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Biochemical separation of a human B cell mitogenic factor

(lymphokines/lymphocyte growth factors/proliferation/differentiation)

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ABSTRACT **Recent studies have established the ability of human B lymphocytes to undergo G_1-phase cell cycle progression and subsequent DNA synthesis upon exposure to factor(s) present in media conditioned by lectin-stimulated mononuclear cells. Procedures for the isolation of such a cytokine have been the focus of the present investigation. Conditioned medium from cells stimulated by lectin for 72 hr was fractionated by ammonium sulfate precipitation, ion-exchange chromatography, and gel filtration chromatography. During the isolation procedure the proliferation-stimulating activity of the column fractions was assayed concurrently on purified human T cells, purified human B cells, and murine thymocytes. T cell and B cell stimulatory factors present in the initial conditioned medium were found to copurify during ammonium sulfate precipitation, DEAE-Sephadex chromatography, and Bio-Gel P-30 gel filtration. However, partial separation of these two activities was achieved after Bio-Gel P-100 gel filtration. Analytic polyacrylamide gel electrophoresis of radiolabeled Bio-Gel P-100 column fractions demonstrated a distinct protein band of 14,000–15,000 daltons in those column fractions predominantly supporting T cell growth and a distinct protein band of 12,000–13,000 daltons for those fractions predominantly supporting B cell growth. The fractions associated with B cell mitogenic activity induced B cell S-phase entry in a proportion of B lymphocytes in the absence of any detectable IgM secretion.**

Soluble growth factors for normal epithelial, fibroblastic, hematopoietic, and lymphoid cells have been described and characterized (1). The best delineated lymphoid cell growth factors have been termed interleukin 1 (IL-1) and interleukin 2 (IL-2) (2). These factors have been demonstrated to function in a bimodal amplification network resulting in the proliferative expansion of activated T cells (3, 4). The role of soluble factors in the induction of B cell proliferation has also recently been investigated (5–10). Studies have confirmed the capability of culturing normal, Epstein–Barr virus-negative, B lymphoblastoid cell lines by using exogenously supplied growth-promoting agents (7, 8). Investigation into the sources for these B cell growth-promoting agents has revealed that conditioned media derived from several culture systems contains the molecule(s) capable of stimulating B cell proliferation. Both lectin-stimulated normal mononuclear cells (derived from human peripheral blood or murine spleen cell preparations) and antigen-restricted normal helper T cells (grown in the presence of irradiated accessory cells) have been shown to produce factors capable of supporting B cell growth (5–7). Similar B cell growth-supporting factors have also been observed in conditioned media from phorbol ester-stimulated EL-4 thymoma cells and lectin-stimulated T hybridoma cells (FS6 14.13) (7, 9). Yet it has been well documented that these conditioned media preparations contain multiple functional biological activities. The question that becomes readily apparent is whether one of the known biologic activities in these media (e.g., IL-1, IL-2, and T cell replacing factor) is inducing B cell proliferation or whether this growth may be dependent upon a unique, previously undefined, biologic signal.

Several observations give some insight into the resolution of this question. Experiments have confirmed that absorption of IL-2 (T cell growth factor, TCGF) from conditioned media capable of stimulating B cell proliferation fails to remove that activity associated with the B cell proliferative response (5, 6, 9). B cell growth may also be maintained by using conditioned media that do not stimulate B cell differentiation (8), thereby making the role of T cell replacing factor conjectural with regard to B cell proliferation (T cell replacing factor is operationally defined here as that factor or group of factors capable of inducing B cell differentiation in T cell-depleted cultures). Furthermore, the ability of clonally derived T cell populations to produce B cell mitogenic factors (7, 9) would seem to militate against macrophage-derived IL-1 being the sole factor involved in the B cell growth stimulation observed. These observations suggest that B cell proliferation may be maintained by a unique T cell-derived product and that this product is evidently distinct from the well-described lymphokines. In the present report, we provide confirmation for this suggestion by the biochemical separation of a specific B cell mitogenic factor. The schema detailed in this report represents efforts directed at the analytic demonstration of the existence of a B cell specific lymphokine and does not address the question of large-scale purification.

MATERIALS AND METHODS

Preparation of Conditioned Media. Human peripheral blood lymphocytes were prepared from several normal donors, pooled, and cultured at 1×10^6 per ml (11) in RPMI 1640 medium supplemented with penicillin, streptomycin, 2 mM glutamine, 0.25% bovine serum albumin, and 0.75% phytohemagglutinin (PHA-M, GIBCO). The cells were grown for 72 hr at 37°C, after which time the conditioned medium was clarified and stored at 4°C until the time of factor isolation.

DEAE-Sephadex Chromatography. A precipitate derived from the conditioned medium by 50–80% saturation with ammonium sulfate was exhaustively dialyzed against 10 mM Tris·HCl/0.2 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$)/2 mM 2-mercaptoethanol, pH 7.5, and loaded onto a DEAE-Sephadex (Pharmacia) column (2.5 × 15 cm) equilibrated with dialysis buffer. The chromatographed material was eluted in stepwise fashion with dialysis buffer containing 0.12 M NaCl. The material eluting from the ion-exchange column was next

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Abbreviations: IL-1, interleukin 1; IL-2, interleukin 2; TCGF, T cell growth factor; BCGF, B cell growth factor; $PhMeSO_2F$, phenylmethylsulfonyl fluoride; E-rosette, sheep erythrocyte rosette.

concentrated and then stabilized with 0.1% polyethylene glycol (M_r 6,000).

Gel Filtration Chromatography. The DEAE-Sephadex eluate derived from 1 liter of crude conditioned medium was next applied to a Bio-Gel P-30 (Bio-Rad) gel filtration column (2.5 × 95 cm). The column was eluted with 10 mM sodium phosphate/0.2 mM $PhMeSO_2F$/2 mM 2-mercaptoethanol/0.15 M NaCl/0.1% polyethylene glycol, pH 7.0. Five-milliliter fractions were collected and utilized for proliferation assays described below. Those fractions possessing B and T cell proliferation-stimulating activity were pooled, concentrated, and applied to a Bio-Gel P-100 gel filtration column (2.5 × 95 cm). The column was again eluted with 10 mM sodium phosphate/0.2 mM $PhMeSO_2F$/2 mM 2-mercaptoethanol/0.15 M NaCl/0.1% polyethylene glycol, pH 7.0. Five-milliliter fractions were collected for subsequent proliferative assay with absorbance recorded at both 230 and 280 nm. For the experiments reported herein each of the above column fractionation procedures was replicated a minimum of five times on five separate preparations of conditioned medium.

The collected B cell specific fractions from four separate Bio-Gel P-100 chromatographic separations were also pooled and applied to a Bio-Gel P-20 gel filtration column (1 × 100 cm). The column was eluted with the gel filtration buffer mentioned above and 1-ml fractions were collected and assayed for their proliferation-stimulating activity on both B and T cells.

Cell Purification. Purified human peripheral blood T cells and monocytes were prepared as described (12). Human peripheral blood B lymphocytes were purified by both negative and positive selection. For the negative selection procedure, the B cells were purified as described (6) from the nonadherent fractions that did not form rosettes with sheep erythrocytes (E-rosettes). The positive selection procedure employed the immunoadherence column technique of Schlossman (13). Briefly, Ficoll/Hypaque-purified mononuclear cells were incubated on glass Petri dishes for 1 hr. Nonadherent cells were removed and 20×10^6 nonadherent cells per ml were layered onto a 10-ml affinity column consisting of rabbit anti-human $F(ab')_2$ fragments coupled to Sephadex. B lymphocytes adherent to the column were subsequently eluted by competitive interaction with a 1% solution of human Ig. The B lymphocytes collected by the elution procedure were further purified by removal of residual E-rosette-positive cells. The B lymphocytes purified by this procedure were ≥95% surface Ig-positive, with ≤4% contamination by nonspecific esterase-positive cells and ≤0.5% contamination by E-rosette-positive cells. Both the above described positive selection procedures and the previously referenced negative selection procedure (6) incorporated the use of a sheep erythrocyte rosetting technique. This resulted in exposure of the B lymphocytes to sheep erythrocytes, a known antigenic stimulus. The residual sheep erythrocytes present in the purified B lymphocyte preparations resulted in the B cell proliferative assays containing 0.01% sheep cells. After either method of purification, a proportion of the human B lymphocytes were activated as judged by morphologic blastogenesis. These blastogenic lymphocytes did not enter the S phase until cultured with appropriate soluble factors (see *Results*).

Proliferative Assays. Assays for cell proliferation were done in microtiter culture in 96-well plates. Cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum for the time periods listed below with and without multiple dilutions of growth factors. The cells were grown at 37°C in a 5% CO_2 humidified atmosphere. Sixteen hours prior to termination, the cells were labeled with 1 μCi (3.7×10^4 becquerels) of [^{3}H]thymidine (6 Ci/mmol, New England Nuclear). The cells were subsequently harvested onto glass-fiber filter supports by using a MASH II microharvester (Microbiological Associates, Bethesda, MD). The cpm for each factor dilution were determined by using a Beckman LS8100 liquid scintillation counter. For determination of the ability of the factor preparations to support long-term growth of cultured T cells, 2×10^5 human T lymphocytes, grown in continuous culture dependent on IL-2 for a minimum of 1 month, were exhaustively washed and then placed in a final volume of 0.2 ml. These cells were grown for a total of 72 hr and tested for S-phase entry with and without multiple dilutions of the factor preparations. For the assessment of B cell proliferation, 2×10^5 purified human B lymphocytes, containing sheep erythrocytes at a final concentration of 0.01%, were grown in 0.2 ml of culture medium for 96 hr. The cells were tested for S-phase entry with and without multiple dilutions of the factor preparations. For assessment of thymocyte comitogenesis, 1×10^6 mouse thymocytes were cultured with concanavalin A (1.25 mg/ml) with and without added factors. The cells were grown for a total of 72 hr.

***In Vitro* Labeling of Column Fractions.** The column fractions, of known absorbance, were reductively methylated according to the procedures of Jentoft and Dearborn (14). The reaction mixture, containing the eluate fraction in a total volume of 1 ml, was composed of 20 mM $NaCNBH_4$, 10 mM Hepes (pH 7.5), 0.35 M NaCl, [^{3}H]formaldehyde (0.83 μCi/mol), and 0.1 mM $PhMeSO_4F$. After 20-hr incubation at 4°C, an aliquot was precipitated with 10% trichloroacetic acid, and the acid-insoluble incorporated radioactivity was used to compute the amounts of material to be analyzed by gel electrophoresis.

Gel Electrophoresis and Autoradiography. Polyacrylamide (15%) slab gel electrophoresis, under denaturing conditions, was performed according to the procedures of O'Farrell (15). After electrophoresis, the slab gel was removed and processed for autoradiography by the original procedure of Bonner and Laskey (16). Subsequent to preparation for autoradiography, the gel slab was loaded into an x-ray film cassette and allowed to expose Kodak RP Royal X-Omat film for ≥ 66 hr. At this time, the film was developed and scanned.

Measurement of IgM Synthesis. Triplicate 6- to 8-day microcultures of 5×10^4 B cells, 2×10^5 exogenously added T cells, 0.01% sheep erythrocytes, with the factor preparations listed in the text, were pooled and centrifuged, and 200 μl of cell-free supernatant was tested for IgM content by using a microenzyme-linked immunosorbent assay according to the procedures of Engvall and Perlmann (17). Values reported herein represent the quantitation of secreted IgM per 10^6 purified B lymphocytes.

RESULTS

Conditioned medium was clarified by centrifugation and a 50–80% saturated $(NH_4)_2SO_4$ precipitate was prepared for subsequent factor isolation. The precipitate was dissolved, exhaustively dialyzed, and loaded onto a DEAE-Sephadex column, which was eluted in a batchwise fashion. The material eluting from the column in buffer containing 0.12 M NaCl was shown to contain activity that was capable of supporting the proliferation of activated T lymphocytes, purified human B lymphocytes, and lectin-activated thymocytes (see Table 1 for B cell proliferative data).

The DEAE-Sephadex eluate possessing the active factors was next concentrated and applied to a gel filtration column (Bio-Gel P-30). This column was eluted and 5-ml fractions were collected and tested for the capacity to support the long-term growth of cultured T cells, to stimulate proliferation in previously quiescent B lymphocytes, and to stimulate the proliferation of lectin-activated thymocytes. Fig. 1 represents a characteristic elution profile from a Bio-Gel P-30 column. The chromatographic pattern indicated that the majority of protein constituents eluted in the void volume. The void volume was followed by the ap-

pearance of both that activity associated with long-term T cell proliferation and that activity associated with B cell proliferation (fractions 30–50). The relatively broad peak of copurifying activities chromatographed with an apparent molecular weight less than 30,000. In addition, a minor peak of absorbance (fractions 75–85, not shown in Fig. 1) appeared at a greater elution volume (i.e., apparent molecular weight of approximately 10,000). Assay of this late-eluting material, in and of itself, revealed a positive response in the thymocyte comitogenic assay with no demonstrable activity in the assay for the stimulation of B cell S-phase entry. The late-eluting material was unable to support the long-term growth of cultured T cells, yet it could induce the production of IL-2 by lectin-activated T lymphocytes. Therefore, the late-eluting fractions (fractions 75–85) possessed those characteristics typically associated with IL-1 (18).

The broad peak of copurifying T and B cell proliferation-stimulating activities (fractions 30–50), separate from the activity associated with thymocyte comitogenesis alone (fractions 75–85), was next concentrated and chromatographed on a second gel filtration column (Bio-Gel P-100). Fig. 2 represents a typical elution profile seen with five different Bio-Gel P-100 filtration runs of five different preparations of conditioned medium. Here one can see that a large proportion of the loaded material again eluted prior to those fractions containing the proliferation-stimulating activity. A separation between the T cell-active fractions and the B cell-active fractions became evident subsequent to this chromatographic run. Although the peaks overlapped, there were several fractions that consistently demonstrated significant B cell proliferation-stimulating activity while lacking demonstrable T cell activity (fractions 59–62). Table 1 summarizes the isolation of B cell mitogenic factor.

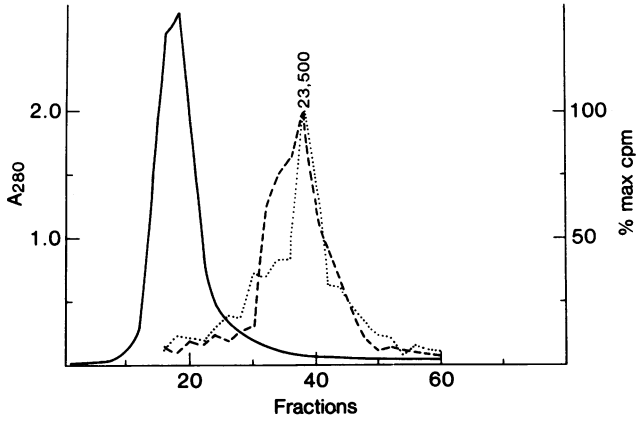

FIG. 1. Bio-Gel P-30 chromatography. Between 60 and 80 A_{280} units were loaded on a column (2.5 × 95 cm). Five-milliliter fractions were collected and absorbance at 230 and 280 nm was measured. Alternate fractions were assayed for the ability to support the long-term growth of cultured human T cells and the ability to stimulate S-phase entry of purified human B cells. Fractions of apparent molecular weight of around 10,000 were also tested for the ability to support thymocyte comitogenesis. ——, A_{280}; ·····, B cell proliferative activity; ----, T cell proliferative activity. B cell and T cell activities are presented as percentage of maximal fraction activity in order to assess factor separation more effectively. The column separation depicted was representative of five different column chromatographic runs on five different preparations of conditioned medium. Representative maximal values of T cell activity measured on long-term T cell blasts in the presence of growth factor were 40,000–45,000 cpm per 2 × 10^5 cells; representative values for T cell activity measured on quiescent T cells stimulated with lectin and growth factor were 18,000–20,000 cpm per 2 × 10^5 cells; representative values for B cell activity measured on purified human B lymphocytes in the presence of growth factor and 0.01% sheep erythrocytes were 10,000–12,000 cpm per 2 × 10^5 cells. Factors were routinely assayed at 1:10 final dilution. Those fractions (75–85) containing thymocyte comitogenic activity alone are not shown in the figure. The apparent molecular weight of the active material is indicated.

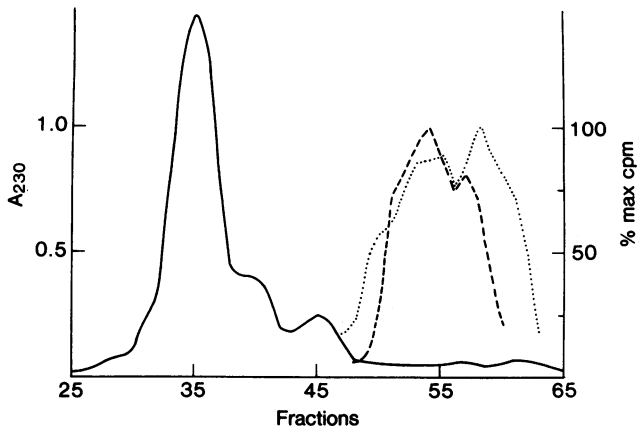

FIG. 2. Bio-Gel P-100 chromatography. Details are the same as described for Fig. 1 except that A_{230} values replace A_{280} values. The maximal activity in the presence of growth factor measured on long-term cultured T cell blasts was 40,000–45,000 cpm per 2 × 10^5 cells. The maximal activity measured on quiescent T cells activated with lectin and growth factor was 18,000–20,000 cpm per 2 × 10^5 cells. The maximal activity as measured on B cells stimulated with growth factor in the presence of 0.01% sheep erythrocytes was 14,000–17,000 cpm per 2 × 10^5 cells.

To determine the nature of the protein constituents associated with the proliferation-stimulating activities, the collected fractions from the Bio-Gel P-100 column were labeled *in vitro* by reductive methylation in the presence of [^{3}H]formaldehyde. These labeled fractions were subsequently electrophoresed under denaturing conditions and the gel was autoradiographed. Fig. 3 represents a scan of the autoradiograph of the labeled fractions. One may note the gradual diminution of a moiety with molecular weight 14,000–15,000 as fraction number increased from 52 through 60. Simultaneously, a molecule of molecular weight 12,000 appeared and increased in magnitude from fractions 54–60. Isolation of fractions 59–62 therefore allowed a significant enrichment of a low molecular weight protein with B cell mitogenic activity with very little T cell mitogenic activity (see Fig. 2).

To further document the observation that the B cell proliferation-stimulating activity (B cell growth factor, BCGF) was due to a moiety distinct from that factor associated with T cell proliferation (T cell growth factor, TCGF) several additional experiments were performed. The first entailed a chromatographic procedure in which those Bio-Gel P-100 fractions possessing B cell proliferation-stimulating activity without significant T stimulating activity were further fractionated on a Bio-Gel P-20 column. This entailed pooling several Bio-Gel P-100 column fractions (59–62), concentrating them, and chromatographing the material. Collection and assay of the eluted fractions revealed that B cell proliferation-stimulating activity could be demonstrated in the absence of T cell proliferation-stimulating activity. The Bio-Gel P-100 fractions were also subject to procedures known to effectively absorb IL-2 (TCGF) from the preparations (Table 2). The Bio-Gel P-100 fractions containing the peak of T cell activity (fractions 50–56) and the fractions containing the peak of B cell activity (fractions 59–62) were separately incubated for 2 hr at 4°C, in the presence of 5.0 × 10^7 activated T cells that had been in continuous culture for at least 30 days. After the absorption, the fractions were assayed for their capacity to stimulate either activated T cell proliferation or B cell proliferation. The results indicated that although absorption of Bio-Gel P-100 fractions 50–56 (i.e., those samples enriched for TCGF) effectively (≥75%) removed that activity

Table 1. Isolation of human B cell mitogenic factor

Purification step*	[^{3}H]Thymidine incorporation,† cpm × 10^{-3}	A_{280}	Total vol,‡ ml	Fold purification§	Yield,¶ %
Conditioned medium	14.2	4.0	1,000	1	100
50–80% $(NH_4)_2SO_4$ precipitate	38.3	5.5	100	2	—
DEAE-Sephadex 0.12 M NaCl eluate	36.0	0.2	300	50	76
Bio-Gel P-30 fractions 32–41	52.0	0.15	50	91	18
Bio-Gel P-100 fractions 58–62	52.1	<0.05	25	730	9
Bio-Gel P-100 peak fraction	84.7	<0.05	5	1,185	3

* The steps listed represent the sequential order of procedures used for fractionating the initial crude conditioned medium. The fractions mentioned in association with the Bio-Gel P-30 and P-100 columns are those tubes in which B cell mitogenic activity was found; the total volume of each fraction was 5 ml. The peak fraction noted in the last step refers to that tube containing the peak of B cell mitogenic activity separable from the peak of T cell mitogenic activity (see Fig. 2).

† The values refer to the incorporated radioactivity per 10^6 purified B cells, with the purification fractions 10% of the final volume of the assay mixture. The cpm reported represent those values of incorporation in cells stimulated with factor minus background incorporation. The data presented are from a single conditioned medium source; a minimum of five replications with different conditioned media gave the same findings. A representative dilutional experiment, examining the efficacy of Bio-Gel P-100 column fractions 58–62, revealed the following data: B cell [^{3}H]thymidine incorporation in the absence of B cell growth factor (BCGF), per 10^6 cells, 4,410 cpm; at 2.5% added BCGF, 24,115 cpm; at 5.0% added BCGF, 63,070 cpm; at 10% added BCGF, 55,125 cpm.

‡ Volume refers to the total volume of the material available after the specified fractionation step with absorbance recorded at 280 nm.

§ Fold purification was calculated by measuring the ability of the fractionation samples to stimulate [^{3}H]thymidine incorporation, normalized per absorbance unit; crude conditioned medium was used as reference standard of unity. For example, cpm per A_{280} at the DEAE-Sephadex step may be calculated to be 180. This value is 50 times greater than cpm per A_{280} in the conditioned medium and represents fold purification (i.e., the fold increment in the capacity to stimulate [^{3}H]thymidine incorporation per arbitrary unit of protein).

¶ Yield was calculated by measuring the ability of the fractionation samples to stimulate thymidine incorporation at a fixed optimal dilution (1:10) per total available volume; crude conditioned medium was considered as 100% of the available activity. The yield of the $(NH_4)_2SO_4$ step has not been included due to potential interference by nondialyzable ammonium sulfate in the samples, resulting in erroneous values. The values of [^{3}H]thymidine incorporation at the fixed dilution of 1:10, which was utilized for the calculation of yield, was chosen due to the consistent observation that at a 10% final volume of factor, the [^{3}H]thymidine incorporation was usually maximal. This method may underestimate the yield calculation, considering that for occasional samples near-maximal incorporation could be achieved at a lower percentage. Therefore, the yield calculation represents the minimum yield possible.

associated with T cell proliferation, the procedure failed to remove any of that activity associated with B cell proliferation. Furthermore, absorption of Bio-Gel P-100 fractions 59–62 (i.e., those samples enriched for BCGF) by T cell blasts also failed to remove any B cell-associated mitogenic activity.

The last series of experiments in this study was directed at the determination of whether the B cell proliferative factor (BCGF) was associated with any capacity to induce a B cell differentiative response (i.e., IgM secretion). For these studies purified B cells were incubated together with exogenously added T cells with and without several growth factor samples from the fractionation procedure described in this report. The BCGF-containing fractions (Bio-Gel P-100 fractions 59–62), the TCGF-containing fractions (Bio-Gel P-100 fractions 50–56), and isolated thymocyte comitogenic activity (Bio-Gel P-30 fractions 75–85) were unable, in and of themselves, to support significant B cell IgM secretion (data not shown). Yet a B cell differentiative response in the presence of exogenously added T cells was promoted by using fractions (i.e., 0.12 M NaCl DEAE-Sephadex eluate) from the early stages of the conditioned medium fractionation scheme. This would appear to indicate either that the promotion of differentiation requires the presence of multiple functional biological activities, as are present in the early stages of the fractionation, or that differentiation is promoted by a unique signal distinct from the purified factors mentioned above.

DISCUSSION

In the present report we have described the analytic separation of a low molecular weight soluble factor that acts as a specific stimulus for S-phase entry in human B lymphocytes. It should be emphasized that questions relating to large-scale purification of such factor(s) have not been addressed in this series of experiments. This is exemplified by the separation characteristics seen with Bio-Gel P-100 chromatography. Although late-eluting fractions may be isolated that possess essentially only BCGF activity, early-eluting fractions may be seen to possess TCGF and BCGF activity. The chromatographic overlap may be due to several different causes, the simplest being that a small amount of BCGF, not detected autoradiographically (see Fig. 3), may be eluting in the early fractions. Yet the separation described in this report does reinforce the concept that B cell growth control may be modulated by soluble factors. Furthermore, one entity capable of stimulating B cell growth appears to be distinct from several other well-described cytokines. The specificity of the isolated factor lies not only in the target cell for its action, but also resides in its specificity of function. As of now, we have observed no tendency for B cells stimulated solely by the purified growth factor preparations to become immunoglobulin-secreting cells. This is not to imply that a growth stimulus plays no role in differentiation. Teleologically, one would presume that *in vivo* differentiation would occur concurrently with the clonal expansion of a specific B cell population. Furthermore, *in vitro* assays measuring differentiation would be enhanced if the differentiating cell were also proliferating. Yet the ability to isolate a factor capable of stimulating proliferation alone argues that on a molecular level these two functions may be readily separated.

As mentioned above, the isolated factor stimulating B lymphocyte S-phase entry possesses target cell specificity. Evidence presented here and elsewhere (9, 10) indicates that, although there are many parallels in the proliferative requirements for both B and T cells, growth stimulation is mediated by separate, unique molecular entities. However, an unanswered question concerns the actual percentage of B cells that are re-

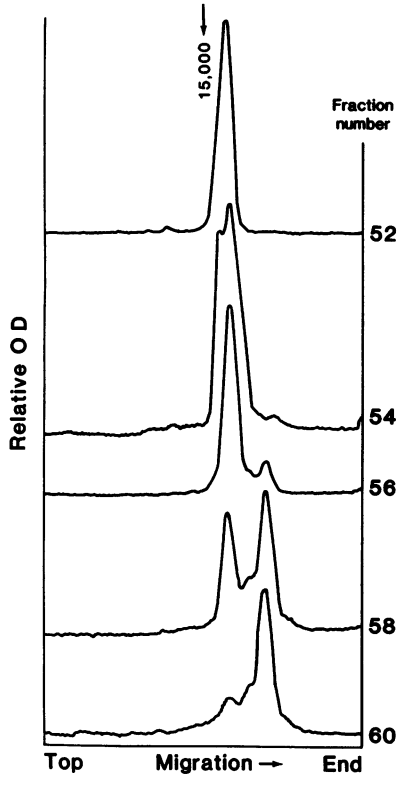

FIG. 3. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of selected Bio-Gel P-100 column fractions. Protein samples from the fractions denoted were radiolabeled and electrophoresed on 15% polyacrylamide gels. Gels were subsequently autoradiographed and scanned.

sponsive to the growth stimulus. At present, growth factor stimulation of B cells either in the presence of sheep erythrocytes or after stimulation by anti-immunoglobulin results in S-phase entry in only a proportion of the cultured population. Whether this limitation in the observed stimulation is due to the specificity of initial obligate activation, the specificity of the growth stimulus employed, the lack of other accessory signals, or simply limitations of *in vitro* culture conditions has not yet been determined. The observation that as-yet-undefined monocyte-derived products may support B cell proliferation (7) suggests that other accessory signals may be required for an optimal proliferative response. Therefore, the exact specifications for this proliferative response require significant clarification.

The cell source for the proliferative factor analytically isolated in the present series of experiments also requires clarification. This factor, by itself, has been shown to be distinct from those factors capable of supporting long-term T lymphocyte proliferation, thymocyte comitogenesis, and B lymphocyte differentiation. Yet it is intriguing to speculate that the cell responsible for secreting that factor ultimately controlling T cell proliferation may also secrete that factor controlling B cell proliferation. Evidence supportive for this speculation may be gleaned from the data compiled on the EL-4 thymoma line (9). In this system one may derive both IL-2 (TCGF) and BCGF from the same stimulated culture population. Definitive evidence for the source of BCGF awaits the demonstration of factor production by normal clonal lymphoid populations, although initial observations suggest that one such factor may be derived from cells of the T cell lineage.

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Table 2. Effect of T cell-mediated absorption on BCGF and TCGF activities

Growth factor*	Assayed for activity with†	Growth factor conc., %	[^{3}H]Thymidine incorporation,‡ cpm × 10^{-3}	
			Unabsorbed	Absorbed with T blasts
Bio-Gel P-100 fractions 50–56	Activated T cells	2.5	51.2	4.9
		5.0	53.7	5.1
		10.0	—	—
Bio-Gel P-100 fractions 59–62	Activated B cells	2.5	9.9	10.9
		5.0	19.2	24.6
		10.0	42.5	59.1

* The growth factors were derived from the Bio-Gel P-100 column fractionation. Fractions 50–56 possess predominantly TCGF activity with some BCGF activity overlap. Fractions 59–62 are the peak of BCGF activity essentially free of any other activity.

† The growth factor preparations were absorbed with T cell blasts that had been in culture for at least 30 days. After absorption, the medium was clarified and tested for its ability to stimulate growth. The activated T cells utilized for the growth assays were derived from cells that had been in continuous IL-2-dependent culture for periods of at least 30 days. The activated B cells were those purified human B cells that had been exposed to sheep erythrocytes.

‡ The results are those from a single experiment; replicates gave the same findings. The values reported are cpm of [^{3}H]thymidine incorporated, per 10^6 cells, by a culture stimulated with factor minus background incorporation in cultures not exposed to growth factor. A — indicates not tested.

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