A recent addition to the lymphokine network is human IL-10 (hIL-10). This novel lymphokine has striking homology to BCRF1 protein, the product of a previously uncharacterized open-reading frame in the Epstein-Barr virus (EBV) genome. To date, IL-10 expression has been described in several T cell clones induced with anti-CD3 and phorbol myristate acetate (PMA), in monocytes stimulated with lipopolysaccharide (LPS), and in murine B-cell lymphomas. We sought to determine whether human B cells express hIL-10 and, if so, its relationship to EBV and to other B-cell lymphokines. We studied 21 EBV-positive B-cell lines derived from patients with acquired immunodeficiency syndrome (AIDS) and Burkitt's lymphoma (n = 6), American Burkitt's (n = 3), African Burkitt's (n = 5), and normal lymphoblastoid cell lines (n = 7), in comparison with seven EBV-negative cell lines. All cell lines were activated with the tumor promoters PMA and teleocidin and were studied by Northern blot analysis, reverse transcription-polymerase chain reaction (RT-PCR), and enzyme-linked immunosorbent assay (ELISA). We demonstrated that EBV-positive cell lines derived from patients with American Burkitt's lymphoma, and especially those from patients with AIDS, constitutively express large quantities of hIL-10 by Northern blot analysis and ELISA (range, 3,101 to 25,915 pg/ml), and that both teleocidin and PMA induce hIL-10 in these cell lines. In contrast, six of seven EBV-negative cell lines did not express hIL-10 even by RT-PCR, and hIL-10 was not triggered by PMA or teleocidin. To assure that the 350 bp amplified by PCR was hIL-10 and not BCRF1, we used PCR primers, which do not amplify a fragment from plasmid templates containing BCRF1. Cloning and sequencing of the 350 bp product also demonstrated that B-cell IL-10 is identical to hIL-10 from the T-cell clone B21. Correlation of hIL-10 with other B-cell lymphokines secreted by these B-cell lines demonstrated that hIL-10 secreter cell lines also constitutively secrete or can be induced to secrete IL-6, although to a much lesser amount. Since both lymphokines influence B-cell growth and differentiation, we suggest that hIL-10 may contribute to the polyclonal B-cell activation and hyperglobulinemia seen in AIDS patients. Finally, several reports support the hypothesis that EBV is an important cofactor in the development of human immunodeficiency virus type 1 (HIV-1)-related B-cell lymphomas. Detection of large quantities of hIL-10 in B-cell lines derived from AIDS patients, the close association between EBV and hIL-10 shown in this report, and the ability of BCRF1 to capture hIL-10 activities, make hIL-10/BCRF1 an attractive candidate as a factor causing B-cell growth and immortalization in patients with AIDS and B-cell lymphomas.

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uncharacterized open-reading frame in the Epstein-Barr virus (EBV) genome.15,22 BCRF1 is similar to mIL-10 and hIL-10 in that it also has CSIF activity, but it differs in some assays where both mIL-10 and hIL-10 have comparable activity, ie, costimulation of growth of murine thymocytes and induction of class II MHC antigens on resting splenic mouse B lymphocytes. In these systems, the activity of BCRF1 is greatly diminished or absent.15 Taken together, these results suggest that the viral cytokine BCRF1 may have captured only a subset of IL-10 activities, implying that IL-10 contains at least two functional epitopes, one of which has been conserved by EBV. Undoubtedly, site-directed mutagenesis based on the homologies of these proteins will help to dissect the structure-function relationships for each of these activities.

To date, hIL-10 expression has been described in several T-cell clones induced with anti-CD3 and phorbol myristate acetate (PMA),13 and its secretion has been detected in monocytes activated by lipopolysaccharide (LPS).23 O'Garra et al24 reported the expression of mIL-10 and its relationship to other cytokines in mouse B-cell lymphoma, but only little information is available on hIL-10 expression in human B cells.13 In this report, we describe the expression of hIL-10 in a wide panel of EBV-positive B-cell lines and from one exceptional EBV-negative cell line. We demonstrate that all B-cell lines derived from patients with acquired immunodeficiency syndrome (AIDS) constitutively secrete large amounts of hIL-10. This raises the possibility that in addition to B-cell-derived IL-6 and TNF-α,25-28 B-cell IL-10 may also play a role in the development of B-cell abnormalities seen in patients with AIDS.

MATERIALS AND METHODS

Cell lines. The study included tumor B-cell lines derived from patients with undifferentiated lymphoma of Burkitt's and non-Burkitt's types (n = 21) and normal lymphoblastoid cell lines (n = 7) (Table 1). PA682BM-1, PA682BM-2, PA682PE-1, PA682PE-2, PA682PB, and AS283A are tumor B-cell lines derived from patients with AIDS and American Burkitt's lymphoma and are designated as AIDS-associated B-cell lines (AABCL), to differentiate them from Burkitt's lymphoma tumor cell lines derived from non-AIDS patients (non-AABCL). The AABCL are EBV-positive/human immunodeficiency virus type 1 (HIV-1)-negative as determined by polymerase chain reaction (PCR) analysis (Jay Levy, personal communication). DW6, JLP(C), and KK124 are EBV-positive tumor B-cell lines derived from patients with American Burkitt's lymphoma (non-AABCL), and MC116, JD38, JD39, ST48, DS179, CA46, and EW36 are EBV-negative cell lines obtained from American patients with undifferentiated lymphoma of Burkitt's and non-Burkitt's types (non-AABCL). AK778, HRI + Raji, Duaudi, and Namalwa are EBV-positive tumor cell lines derived from patients with African Burkitt's lymphoma (non-AABCL). All tumor B-cell lines except KK124 contain an 8;14 or 8;22 chromosomal translocation or a 14q+ chromosomal abnormality as previously described.29-33 The lines were positive for B-lymphocyte markers only.29-35 A wide panel of B and T surface markers was employed, including CD3, CD4, CD8, CD9, CD10, CD19, CD20, CD21, CD25, HLA-DR, and surface immunoglobulins (μ, γ, α, δ, κ, λ). Of the seven normal lymphoblastoid B-cell lines, three EBV-positive cell lines were obtained from peripheral lymphocytes of patients with infectious mononucleosis (IM), and three EBV-transformed cord blood (CB) B-lymphocyte cell lines were derived as previously described.34 The PACB-PE is an EBV-transformed B-cell line obtained by transfection of normal B cells with supernatant derived from the AABCL, PA682PE-1. The cell lines were maintained in suspension culture in RPMI 1640 plus 10% fetal calf serum (FCS; Whittaker Bioproducts, Walkersville, MD) at 37°C, in 5% CO₂, and were subcultured every 3 to 4 days. These cell lines have been previously studied for lymphokine secretion and lymphokine receptor expression.35-38

Exposure of cells to tumor promoters. Cells obtained on the fourth day following subculture were resuspended in fresh medium at 5 x 10^6 cells/mL and incubated in 75-cm² tissue culture flasks (Corning, Park Ridge, IL) at 37°C for 1 to 4 days in the presence or absence of the tumor promoters teleocidin (kindly provided by Dr Fujiki, National Cancer Institute, Tokyo, Japan) or PMA (Sigma, St. Louis, MO). Teleocidin is known to share many biological activities with PMA and similarly to PMA exerts its effect via activation of protein kinase C.39 Although teleocidin binds to the same specific cell surface receptors as PMA, it is structurally different.39 In contrast to PMA, which may mimic cytokine activity in biological assays, no such interference was observed with teleocidin.2 A broad range of concentrations of teleocidin (0.625 to 10 ng/mL) and PMA (5 to 100 ng/mL) were screened. Optimal results were obtained with 10 ng teleocidin/mL and with 50 ng PMA/mL. Cultures (control, teleocidin-activated, and PMA-activated) were harvested at designated time points and supernatants were collected and stored at −70°C until assayed by enzyme-linked immunosorbent assay (ELISA) for lymphokine activity. The cells were immediately processed for RNA studies.

RNA extraction and analysis. Total cellular RNA was extracted from control, teleocidin-activated, and PMA-activated cells using a guanidinium isothiocyanate/phenol chloroform methodology as previously described.39 Total RNA was prepared and 10-μg samples were analyzed by electrophoresis in 1% agarose formaldehyde gels followed by Northern blot transfer40 to GeneScreen Plus membranes (NEN, Bannockburn, IL). The gels were stained with ethidium bromide to confirm that approximately equivalent amounts of RNA were loaded in each gel lane. RNA blots were prehybridized at 42°C for at least 2 hours in solution containing 50% formamide, 5 x SSC (standard saline citrate, 1 x SSC = 150 mmol/L NaCl + 15 mmol/L Na citrate), 1% sodium dodecyl sulfate (SDS), 50 bp Denhardt's solution (0.02% ficoll, 0.02% polyvinylpyrrolidone (PVP), and 0.02% bovine serum albumin), and 5 x 10^6 cpm of salmon sperm DNA/mL. Hybridizations for at least 18 hours at 42°C were performed in solution in which the DNA and SDS were replaced with 0.02 mg Escherichia coli rRNA/mL and 100 cpm or greater of random primed probe. The probe consisted of plasmid DNA encoding hIL-10 (kindly provided by DNAX, Palo Alto, CA) radiolabeled by the Random Primer Labeling Kit (Boehringer-Mannheim, Indianapolis, IN) with [α-32P]dCTP (3,000 cimol, Amersham, Arlington Heights, IL). Filters were washed twice in 2 x SSC and 0.1% SDS at 25°C for 15 minutes, twice in 2 x SSC and 0.1% SDS at 60°C for 30 minutes, and twice in 0.1 x SSC and 0.1% SDS at 50°C for 30 minutes. Membranes were placed at −70°C to expose Kodak XAR film (Eastman Kodak, Rochester, NY). For several cell lines, poly(A+) RNA was prepared using the Invitrogen Fast Track mRNA isolation kit (Invitrogen, San Diego, CA) following the manufacturer's directions. mRNA expression was quantitated by scanning with an Ultrascan XL densitometer (Pharmacia LKB, Uppsala, Sweden).

Amplification of RNA using reverse transcription-PCR. We used the reverse transcription (RT)-PCR technique in an attempt to detect the expression of hIL-10 in B-cell lines where no evidence for hIL-10 mRNA was shown by Northern blot analysis. Using a
E-CELL INTERLEUKIN-10

Perkin Elmer thermal cycler and GeneAmp Perkin Elmer PCR Kit (Perkin Elmer, Norwalk, CT). cDNAs were prepared by reverse transcription of 1 μg total RNA, or 0.1 μg of poly(A) RNA following the manufacturer’s directions. Then, 5 to 20 μL of the cDNA reaction were then denatured with alkali, neutralized, and transferred to a total volume of 100 μL. The mixture was overlaid with mineral oil and then amplified over 30 cycles, primer annealing at 55°C for 20 seconds, and extending at 72°C for 30 seconds, and extending at 72°C for 30 seconds. The cDNAs were prepared by reverse transcription of 1 μg total RNA, or 0.1 μg of poly(A) RNA following the manufacturer’s directions. Then, 5 to 20 μL of the cDNA reaction was amplified with 2.5 U of 

The relative density of the bands in Northern blots was measured and expressed as percentage of the maximum expression of hIL-10. Strong hIL-10 message has been found in the American Burkitt’s lymphoma cell lines, the African cell line AK778, and the EBV-negative cell line MC116. By RT-PCR analysis, hIL-10 gene expression has been detected in all EBV-positive cell lines, but in only one (MC116) of the EBV-negative cell lines. Pos, positive; Neg, negative; UL, undifferentiated lymphomas; AABCL, AIDS-associated E-cell lines; CB, cord blood; IM, infectious mononucleosis.

### Table 1. hIL-10 Expression Studied by Northern Blot Analysis and RT-PCR

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>EBV Status</th>
<th>Country of Origin</th>
<th>Histology of Original Tumor</th>
<th>Tissue Source</th>
<th>Relative Absorbance Units (%)</th>
<th>RT-PCR Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AABCL</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Bone marrow</td>
<td>3.3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>PA682BM-1</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Bone marrow</td>
<td>51.7</td>
<td>+</td>
<td></td>
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<tr>
<td>PA682BM-2</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Pleural effusion</td>
<td>45.6</td>
<td>+</td>
<td></td>
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<tr>
<td>PA682PE-1</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Peripheral blood</td>
<td>14.3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>PA682PE-2</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Peripheral blood</td>
<td>43.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>AS283A</td>
<td>Pos</td>
<td>USA</td>
<td>Burkitt’s Ascitic fluid</td>
<td>15.3</td>
<td>+</td>
<td></td>
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<tr>
<td>Non-AABCL</td>
<td>Dw6</td>
<td>USA</td>
<td>Burkitt’s Pleural effusion</td>
<td>10.9</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>JLP(C)</td>
<td>Pos</td>
<td>USA</td>
<td>Ul</td>
<td>18.0</td>
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<td></td>
</tr>
<tr>
<td>KK124</td>
<td>Pos</td>
<td>Africa</td>
<td>Burkitt’s Ascitic fluid</td>
<td>1.2</td>
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<td></td>
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<tr>
<td>MC116</td>
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<td>USA</td>
<td>Ul</td>
<td>76.5</td>
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<td></td>
</tr>
<tr>
<td>JD38</td>
<td>Neg</td>
<td>USA</td>
<td>Ul</td>
<td>—</td>
<td>—</td>
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<tr>
<td>JD39</td>
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<td>USA</td>
<td>Ul</td>
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<td>Neg</td>
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<td>Burkitt’s Ascitic fluid</td>
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<tr>
<td>DS179</td>
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<td>USA</td>
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<tr>
<td>CA46</td>
<td>Neg</td>
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<td>Burkitt’s Ascitic fluid</td>
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</tr>
<tr>
<td>EW36</td>
<td>Neg</td>
<td>USA</td>
<td>Ul</td>
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<tr>
<td>Non-AABCL</td>
<td>AK778</td>
<td>Africa</td>
<td>Burkitt’s Ascitic fluid</td>
<td>22.6</td>
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<tr>
<td>HRI+2</td>
<td>Pos</td>
<td>Africa</td>
<td>Burkitt’s Ascitic fluid</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>RAJ</td>
<td>Pos</td>
<td>Africa</td>
<td>Burkitt’s Ascitic fluid</td>
<td>1.9</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>DAUDI</td>
<td>Pos</td>
<td>Africa</td>
<td>Burkitt’s Orbital tumor</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>NAMALVA</td>
<td></td>
<td></td>
<td></td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>Normal lymphoblastoid cell lines</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-AABCL</td>
<td>CB23</td>
<td>USA</td>
<td>Cord blood</td>
<td>&lt;1.0</td>
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<td></td>
</tr>
<tr>
<td>CB33</td>
<td>Pos</td>
<td>USA</td>
<td>Cord blood</td>
<td>—</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CB34</td>
<td>Pos</td>
<td>USA</td>
<td>Cord blood</td>
<td>—</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IM1178</td>
<td>Pos</td>
<td>USA</td>
<td>Peripheral blood</td>
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<td>IM-B182</td>
<td>Pos</td>
<td>USA</td>
<td>Peripheral blood</td>
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<td>—</td>
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<tr>
<td>IM-E182</td>
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<td>USA</td>
<td>Peripheral blood</td>
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<td>—</td>
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<tr>
<td>PACB-PE</td>
<td>Pos</td>
<td>USA</td>
<td>PA682PE-1</td>
<td>&lt;1.0</td>
<td>+</td>
<td></td>
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</table>

The constitutive expression of hIL-10 detected in Northern blot analysis and RT-PCR in 28 B cell lines is summarized. Using a densitometer, the absorbance of the bands in Northern blots was measured and expressed as percentage of the maximum expression of hIL-10. Strong hIL-10 expression has been detected in the American Burkitt’s lymphoma cell lines, the African cell line AK778, and the EBV-negative cell line MC116. By RT-PCR analysis, hIL-10 gene expression has been detected in all EBV-positive cell lines, but in only one (MC116) of the EBV-negative cell lines. Pos, positive; Neg, negative; UL, undifferentiated lymphomas; AABCL, AIDS-associated E-cell lines; CB, cord blood; IM, infectious mononucleosis.

Specificity of amplification was confirmed by hybridization to an oligonucleotide probe spanning nucleotides 429 to 498 (5’-CAGGTGAAGAATGCTCCTTATAAGTCCACGAAGAACCACATC-3’) that was end-labeled by the enzyme bacteriophage T4 polynucleotide kinase using [7-32P] adenosine triphosphate (ATP) as previously described.42

Cloning and sequencing RT-PCR products. RT-PCR products from amplifications using primers 1 and 2 were blunt end-cloned using T4 DNA ligase (Gibco BRL, Gaithersburg, MD) into the HindII site of pBluescript II (Stratagene, La Jolla, CA) after passage of the RT-PCR reaction mixture over a nuc-trap column (Stratagene) to remove the unincorporated nucleotides. White colonies growing on LB agar containing 100 μg ampicillin/mL, 40 μg isopropyl-β-D-galactoside/mL (IPTG, BRL), and 40 μg 5-bromo-4-chloro-3-indolylo-β-D-galactoside/mL (X-GAL, BRL) were selected and grown overnight in a total volume of 100 μL. Plasmid minipreparations were performed following boiling lysis of the bacteria from 1.5 mL of overnight culture following the protocol for Stratagene resin (Stratagene) miniprep as described by the manufacturer. Plasmids containing the appropriate size inserts, as determined by electrophoresis in 1.5% agarose minigels after cutting with PvuII, were
sequenced. The SK and T7 primers for pBluescript II were used to obtain sequence information for both strands of the insert from double-stranded plasmid DNA as described in the Stratagene DSK sequencing kit. Dideoxynucleotide chain-terminated products were separated on 6% acrylamide wedge-sequencing gels containing 7 mol/L urea.

ELISA. Detection of hIL-10 and hIL-6 used monoclonal antibodies for these lymphokines as previously described. Although specific monoclonal antibodies for hIL-10 have been used, these antibodies cross-react with BCRF1 (Kevin Moore, personal communication).

RESULTS

Expression of hIL-10 mRNA by Northern blot analysis. Twenty-eight B-cell lines were studied for hIL-10 mRNA expression using Northern blot analysis. Since PMA has been shown to induce hIL-10 in several T-cell clones, all cell lines were also activated with PMA 50 ng/mL or teleocidin 10 ng/mL. Constitutive expression of substantial amounts of hIL-10 mRNA was shown in the AABCL PA682BM-1, PA682BM-2, PA682PE-1, PA682PE-2, PA682PB, and AS283A (Fig 1; Table 1). Only the single band of 1.5 kb was seen. In the three non-AABCL which were also derived from patients with American Burkitt’s lymphoma, a substantial amount of constitutively produced hIL-10 mRNA was detected in JLP(C) and DW6 cell lines, whereas only little message was found in unstimulated KK124 cells (Table 1). However, following activation with the tumor promoters PMA and teleocidin, a threefold to 18-fold increase in hIL-10 mRNA level was observed in these three non-AABCL (data not shown). Whereas all of the EBV-positive cell lines derived from patients with American Burkitt’s lymphoma expressed hIL-10 mRNA, only two of the five cell lines obtained from patients with African Burkitt’s lymphoma, and one of the seven EBV-negative cell lines (MC116 cell line) constitutively expressed hIL-10 (Fig 1; Table 1). Neither teleocidin nor PMA induced hIL-10 mRNA expression in any of the other three African cell lines or the other six EBV-negative lines. Finally, we examined the seven normal lymphoblastoid cell lines for hIL-10 mRNA. None of these cell lines was found to constitutively express hIL-10 mRNA by Northern blot analysis and neither teleocidin nor PMA induced its expression.

RT-PCR analysis. We next tried to determine whether hIL-10 mRNA was expressed in cell lines in which it was not detected by Northern blot analysis, while also confirming that the message we were detecting was hIL-10 and not BCRF1. Total RNAs obtained from all cell lines (control, teleocidin-activated, PMA-activated) were therefore analyzed by RT-PCR amplification. As shown in Fig 2A, all of the AABCL expressed hIL-10. An additional cell line included in this blot is the EBV-negative cell line EW36, in which no amplification could be detected. To further confirm that the amplified 350 bp fragment was hIL-10, samples obtained from the PCR reaction were run on 1.5% agarose gel, blotted using the Southern technique, and then detected with a [γ²P] ATP-labeled oligonucleotide designed to hybridize to the hIL-10 sequence in between the PCR primers. The data obtained using this procedure (Fig 2B) corresponded to that of the ethidium bromide staining (Fig 2A). With RT-PCR amplification, we also demonstrated that the four African cell lines, the cord blood-transformed lymphoblastoid cell lines, and the infectious mononucleosis cell lines, which did not express hIL-10...
mRNA by Northern blot analysis, expressed the 350 bp fragment by RT-PCR. However, with the exception of MC116 cells, which expressed hIL-10 mRNA, no amplification of hIL-10 mRNA has been detected in the EBV-negative cell lines (Table 1).

Since RT-PCR amplification for hIL-10 suggested that all EBV-positive cell lines express the hIL-10 gene, we assumed that the questionable weak messages for hIL-10 in the PACB-PE and CB23 cell lines were due to extremely low expression of hIL-10. Thus, poly(A⁺) RNA was prepared from these two cell lines, and from two AABCL that served as controls. Using these poly(A⁺) RNA, a weak hIL-10 message in PACB-PE and a strong message in CB23 have been demonstrated by Northern blot (Fig 1) and RT-PCR (Fig 3). This further confirmed that all the EBV-positive cell lines express hIL-10, although different levels of hIL-10 expression exist.

Cloning and sequencing B-cell IL-10. We have cloned and sequenced the hIL-10 RT-PCR fragment from four representative B-cell lines: PA682BM-1, PA682BM-2, JLP(C), and MC116. The two EBV-positive AABCL (PA682BM-1 and PA682BM-2) were selected since they represent American Burkitt’s lymphoma in AIDS patients; the EBV-positive cell line JLP(C) represents American Burkitt’s lymphoma derived from a non-AIDS patient (non-AABCL); and the EBV-negative cell line MC116 represents undifferentiated lymphoma of Burkitt’s type obtained from an American patient. If EBV induces B cells to secrete a cytokine related to but not identical to hIL-10, the “IL-10” expressed by MC116 cell line would be predicted to be different from that of JLP(C). If HIV-1 can induce a similar phenomenon, the AABCL products could conceivably have had a different sequence from that of JLP(C). Samples obtained from the RT-PCR reaction were cloned and sequenced simultaneously. All four B-cell lines showed the same hIL-10 sequence, which was also identical to the hIL-10 sequence described in the T-cell clone B-21.15

Constitutive secretion of hIL-10 and its relationship to hIL-6 secretion. The data obtained on hIL-10 secretion corresponded to that determined in Northern analysis in most cell lines (Table 2). The AABCL were found to constitutively secrete large amounts of hIL-10 (range, 3,101 to 3,395 pg/mL) and teleocidin-induced hIL-10 synthesis in these cells (range, 3,095 to 41,315 pg/mL). PMA also seems to have some inducing effect, but was less potent than teleocidin. Constitutive secretion of hIL-10 was also detected in the two non-AABCL JLP(C) and DW6, the EBV-negative cell line MC116, and the African cell line
**DISCUSSION**

In this study, we investigated a wide panel of tumor B-cell lines, as well as normal lymphoblastoid cell lines for hIL-10 expression, and the relationship of this novel lymphokine to EBV and to other B-cell lymphokines. Our data demonstrate that EBV-positive cell lines derived from patients with American Burkitt's lymphoma, and especially those obtained from patients with AIDS, constitutively express large amounts of hIL-10 as shown by Northern blot analysis and ELISA. Much lower amounts are found in African Burkitt's lymphoma cell lines (excluding AK778 cell line) and normal lymphoblastoid lines in which constitutive expression of hIL-10 gene is detected only by RT-PCR amplification. In contrast to hIL-10 expression in all of the EBV-positive cell lines, the EBV-negative cell lines (excluding the cell line MC116) do not express hIL-10 even by RT-PCR analysis. It is not clear why only the EBV-positive cell lines constitutively secrete hIL-10, nor why this phenomenon is even more prominent in the B-cell lines derived from the AIDS patients.

Since hIL-10 shows 80% homology to BCRF1, it can be argued that the message detected and the lymphokine measured is not hIL-10, but rather BCRF1. However, the only previous detection of BCRF1 by Northern blot was seen after 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulation of the EBV-producing B lymphocyte line B95-8, but not in unstimulated B95-8 cells. The size of the predominant message seen in this case was 0.8 kb, which corresponds to that expected for the predicted open-reading frame for BCRF1 in the EBV sequence. A minor band at 1.6 kb was found using two different EBV BCRF1 fragments containing extensive homology to hIL-10. This 1.6-kb band corresponds fairly well to the 1.5-kb message we find for hIL-10 and to the approximate 1.4 kb mIL-10 mRNA. Other groups have been unable to detect BCRF1 expression by Northern or dot blots. Even using the exquisitely sensitive technique of RT-PCR, Hsu et al were only able to detect BCRF1 expression in four of seven EBV+ cell lines. This suggests that the large amount of message detected and consequently the lymphokine secreted from our B-cell lines is indeed hIL-10. This will be conclusively determined once monoclonal antibodies for hIL-10 that do not cross-react with BCRF1 have been generated.

The high level of hIL-10 secreted by the AABCL might be of special importance to understanding B-cell abnormalities seen in patients with AIDS. In comparison to purified monocytes induced by LPS, the amounts of hIL-10 secreted by these B-cell lines is much higher. The maximum level of hIL-10 secreted by monocytes was detected 48 hours after stimulation and did not exceed 52 ng/mL and the IL-6 level was 215 ng/mL. Current studies of other cell types will help us to determine if the large amount of hIL-10 produced by these human B-cell lines is unique.
with reduced proliferative responses of B cells to different mitogens and antigens.\textsuperscript{40} suggests that various signals are involved in inducing B-cell abnormalities.\textsuperscript{51-54} Recently, much attention has also been directed to the possible role and effects of HIV-1 and B-cell lymphokines.\textsuperscript{26,55} Sastry et al\textsuperscript{55} demonstrated that HIV-1 tat gene introduced into Raji B cells by retroviral-mediated transfection, induced the expression of TNF-\(\alpha\) and IL-6. These B cells were found to induce the production of TNF-CY. and IL-6. These B cells were found to induce the proliferation of adult and fetal thymocytes to IL-2, IL-4, and IL-7.\textsuperscript{56,58} Since hIL-10 and IL-2-like molecule, IL-4, TNF, IL-6, and IL-7), thus indicating that mIL-10 shares greater than 80% homology and several functional activities.\textsuperscript{15,22} we predict that hIL-10 will also function as growth cofactor with one (or more) of the several lymphokines secreted by our B-cell lines (IL-1, IL-2-like molecule, IL-4, TNF, IL-6, and IL-7), thus indirectly leading to feedback stimulation of B cells. (4) Finally, several reports support the hypothesis that EBV is an important cofactor in the development of HIV-1-related B-cell lymphomas.\textsuperscript{59-62} IL-10 is known to impair the synthesis of T-cell IFN-\(\gamma\) and IL-2.\textsuperscript{63,64} Besides its antiviral activities, these lymphokines are known to support the expansion of immune-activated T cells in autocrine fashion. Karp and Broder\textsuperscript{62} therefore suggested that excess IL-10 may permit viral replication to go unchecked (particularly, with reduced proliferative responses of B cells to different mitogens and antigens).\textsuperscript{40} suggests that various signals are involved in inducing B-cell abnormalities.\textsuperscript{51-54} Recently, much attention has also been directed to the possible role and effects of HIV-1 and B-cell lymphokines.\textsuperscript{26,55} Sastry et al\textsuperscript{55} demonstrated that HIV-1 tat gene introduced into Raji B cells by retroviral-mediated transfection, induced the expression of TNF-\(\alpha\) and IL-6. These B cells were found to induce the production of TNF-CY. and IL-6. 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Karp and Broder\textsuperscript{62} therefore suggested that excess IL-10 may permit viral replication to go unchecked (particularly,
EBV and possibly also HIV-1), which in turn promotes the cascade of events that culminate in the establishment of clonal B-cell malignancy. Detection of high levels of hIL-10 in the B-cell lines derived from patients with AIDS and Burkitt's lymphoma, the high titer of EBV detected in these patients, and the close association between EBV and hIL-10 shown in this report, make hIL-10 an attractive candidate as a factor leading to dysregulation of B-cell growth and function in patients with AIDS and lymphomas.

Since hIL-10 expression was found in only a single EBV-negative cell line, and given the high degree of homology of BCRF1 to hIL-10, we cloned and sequenced EBV-negative cell lines, and EBV-positive cell lines, demonstrates that in each of the differently derived cell lines, it is the same gene that is being expressed. It also suggests that in addition to EBV, other mechanisms influence hIL-10 expression.

The sum of these studies provides indirect evidence that B-cell-derived hIL-10 may be an important part of the cytokine network that determines the function of both B cells and T cells. Future experiments will aim at exploring whether hIL-10 and/or BCRF1 contribute to immortalization of B cells by EBV and at clarifying the role of hIL-10 in the immune response.

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Human B-cell interleukin-10: B-cell lines derived from patients with acquired immunodeficiency syndrome and Burkitt's lymphoma constitutively secrete large quantities of interleukin-10 [see comments]

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