Epstein-Barr Virus Transformed B Lymphocytes Produce Interleukin-5

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Interleukin-5 (IL-5) has previously been isolated only as a product of T lymphocytes. We have found that Epstein-Barr virus transformed B lymphocytes produce large amounts of IL-5 activity in culture supernatants, inducing proliferation of murine BCL₁ cells, and supporting the selective growth of eosinophil colonies in semi-solid culture. Production of IL-5 messenger RNA by transformed B-cell lines was verified by Northern analysis using a 3.2-kilobase cloned DNA fragment containing the full-length human IL-5 gene, and immunoreactive IL-5 was detected in B-cell culture supernatants. These findings suggest a possible expanded role for the B cell in the induction of eosinophilia, and should serve as a focus for additional investigation into possible roles for IL-5 in human B-cell proliferation and differentiation.

INTERLEUKIN-5 (IL-5) is a hematopoietic growth factor that was initially studied in murine systems, where it was found to induce proliferation and differentiation of eosinophil precursors and promote proliferation and antibody production by B cells.¹ The gene coding for IL-5 was isolated from a murine T-cell hybridoma, molecularly cloned, and used to locate and clone the human IL-5 gene from a T-cell leukemia line.² ³ The murine and human IL-5 genes have 77% homology,⁴ and recombinantly produced human IL-5 has been found to induce the growth of human eosinophil colonies,¹ ² but has lacked stimulatory effects in established human B-cell functional assays.¹

Infection of human B cells by the Epstein-Barr virus (EBV) results in a permanent state of activation, in which the B cell is induced to express a number of activation antigens and to secrete substances with apparent growth factor activity.⁵ It is thought that an autocrine mechanism fuels the perpetual proliferation of EBV-transformed B cells in vitro.⁶ We have found that EBV transformation of human B cells consistently results in the induction of production and secretion of large amounts of IL-5. This phenomenon may have physiologic relevance, since it suggests a possible expanded role for the B cell in the promotion of eosinophilia during type 1 immune responses. In addition, these findings may serve as a focus for further investigation into possible activities of IL-5 on human B cells.

MATERIALS AND METHODS

Establishment of EBV-transformed B-cell lines. Peripheral blood was obtained after informed consent on several occasions from each of two subjects with known previous exposure to EBV. We had determined previously that spontaneous EBV-transformed B-cell lines would emerge from suspension cultures of peripheral blood mononuclear cells of these subjects.⁷ The mononuclear fraction was isolated after centrifugation of the buffy layer over Ficoll-Hypaque, and the cells were resuspended at 4 × 10⁷/mL in complete medium consisting of RPMI-1640 containing 7.5% fetal calf serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 5 × 10⁻³ mol/L 2-mercaptoethanol, and 40 μg/mL gentamicin. Suspensions cultures were maintained in 75-cm² tissue culture flasks and fed periodically by the replacement of one third of the medium. Transformed B-cell lines were seen to emerge at about 6 weeks, after which time they rapidly became the only viable elements in the cultures. The identity of the emerging cells was confirmed by flow cytometric analysis for the B-cell specific antigens CD20 and CD19, and the EBV-induced antigen CD23. Presence of EBV in the cell lines was confirmed by immunofluorescent labeling with anti-EBNA (Chemicon International Inc, El Segundo, CA). All EBV-transformed cell lines studied for growth factor activity had been maintained continuously in culture for more than a year. The purity of the populations was verified by flow cytometric analysis demonstrating absence of cell types other than B cells. In some instances, immunoglobulin gene rearrangement studies were performed, documenting the presence after long-term culture of pure B-cell clones.

Detection of IL-5 activity in B-cell culture supernatants. Culture supernatants were tested in two assays standardly used to detect IL-5 activity: the induction of proliferation of the murine B-cell line BCL₁,⁸ and the support of selective growth of eosinophil colonies in semi-solid cultures of human bone marrow.⁹ These assays are quite specific for the detection of IL-5 activity, as no human growth factor other than IL-5 has been shown to stimulate BCL₁ cells, and other lymphokines capable of stimulating eosinophil colony growth, such as granulocyte-macrophage colony-stimulating factor and IL-3, also stimulate the growth of other hematopoietic lineages under the culture conditions used.¹

BCL₁ stimulation assay. BCL₁ cells, freshly obtained after in vivo passage,¹⁰ were used for all assays. BCL₁ cells, 1 × 10⁶, were cultured in 96-well flat-bottomed tissue culture plates in either 0.2 mL complete medium alone or with the addition of 25% vol/vol medium conditioned by EBV-transformed B-cell lines. Quadruplicate cultures were incubated for 48 hours at 37°C before being labeled with 0.5 μCi ³H-thymidine for an additional 6 hours. The cultures were harvested onto glass fiber filters and the relative amount of radiolabel incorporation was determined by liquid scintillation counting. IL-5 activity present in B-cell supernatants was quantified by comparison to a reference curve constructed by culturing BCL₁ cells as above in varying concentrations of recombinant human IL-5 (generously provided by Steven Clark, Genetics Institute, Cambridge, MA).

Eosinophil colony formation assay. Bone marrow from four normal volunteers was aspirated into heparinized syringes. The mononuclear cell fraction was cultured using standard methodology¹ in Iscove’s Modified Dulbecco’s medium with 0.8% methylcellulose (final concentration), containing 20% heat-inactivated fetal calf serum, 5 × 10⁻³ mol/L 2-mercaptoethanol, and 2 mmol/L L-glutamine. Colonies were scored on day 14 and were identified by characteristic morphology by inverted microscopy, with confirm-
tion by Wright-Giemsa staining of colonies picked off the semi-solid culture and allowed to dry onto a glass microscope slide. Background colony numbers were obtained from cultures not supplied with any exogenous growth factor. Positive controls were incubated with 10 U of recombinant human (rh) IL-5. Experimental cultures contained 50 μL of sterile-filtered medium conditioned by EBV-transformed B-cell lines. All cultures were performed in quadruplicate, except unconditioned cultures used for comparison.

Northern analysis for IL-5 messenger RNA (mRNA) expression by B-cell lines. Northern analysis was performed using standard methodology.10 Cells were lysed in 4 mol/L guanidinium solution and the lysate was ultracentrifuged through 5.5 mol/L cesium chloride and 0.1 mol/L EDTA for 18 hours. The RNA pellet was resuspended in 10 mmol/L Tris.Cl (pH 7.4), 5 mmol/L EDTA, and 1% sodium dodecyl sulfate, ethanol-precipitated, and dissolved in diethylpyrocarbonate-treated water. Total cellular RNA (15 μg/lane) was electrophoresed through a 1.3% denaturing agarose gel. Visualization of the 28s and 18s ribosomal RNA bands by ethidium bromide staining was used to ascertain that similar amounts of RNA were loaded in each lane before blotting to a nylon transfer membrane (Nytran, Schleicher & Schuell, Keene, NH). A 3.2-kilobase (kb) cloned DNA fragment containing the full-length human IL-5 gene11 (generously provided by Steven Clark) was 32P-labeled by the random oligonucleotide primer method,12 and hybridized with the blot. The blot was then washed to greater than 90% stringency and autoradiographed at -70°C using an intensifying screen.

Western analysis for IL-5. Separation by polyacrylamide gel electrophoresis was performed using the PhastSystem (Pharmacia, Inc, Piscataway, NJ), with an 8% to 25% gradient, followed by diffusion blotting at 70°C of separated proteins to nitrocellulose. The filter was blocked in phosphate-buffered saline containing 20% fetal calf serum. The primary antibody was the rat monoclonal anti-IL-5, TRFK-5 (gift of Donna Rennick, DNAX, Palo Alto, CA). As this antibody is known to react preferentially with dimeric IL-5 (personal communication, Dr Michael Grace, Schering Corp, November 1989), electrophoresis conditions were nonreducing. The secondary antibody was a goat anti-rat immunoglobulin (Ig) G alkaline phosphatase conjugate (BRL, Inc, Gaithersburg, MD), and the substrate was 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt and nitroblue tetrazolium chloride (BRL).

RESULTS

BCL1 proliferation assay. Culture supernatants of five EBV-transformed B-cell lines markedly augmented the proliferation of murine BCL1 cells in short-term culture (Fig 1). In a repeat experiment, the activity of B-cell supernatants on BCL1 cells was completely removed by preincubation of the supernatants for 2 hours at 22°C with the monoclonal IL-5 neutralizing antibody, TRFK-513 (Fig 2).

Eosinophil colony formation assay. Eosinophil colonies were obtained in bone marrow cultures supplemented with EBV-transformed B-cell conditioned medium in numbers comparable with those obtained in cultures receiving 10 U rhIL-5. Only rare colony forming unit-granulocyte macrophage (CFU-GM) or CFU-M colonies were seen in cultures receiving no supplementation, and these colonies were present in similar numbers in supplemented cultures (Table 1).

Northern analysis for IL-5 mRNA. The DNA probe containing the full-length human IL-5 gene specifically hybridized to a 3.2-kb fragment of RNA in all four EBV-transformed B-cell lines tested (Fig 3).

Western analysis for IL-5. Immunoreactive bands were found at a molecular weight of approximately 45 Kd, as has been reported for native human IL-5,14 in culture supernatants of 3 of 4 EBV-transformed B-cell lines studied (Fig 4).

DISCUSSION

The finding that B cells can be induced to produce IL-5 by EBV infection is cause for re-evaluation of the interactions

| Bone Marrow Colony Formation in Response to rhIL-5 and EBV-Transformed B-Cell Culture Supernatant |
|----------------------------------|----------------------------------|----------------------------------|
| Bone Marrow | Unsupplemented | +10 U rhIL-5 | +B-Cell CM |
| CFU-Eo | 0 | 5.4,8.4 | 8.4,9.8 |
| CFU-GM or M | 1 | 0,2,0.1 | 1,0,1.0 |
| CFU-Eo | 0 | 8,7,6,9 | 8,10,10,12 |
| CFU-GM or M | 2 | 1,2,1.0 | 1,0,0.2 |
| CFU-Eo | 0 | 5,8,6.8 | 9.1,15,10 |
| CFU-GM or M | 2 | 1.0,0.0 | 0,2,0,0 |
| CFU-Eo | 0 | 5,4,5.3 | 6.8,10 |
| CFU-GM or M | 2 | 0,1,0.0 | 1,0,0 |

Cultures supplemented with rhIL-5 or EBV-transformed B-cell conditioned medium were performed in quadruplicate. Single unconditioned cultures were done for comparison.

Abbreviation: CM, conditioned medium.
that occur in the induction of eosinophilia and in B-cell activation, growth, and differentiation. Transformation of B cells by EBV results in the induction of expression of several cell-surface antigens, including the CD23 structure that functions as a low-affinity IgE receptor. It is now known that a variety of activation stimuli, including *Staphylococcus aureus* Cowan strain I, TPA (12-o-tetradecanoylphorbol 13-acetate), and IL-4 will induce normal B lymphocytes to express the CD23 activation antigen. Thus, B-cell expression of an IgE receptor seems to be a general feature of B-cell activation, and may be coupled, at least in EBV-transformed cells, with the production and secretion of large amounts of IL-5. If IL-5 production is found to be inducible in B cells by activation stimuli other than EBV, it will be necessary to consider the possibility that B cells may be capable of amplifying the IL-5 signal in the induction of eosinophilia in type 1 immune responses.

In murine systems, IL-5 has been shown to induce B cells to produce Ig and cause augmented proliferation of B cells costimulated with a mitogen such as dextran sulfate. Murine B cells have been found to display an IL-5 receptor that is upregulated in response to lipopolysaccharide stimulation. Although no B-cell growth activity of IL-5 has yet been convincingly detected in human systems, the finding that human B cells may be induced to produce and secrete IL-5 provides a focus for future investigations of the interaction of IL-5 with human B lymphocytes, including possible autocrine mechanisms operative during B-cell proliferation and differentiation.

REFERENCES


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**Fig 3.** Northern analysis for IL-5 mRNA showing specific bands at 3.2 kb for four distinct EBV-transformed B-cell lines (lanes b through e). (Lane a) WS1 human fibroblast cells (negative control). (Right) Ethidium bromide stained gel showing comparable amounts of total cellular RNA loaded into each lane.

**Fig 4.** Western analysis for immunoreactive IL-5. (Lane 1) Culture supernatant of normal human peripheral blood mononuclear cells. (Lane 2) Recombinant human IL-5. (Lanes 3 and 4) Culture supernatant of two EBV-transformed B-cell lines. In additional experiments, 3 of 4 cell lines tested produced detectable IL-5.
B CELLS PRODUCE IL-5


Epstein-Barr virus transformed B lymphocytes produce interleukin-5

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