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Immunology

T-cell-replacing factor (interleukin 5) induces expression of interleukin 2 receptors on murine splenic B cells

(B-cell activation/lymphokines/receptor modulation)

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ABSTRACT **Small, resting B lymphocytes express few, if any, interleukin 2 (IL-2) receptors, but activated B cells may express such receptors. This paper examines the requirements for receptor expression. Normal murine splenocyte populations were enriched for B cells and cultured at relatively low density. IL-2 receptor expression was studied by measuring the binding of the anti-IL-2 receptor monoclonal antibody PC61. Lymphoblasts arising through stimulation by *Escherichia coli* lipopolysaccharide failed to express IL-2 receptors. B cells cultured with conditioned medium from concanavalin A-stimulated EL4 thymoma cells, with or without LPS, displayed IL-2 receptors. This bioactivity of EL4 conditioned medium could not be replaced by any concentration of B-cell-stimulatory factor 1 (IL-4), IL-1, IL-2, or IL-3 tested. However, the recently cloned lymphokine T-cell-replacing factor (IL-5) was a potent inducer of IL-2 receptor expression, as was the probably identical material known as eosinophil differentiation factor. The receptors so induced appeared to be functional, as receptor-expressing (but not control) lymphoblasts, responded to IL-2 by proliferation, indicative of high-affinity-receptor expression.**

Interleukin 2 (IL-2) is a T-cell-derived cytokine of 133 amino acids with growth and differentiation activity on both T (1) and B (2) lymphocytes, mediated via a specific cell surface receptor. The expression of cell surface receptors for IL-2 by activated T cells has long been established (3), and under appropriate conditions B cells can also express receptors for and respond to IL-2 (4). For example, certain B-cell leukemias, notably hairy-cell leukemias, express plentiful IL-2 receptors (5), and the existence of small numbers of IL-2 receptors on normal B cells has been reported (6). However, to achieve high numbers of receptors on normal B cells, some form of activation of the cell is required. Zubler, Lowenthal, and their colleagues (7, 8) demonstrated that splenic B cells, when cultured in the presence of lipopolysaccharide (LPS), anti-immunoglobulin antibody-coated beads, and 10% EL4 thymoma cell conditioned medium (EL4-BGDF) as a source of T-cell-derived factors, expressed IL-2 receptors in similar numbers and of similar affinity to those present on concanavalin A (Con A)-activated T cells. This was a clear indication that one or more lymphokines present in EL4-BGDF may be important in the induction of the expression of IL-2 receptors on normal B cells. In addition, Nakanishi *et al.* (9) reported that B cells stimulated with anti-IgM and B-cell-stimulatory factor (BSF-1) expressed IL-2 receptors and that the addition of the B-cell-active lymphokine B151-TRF ("T-cell-replacing" factor from the B151 T-cell hybrid line) led to an enhancement of this expression (9). More recently, molecular cloning of both human and murine TRF (10) has been achieved (11, 12). The cloned material has been shown to retain this differentiative capacity and to synergize with IL-2 in the induction of immunoglobulin production by human B cells (12).

The nomenclature of B-cell-active factors is in a state of flux; TRF has provisionally been named IL-5, and its bioactivity appears to be identical to that of the eosinophil differentiation factor (EDF) studied by Sanderson *et al.* (13). The designation of BSF-1 as IL-4 has also gained wide support; until an international nomenclature has been agreed upon we intend to follow this classification—i.e., BSF-1 is IL-4 and TRF is IL-5.

The concept of the binding of one factor to its specific receptor leading to the up-regulation of expression of the receptor for a second, distinct factor has already been established. In the T-cell system, binding of IL-1 to its receptor has been shown to be important in the generation and maintenance of cell surface receptors for IL-2 (3). Similarly, binding of IL-3 to its receptor on hematopoietic-lineage cells leads to the up-regulation of expression of receptors for IL-2 (14), as does the binding of interferon γ to monocytes (15).

Given such cross up-regulation of receptor expression, we investigated the role of EL4-BGDF and of defined lymphokines in the up-regulation of IL-2 receptor expression by normal murine splenic B cells. We report here that EL4-BGDF activity alone can lead to the up-regulation of functionally active IL-2 receptors. Of the other lymphokines tested, only the recently cloned IL-5 was similarly active.

MATERIALS AND METHODS

Lymphokines. EL4-BGDF was a 10× concentrate of medium conditioned by Con A-stimulated EL4 thymoma cells as described (16). Recombinant murine IL-1 was prepared as described (17) and kindly provided by Hoffmann–La Roche. Recombinant human IL-2 was prepared as described (18) and kindly provided by Cetus Immune (Palo Alto, CA). BSF-1 (IL-4) was affinity-purified as described (19) and was a kind gift of W. E. Paul. Native granulocyte/macrophage colony-stimulating factor (GM-CSF) was purified as described (20) and was a kind gift from N. A. Nicola (Walter and Eliza Hall Institute). Native multi-CSF, a molecule whose structure and activity are indistinguishable from those of IL-3, was prepared as described (21) and was a kind gift from N. A. Nicola. Semipurified EDF was prepared as described (13) and was a kind gift from C. Sanderson. Interferon γ was obtained from Genentech (South San Francisco, CA).

Purified B151-TRF (IL-5) was prepared from a batch of B151-T4 conditioned medium as previously described (10).

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Abbreviations: BSF-1, B-cell-stimulatory factor 1 (interleukin 4); CSF, colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; EDF, eosinophil differentiation factor; EL4-BGDF, EL4 thymoma cell conditioned medium; FACS, fluorescence-activated cell sorter; FITC-GAR, fluorescein isothiocyanate-conjugated goat anti-rat immunoglobulin; IL, interleukin; rIL-5, recombinant interleukin 5; LPS, *Escherichia coli* lipopolysaccharide; TRF, T-cell-replacing factor.

The medium was fractionated by ammonium sulfate precipitation. The precipitate obtained at 50–80% saturation was suspended in and dialyzed against 10 mM Tris/HCl buffer (pH 8.5) and then was applied to a DE52 (Whatman) column using a linear gradient of NaCl (0–0.25 M) in 10 mM Tris/HCl buffer (pH 8.5) for elution. Eluate fractions possessing B151-TRF activity were dialyzed against 25 mM Bistris/HCl buffer (pH 6.3) and applied to a Mono P column (Pharmacia). The samples were eluted with 10% Polybuffer (Pharmacia) at a pH range between 6.3 and 4.0. B151-TRF-active material from the Mono P column was applied to a Superose 12 column (Pharmacia) for gel-permeation chromatography using 0.15 M NaCl/10 mM Hepes (pH 7.0). Finally, B151-TRF-active fractions after gel permeation were applied to a Vydac Protein C_4 column (The Separations Group, Hesperia, CA) for reversed-phase HPLC and were eluted with a linear gradient of acetonitrile (0–80%) in 0.1% trifluoroacetic acid. Batches of purified material were made up in culture medium to yield approximately equivalent bioactivities and were used in culture at appropriate, arbitrary dilutions.

Recombinant murine TRF (rIL-5) was prepared as described (11). The cDNA for murine TRF (pSP6K-mTRF23) was cleaved with *Sal* I to linearize plasmid DNA, and mRNAs were synthesized using bacteriophage SP6 RNA polymerase. The synthesized RNAs were injected into *Xenopus* oocytes. Incubation media were collected after 36 hr.

IL-2 Indicator Cell Line. CTLL cells (22) are a continuously growing IL-2-dependent line and were harvested 4 days after the last feeding.

Culture Media and General Culture Methods. Cells were cultured in RPMI 1640 medium (Flow Laboratories, Sydney, Australia) supplemented with 5% fetal bovine serum (Flow Laboratories) and 0.1 mM 2-mercaptoethanol. Where indicated, *Escherichia coli* lipopolysaccharide (LPS; Sigma; final concentration 75 μg/ml) and/or various factors or 20% EL4-BGDF were added to the culture medium. Cultures (200 μl) were set up in 96-well flat-bottomed plates (Disposable Products, Sydney, Australia) with, unless otherwise stated, 2.5×10^5 cells per well. Cells were incubated between 1 and 5 days, as indicated, at 37°C in the presence of 10% CO_2.

Preparation of Murine B Cells. Mice from the inbred strain CBA/CaH/WEHI were used as spleen donors at 8–10 weeks of age. Mice were held as specific-pathogen-free until shortly before they were killed. A cell population enriched for B cells was obtained from splenocytes by use of a complement-mediated cell-lysis step (23). In brief, splenocytes were suspended in medium (RPMI 1640 plus 1% fetal bovine serum) to which the monoclonal antibodies anti-Thy-1.2 and anti-MAC1 had been added. After being held under these conditions on ice for 40 min, DNase (Boehringer Mannheim) and newborn rabbit complement (that had been adsorbed with mouse spleen, blood, and thymus) were added. The cells were incubated at 37°C for 20 min, collected, and subjected to density separation in a metrizamide density gradient (Nyegaard, Oslo). The population representing live cells was collected, washed, and used for culture purposes. The cells were analyzed for surface IgM by staining with fluorescein-labeled goat anti-mouse IgM (μ heavy chain-specific) (Southern Biotechnology Associates, Birmingham, AL) and by fluorescence-activated cell sorter (FACS II, Becton Dickinson) analysis. Following the above fractionation, 87.6% of the cells were surface IgM-positive and <1% were Thy-1.2-positive, whereas fractionated cells after culture with LPS and 20% EL4-BGDF were 99.6% surface IgM-positive and <1% Thy-1.2-positive. In addition, fractionated cells were found to be unresponsive to Con A stimulation.

FACS Analysis of IL-2 Receptor Expression. Cells were collected and IL-2 receptor expression was assessed by an indirect immunofluorescence technique. The first reagent was the rat monoclonal antibody PC61, which recognizes determinants on the murine IL-2 receptor (24); the second reagent was a goat polyvalent anti-rat immunoglobulin (mouse-adsorbed) labeled with fluorescein isothiocyanate (Kirkegaard and Perry, Gaithersburg, MD) (FITC-GAR). Prior to FACS analysis, cells were suspended in medium supplemented with 0.03% propidium iodide, and analysis involved appropriate channel settings to exclude dead cells from the sample population.

The number of cells that bound significant amounts of rat monoclonal antibody PC61 was calculated. This involved using information gained from fluorescence histograms obtained via FACS analysis as a measure of cells with a high degree of specific staining for PC61 and subtracting the percentage of cells with similar levels of staining when the second antibody (FITC-GAR) alone was used. As consistently less than 3% of the cells scored greater than 80 channel units on the green fluorescence axis with the second antibody alone, this was chosen as the level above which cells would be considered to be significantly stained. Consequently, percentages referring to the number of positive cells in culture were calculated by subtracting the percentage of cells scored greater than 80 channel units with second-antibody staining alone from the percentage greater than 80 channel units when both PC61 and the second antibody were added. This method of scoring always gave a percentage of positive cells less than that obtained by subtraction of the histogram for FITC-GAR staining alone from the histogram for staining with PC61 and FITC-GAR. We chose to use the former method because it avoided the problem of scoring lightly stained cells as positive.

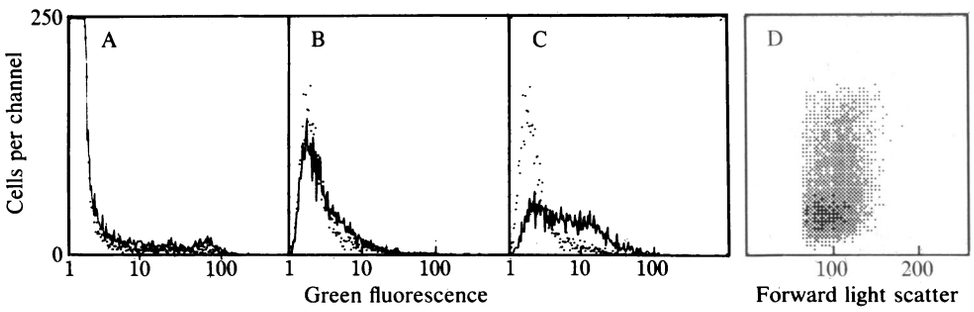

FIG. 1. Immunofluorescence staining of B cells. Purified B cells were stained either before or after culture as indicated and analyzed on a FACS II. (*A*) Fluorescence histograms of B cells, prior to culture, stained with FITC-GAR alone (broken line) or for IL-2 receptor expression by sequential reaction with monoclonal antibody PC61 and FITC-GAR. (*B*) Fluorescence histograms of B cells cultured with LPS for 3 days. Cells were then harvested and stained for IL-2 receptor expression or with FITC-GAR alone. (*C*) Fluorescence histograms of B cells cultured with LPS and 20% EL4-BGDF for 3 days. Cells were then harvested and stained for IL-2 receptor expression or with FITC-GAR alone. (*D*) Contour plot of B cells cultured for 3 days with 20% EL4-BGDF and then stained for IL-2 receptor expression. Ordinate indicates staining with PC61 and FITC-GAR.

Table 1. IL-2 receptor expression by cultured B cells

Culture conditions*	% positive cells†	Mean channel number†	Positive cells per well,‡ no. × 10^{-4}
Pre-culture	1.1 ± 0.3	19.0 ± 3.3	0.3
Medium	0.0 ± 0.5	21.7 ± 2.1	0.0
LPS	1.5 ± 0.5	39.8 ± 3.1	0.7
EL4-BGDF	35.7 ± 6.0	118.3 ± 25.7	7.1
LPS + EL4-BGDF	29.3 ± 2.8	92.2 ± 5.7	14.6

*Cells were cultured for 3 days, harvested, and stained for FACS analysis of IL-2 receptor expression.
†Mean ± SEM of three experiments.
‡Prior to staining for IL-2 receptors, an aliquot of cells was removed and stained with eosin, the cells were counted, and the number of live cells per well was calculated. From the FACS data the number of IL-2 receptor-expressing cells per well was then calculated: total number of positive cells per well = (number of live cells per well) × (% positive cells/100%).

RESULTS

EL4-BGDF Leads to IL-2 Receptor Expression. B cells were cultured without stimulation or with LPS, 20% EL4-BGDF, or LPS plus 20% EL4-BGDF, and the expression of IL-2 receptors on cells from replicate wells was assayed on day 3 (Fig. 1; Table 1). Culture without stimulation or with LPS alone had little demonstrable effect on receptor expression; however, stimulation with 20% EL4-BGDF, either with or without the addition of LPS, led to a marked increase in receptor expression. To examine whether EL4-BGDF alone leads to an increase in the total number of IL-2 receptor-expressing cells over cultures in medium alone or with LPS, the total number of live cells per culture was counted and the number of IL-2 receptor-positive cells per well was calculated (Table 1). The cultures supplemented with the mitogen LPS contained far more cells than those with 20% EL4-BGDF or medium alone. However, the number of IL-2 receptor-expressing cells by day 3 in the culture with 20% EL4-BGDF alone was 10 times that in the culture with LPS alone, whereas the culture with LPS plus 20% EDL4-BGDF had almost 21 times as many IL-2 receptor-expressing cells as the culture with LPS alone.

Activity of EL4-BGDF Is Dependent Both on Its Concentration and on Cell Number. To examine whether the induction of IL-2 receptors on cultured B cells was dependent on the concentration of EL4-BGDF added to the culture, cultures were supplemented with various amounts of EL4-BGDF, from 0% to 30% (Table 2). The degree of IL-2 receptor expression was directly related to the concentration of EL4-BGDF in culture. The effect of cell number per culture on IL-2 receptor expression was also investigated. Cultures of from 10^6 to 10^4 cells per well were incubated with a constant concentration of EL4-BGDF (Table 3). An inverse relationship between cell number and IL-2 receptor expression was noted, raising the possibility that in dense cultures, the relevant bioactivity was being adsorbed and/or degraded. Cell proliferation was noted to be poor in the least dense cultures, and thus a standard number of 2.5×10^5 cells per 200-μl well (1.25×10^6 cells per ml) was chosen for most of this work.

Table 2. IL-2 receptor expression increases with EL4-BGDF concentration

% EL4-BGDF	% positive cells
0	1
5	3
10	5
15	20
20	22
30	30

Cells were cultured with LPS and various concentrations (% by volume) of EL4-BGDF for 3 days, harvested, and stained for FACS analysis of IL-2 receptor expression.

Table 3. IL-2 receptor expression is decreased in dense cultures

Cells per well, no. × 10^{-4}	% positive cells
100	17
50	22
10	28
1	35

Cells in various numbers per 200-μl well were cultured for 3 days in medium with LPS plus 20% EL4-BGDF, harvested, and stained for FACS analysis of IL-2 receptor expression.

Activity of EL4-BGDF Is Mediated by a Molecule of ≈40 kDa. To investigate further the nature of this activity in EL4-BGDF, 500 ml of unconcentrated EL4-BGDF was concentrated 100-fold and run on a Sephadex G-100 (Pharmacia) gel-filtration column. Eighty fractions were obtained from the column, with fractions 32–77 containing protein. These fractions were tested for IL-2 receptor-inducing activity (Fig. 2). A broad single peak of activity was noted in fractions 52–72 (corresponding to 30–55 kDa), with a peak of activity in fraction 65 (≈40 kDa).

rIL-5 Leads to IL-2 Receptor Expression. Several factors known to be present in EL4-BGDF were tested at various concentrations, either alone or in combination, to see whether this activity was mediated by any known factor (Table 4). Of the various factors tried, only purified IL-5, rIL-5, and semipurified EDF had any activity. rIL-5 was further tested for activity with titration from 0.1% to 1.5% concentration in the culture medium, and the number of positive cells per well was calculated (Table 5) as described for Table 1.

To compare the expression of IL-2 receptors on IL-5-stimulated B cells and on cells from the IL-2-dependent T-cell line CTLL, we carried out indirect immunofluorescence studies using PC61 and FITC-GAR (Fig. 3). From the mean fluorescence intensities, it was estimated that CTLL cells

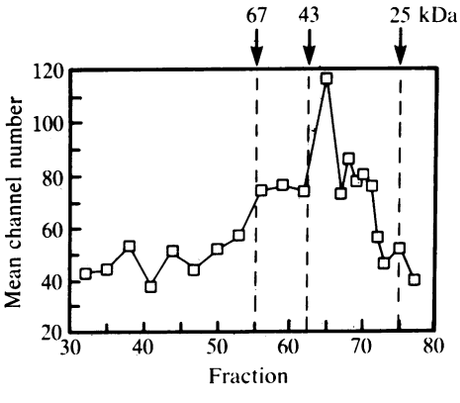

FIG. 2. Gel filtration of EL4-BGDF. A Sephadex G-100 column was calibrated by chromatography of standard proteins (molecular masses in kDa as indicated). Then 5 ml of 100× EL4-BGDF was run through the column with mouse-tonicity phosphate-buffered saline (0.15 M NaCl/0.02 M sodium phosphate). B cells were cultured for 3 days in medium supplemented with 20-μl aliquots from various fractions. For fractions 32–64, cells were cultured in medium supplemented with 20 μl from three successive fractions; for fractions 65–71, cells were cultured in medium supplemented with 20 μl from individual fractions; for fractions 72–77, cells were cultured in medium supplemented with 20 μl from two successive fractions. Cells were then harvested and stained for FACS analysis of IL-2 receptor expression.

Table 4. Induction of IL-2 receptors by defined lymphokines

Culture conditions	% positive cells	Culture conditions	% positive cells
IL-1, u/ml		IFN-γ, u/ml	
100	2	10	5
200	0	20	5
IL-2, u/ml		IL-1 + IL-2*	5
100	3	IL-4 + IL-1*	8
200	2	IL-4 + IL-2*	7
300	2	EDF, % (vol/vol)	
Multi-CSF, dilution		1	7
1:33	2	5	12
1:16	1	10	13
IL-4, u/ml		IL-5,† % (vol/vol)	
10	2	2.5	8
20	2	5	17
GM-CSF, ng/ml		7.5	18
1	4	rIL-5, % (vol/vol)	
10	6	0.1	1
50	3	0.2	6
Con A (5 μg/ml)	1	0.4	14

Cells were cultured for 3 days in medium containing various factors as indicated, harvested, and stained for FACS analysis of IL-2 receptor expression. IFN-γ, interferon γ; u, units.
*IL-1, 100 u/ml; IL-2, 100 u/ml; IL-4, 10 u/ml.
†Purified from B151-T4 conditioned medium.

bound 7-fold more PC61 per cell than did B cells cultured for 3 days in medium supplemented with 1.0% rIL-5.

EL4-BGDF-Induced IL-2 Receptors Are Functional. To address the question of the functional capabilities of EL4-BGDF-induced IL-2 receptors, we tested cells that had been cultured for 3 days under various conditions for proliferation in response to IL-2 stimulation with a [^{3}H]thymidine incorporation assay (Fig. 4). Cells from cultures supplemented with EL4-BGDF or with EL4-BGDF plus LPS were found to respond to increasing concentrations of IL-2 with increasing incorporation of [^{3}H]thymidine. Cells from cultures with LPS or medium alone showed no such response, demonstrating the functional capacity, with respect to proliferation, of EL4-BGDF-induced IL-2 receptors.

DISCUSSION

This report demonstrates that EL4-BGDF acting alone can induce functional IL-2 receptors on murine B cells and that this action can be replaced by IL-5. Because gel filtration of EL4-BGDF yielded only a single peak of IL-2 receptor-inducing activity around the 40-kDa region, and because IL-5 is known to have a molecular mass of ≈45 kDa (11), it appears likely that the activity of EL4-BGDF is mediated by IL-5. Because up to 50% of cultured cells were IL-2 receptor-positive, and because the starting population was an enriched B-cell population from specific-pathogen-free mice, it seems likely that the targets for factor action were small, resting B cells. It is unlikely that this action was mediated via an action on contaminating non-B cells. The B-cell preparation was unresponsive to Con A stimulation, and <1% of the cells were Thy-1.2-positive, so that a T-cell-based cascade seems an unlikely explanation. In addition, the few surface immu-

Table 5. Induction of IL-2 receptors by rIL-5

% rIL-5	% positive cells	Positive cells per well,* no. × 10^{-4}
0.1	2	0.5
0.2	10	2.3
0.4	18	4.1
0.8	35	8.0
1.0	28	7.3
1.5	49	13.2

Cells were cultured for 3 days in medium supplemented with various concentrations of rIL-5, harvested, and stained for FACS analysis of IL-2 receptor expression.
*Calculated as for Table 1.

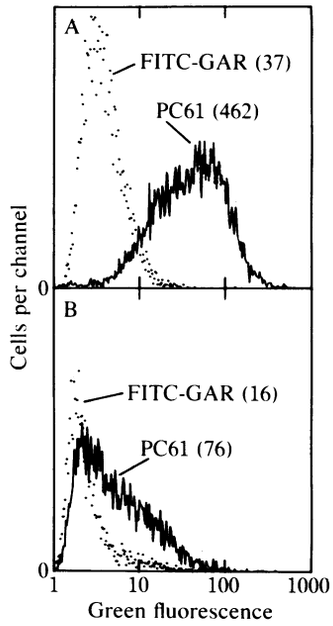

FIG. 3. Comparison of immunofluorescence staining of the continuous IL-2-dependent T-cell line CTLL harvested 4 days after the last feeding (*A*) and IL-5-stimulated B cells (*B*). B cells were cultured for 3 days in medium supplemented with 1.0% rIL-5. Mean fluorescence intensities were calculated and are indicated next to each histogram. Relative staining was calculated according to the following formula: relative staining = (mean fluorescence of CTLL cells stained with PC61 and FITC-GAR − mean fluorescence of CTLL cells stained with FITC-GAR)/(mean fluorescence of stimulated B cells stained with PC61 and FITC − mean fluorescence of stimulated B cells stained with FITC-GAR). This is (462 − 37)/(76 − 16) = 7.1.

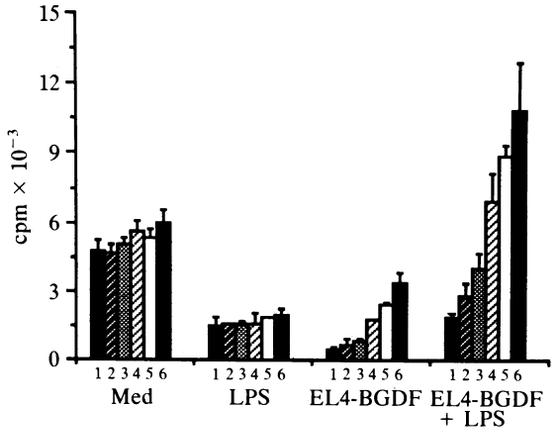

FIG. 4. Cells were cultured for 3 days in medium alone (Med) or with LPS, 20% EL4-BGDF, or 20% EL4-BGDF plus LPS. They were then harvested, washed twice in fetal bovine serum, counted, and cultured (2 × 10^4 live cells per 200 μl in 96-well culture plates) either in fresh medium alone (bars 1) or in medium containing 5 (bars 2), 10 (bars 3), 50 (bars 4), 100 (bars 5), or 1000 (bars 6) units of IL-2 per ml. After 24 hr, 1 μCi (37 kBq) of [^{3}H]thymidine was added to each well. Sixteen hours later the cells were harvested and [^{3}H]thymidine incorporation was measured by scintillation counting. Each bar represents the mean + SEM of five replicate wells.

noglobulin-negative cells that were present in the preparation died rapidly in culture, and a relatively low cell density was used. However, the possibility of a lymphokine cascade cannot be formally excluded.

Cells activated to express IL-2 receptors clearly responded to IL-2 stimulation with an increase in DNA synthesis (Fig. 4). We therefore believe that induced receptors are functional and probably of physiological importance in an ongoing immune response.

This report emphasizes the complex interactions between cell surface receptors for different factors. As demonstrated by Walker *et al.* (25), the binding of a ligand to its receptor can lead to the altered expression of receptors for differing ligands, with either up- or down-regulation of expression possible. This dynamic nature of receptor expression most likely reflects the regulatory role that they play in cell activation, proliferation, and differentiation, with receptors for factors acting late in this pathway being expressed on already activated cells, and receptors for those that act early in the pathway being expressed by resting cells. It was therefore with initial surprise that we discovered that IL-5, previously reported as a late-acting factor, mediated an activity on resting B cells. Clearly then, IL-5 is not exclusively a late-acting factor. In fact, separate studies to be reported elsewhere (M. R. Aldersen, B. L. Pike, N.H., A. Tominaga, K.T., and G.J.V.N.) show that IL-5 is a potent B-cell growth and differentiation factor when acting with antigen on single B cells. We had expected that a known early-acting factor, BSF-1 (IL-4) would mediate receptor induction, yet BSF-1 was completely inactive in this assay. As IL-2 receptors are not expressed at an appreciable level on resting B cells, but only after initial activation (e.g., with IL-5), IL-2 would be expected to be inactive on resting B cells but to exert its proliferative activity on already activated cells. This possibility of an activation cascade of receptors and therefore of factor responsiveness could explain why several investigators have been unable to ascribe a B-cell growth factor activity to IL-2 (26). In this respect it is interesting that LPS acting alone, although able to induce growth and differentiation (27, 28), does not induce IL-2 receptor expression. LPS-induced activation and proliferation therefore in some way bypass the normal requirements for B-cell factors.

This work raises the wider question of the control of expression of the receptors for the various factors now known to guide B-cell growth and differentiation, which include IL-1 (29), IL-2 (2), IL-4 (30), IL-5 (10), and B151-TRFII (31). IL-4 receptors have been shown to be constitutively expressed (32), and our results suggest that EL4-BGDF- or LPS-activated cells develop IL-5 receptors. As our single-cell studies have shown IL-1 and IL-2 to be inactive on B cells except in the concomitant presence of an effective "T-independent" antigen, it is probable that receptors for IL-1 and IL-2 always have to be induced, whether by factors or by some property of the carriers of effective "T-independent" antigens. In that sense, the failure of LPS to induce IL-2 receptors is of special interest. The Zubler group (7) has postulated a three-signal model of B-cell activation for IL-2 receptor expression, crosslinking of the surface immunoglobulin receptors, mitogenic stimulation, and the synergistic action of lymphokines. The demonstration that LPS plus IL-5 can induce the expression of a receptor that does not appear on lymphoblasts stimulated by LPS alone suggests that the signaling process may be more complex still. Progressive unraveling of the regulation of receptor expression should contribute to the resolution of this complexity.

Note Added in Proof. Since this manuscript was submitted, Nakanishi *et al.* (33) have reported the up-regulation of IL-2 receptor expression (in a cloned subline of the murine B-cell leukemia line BCL_1) by IL-5, complementing the present studies on normal B cells.

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1. Smith, K. A. (1980) *Immunol. Rev.* **152,** 337–357.
2. Pike, B. L., Raubitschek, A. & Nossal, G. J. V. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7917–7921.
3. Smith, K. A. (1984) *Annu. Rev. Immunol.* **2,** 319–333.
4. Tsudo, M., Uchiyama, T. & Uchino, H. (1984) *J. Exp. Med.* **160,** 612–617.
5. Korsmeyer, S. J., Greene, W. C., Cossman, J., Su-Ming Hsu, Jensen, J. P., Neckers, L. M., Marshall, S. L., Bakhshi, A., Depper, J. M., Leonard, W. J., Jaffe, E. S. & Waldmann, T. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4522–4526.
6. Armitage, R. J. & Cawley, J. C. (1986) *Clin. Exp. Immunol.* **63,** 298–302.
7. Zubler, R. M., Lowenthal, J. W., Erard, F., Hashimoto, N., Devos, R. & MacDonald, H. R. (1984) *J. Exp. Med.* **160,** 1170–1183.
8. Lowenthal, J. W., Zubler, R. H., Nabholz, M. & MacDonald, H. R. (1985) *Nature (London)* **315,** 669–672.
9. Nakanishi, K., Malek, T. R., Smith, K. A., Hamaoka, T., Shevach, E. M. & Paul, W. E. (1984) *J. Exp. Med.* **160,** 1605–1621.
10. Takatsu, K., Harada, N., Hara, Y., Takahama, Y., Yamada, G., Dobashi, K. & Homaoka, T. (1985) *J. Immunol.* **134,** 382–389.
11. Kinashi, T., Harada, N., Severinson, E., Tanabe, T., Sideras, P., Azuma, C., Tominaga, A., Bergstedt-Lindqvist, S., Takashi, M., Konishi, M., Matsuda, F., Yaoita, Y., Takatsu, K. & Honjo, T. (1986) *Nature (London)* **324,** 70–73.
12. Azuma, C., Toshizumi, T., Konishi, M., Kinashi, T., Noma, T., Matsuda, F., Yaoita, Y., Takatsu, K., Hammarström, L., Edvard Smith, C. I., Severinson, E. & Honjo, T. (1986) *Nucleic Acids Res.* **14,** 9149–9158.
13. Sanderson, C. J., O'Garra, A., Warren, D. J. & Klaus, G. G. B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 437–440.
14. Brichenall-Sparks, M. C., Farrar, W. L., Rennich, D., Kilian, P. L. & Ruscetti, F. W. (1986) *Science* **233,** 455–458.
15. Rambaldi, A., Young, D. C., Herrmann, F., Cannistra, S. A. & Griffin, J. D. (1987) *Eur. J. Immunol.* **17,** 153–156.
16. Pike, B. L., Vaux, D. L., Clark-Lewis, I., Schrader, J. W. & Nossal, G. J. V. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6350–6354.
17. Lomedico, P. T., Gubler, U., Hellman, C. P., Dukovich, M., Giri, J. G., Yu-Ching, P., Collier, K., Semionow, R., Oshua, A. & Mizel, S. B. (1984) *Nature (London)* **312,** 458–462.
18. Rosenberg, S. A., Grimm, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K. & Mark, D. F. (1984) *Science* **223,** 1412–1415.
19. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315,** 333–336.
20. Burgess, A. W., Camakaris, J. & Metcalf, D. (1977) *J. Biol. Chem.* **252,** 1998–2003.
21. Clark-Lewis, I., Kent, S. B. H. & Schrader, J. W. (1984) *J. Biol. Chem.* **259,** 7488–7494.
22. Gillis, S., Fern, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
23. Shortman, K., Linthicum, D. S., Battye, F. L., Goldschneider, I., Liabenf, A., Goldstein, P., Clark, E. A. & Lake, P. (1979) *Cell Biophys.* **1,** 255–270.
24. Ceredig, R., Lowenthal, J. W., Nabholz, M. & MacDonald, H. R. (1985) *Nature (London)* **314,** 98–100.
25. Walker, F., Nicola, N. A., Metcalf, D. & Burgess, A. W. (1985) *Cell* **43,** 269–276.
26. Leanderson, T. & Julius, M. H. (1986) *Eur. J. Immunol.* **16,** 182–187.
27. Wetzel, G. D. & Kettman, J. R. (1981) *J. Immunol.* **126,** 723–728.
28. Pike, B. L. & Nossal, G. J. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3395–3399.
29. Pike, B. L. & Nossal, G. J. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8153–8157.
30. Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T. & Paul, W. E. (1982) *J. Exp. Med.* **155,** 914–922.
31. Ono, S., Hayashi, S., Takahama, Y., Dobashi, K., Kotah, Y., Nakaniski, K., Paul, W. E. & Hamaoka, T. (1986) *J. Immunol.* **137,** 187–196.
32. Ohara, J. & Paul, W. E. (1987) *Nature (London)* **325,** 537–540.
33. Nakanishi, K., Hashimoto, T., Hiroishi, K., Matsui, K., Yoshimoto, T., Morse, H. C., III, Furuyama, J., Hamaoka, T., Higashino, K. & Paul, W. E. (1987) *J. Immunol.* **138,** 1817–1825.