrated with murine lymphocytes (16). Further evidence for the role of BCGF in this proliferative response is provided by its specificity of action and function (3, 5).

The concept that BCGF has a stimulatory effect on the induction of S-phase entry in a subpopulation of normal human B cells is reinforced by the observation that these cells may specifically absorb the growth factor. Prior experimentation had demonstrated that long-term cultures of T cells could effectively absorb TCGF but were incapable of removing that proliferation-stimulating activity specific for B cells (3–5). In the present report, this observation has been extended to include the data that activated B lymphocytes, and those B lymphocytes grown in long-term growth-factor-dependent culture, specifically absorb BCGF from a factor preparation. This would suggest the presence, on activated B cells, of receptors for the B-cell-specific factor in a manner analogous to the receptors present on T lymphocytes for TCGF (17).

The ability to absorb BCGF and the ability to mitogenically respond to its presence has allowed for the development of long-term factor-dependent populations of human B lymphocytes. To this date, there has been no evidence for viral transformation of these cells. The capability of maintaining human B cells in extended culture has allowed the development of a rapid microassay specific for human BCGF. The microassay on human B cells is directly analogous to those assays for TCGF using continuously cultured T cells (18). This microassay may effectively be utilized for unit quantitation by means of probit analysis (18). The availability of a microassay for BCGF activity will be of significant utility in future studies on both the biology and the biochemistry of this unique lymphokine.

Note Added in Proof. Refs. 19 and 20 have appeared since submission of this manuscript. Both of these papers have confirmed the effectiveness of anti- μ stimulation of human B cells in terms of inducing subsequent receptivity to BCGF.

We acknowledge the excellent technical assistance of Rosalia Sandler, Suzan VanLaningham, and Deborah Valerga and the manuscript preparation of Julie Wiley. This work was done in partial fulfillment of the doctoral dissertation requirements of J.M. and S.M. This work was supported in part by National Institutes of Health Grant CA21927, a grant from the Faisal Foundation, the Kleberg Foundation, and an institutional grant from the Interferon Research Fund.

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