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Production of the Immunosuppressive Cytokine Interleukin-10 by Epstein-Barr-Virus-Expressing Pyothorax-Associated Lymphoma

Possible Role in the Development of Overt Lymphoma in Immunocompetent Hosts

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Malignant lymphomas frequently develop in the pleural cavity of patients with long-standing pyothorax. Thus, the term pyothorax-associated lymphoma (PAL) has been proposed for this type of tumor. Most PALs are diffuse lymphomas of B cell type and contain Epstein-Barr virus (EBV) DNA. We have established two lymphoma cell lines from the biopsy specimens of PAL cases, OPL-1 and OPL-2. Both cell lines contain EBV DNA, but only OPL-1 expresses EBV nuclear antigen 2, which works as a target molecule for the cell-mediated immune response. As systemic immunodeficiency is unlikely to be present in PAL patients, PAL from which OPL-1 derived was not expected to be fully developed. In this study, we examined the expression of immunosuppressive factors in OPLs. Only OPL-1, not OPL-2, expressed interleukin-10 (IL-10) mRNA and secreted IL-10 into culture supernatant. Both OPL-1 and OPL-2 expressed transforming growth factor (TGF)-β1 mRNA; however, neither expressed latent TGF-β-binding protein mRNA at a detectable level by Northern blot analysis. Because TGF-β expresses its functions in cooperation with latent TGF-β-binding protein, the biological functions of TGF-β1 could be negligible. Neither cell line expressed at a detectable level EBV BCRF-1 mRNA, a viral gene product that is partly homologous to human IL-10 and shares biological activities of IL-10. Although IL-10 is reported to promote the growth of activated or neoplastic B cells, OPL-1 did not respond to human recombinant IL-10 by growing faster. As OPL-1 expresses a target antigen for the host cytotoxic T-cell response, the production of an immunosuppressive cytokine, IL-10, might contribute to the development of overt lymphoma by inducing locally immunosuppressive circumstances. The present study suggests that an immunosuppressive cytokine plays a role in lymphomagenesis of immunocompetent patients. (Am J Pathol 1997, 150:349–357)

Epstein-Barr virus (EBV) is identified as an etiological agent of the African type of Burkitt's lymphoma and nasopharyngeal carcinoma in southern China.[1] Latent infection genes of EBV including EBV nuclear antigens (EBNAs) and latent membrane proteins are expressed in latently infected and immortalized B cells.[2] Burkitt's lymphoma cells only express EBNA-1, which is not recognized as a target by virus-specific cytotoxic T lymphocytes (CTLs) and thus are successfully able to evade immune surveillance.[3] In the course of *in vitro* cultivation, however, Burkitt's cells express all genes associated with latent EBV infection.[4] These genes include EBNA-2

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Table 1. *EBV- and Growth-Related Characteristics of OPLs*

	EBV genome		EBV latent genes		Colony formation on soft agar
	Type	Clonality	EBNA-2	LMP-1	
OPL-1	B	+	+	−	−
OPL-2	A	+	−	−	+

and -3s and latent membrane proteins, which serve as target viral antigens for the elimination of infected cells by CTLs.[5] It is speculated that Burkitt's lymphoma cells that express these antigens are eliminated by CTLs *in vivo*.[2]

EBV infection in immunocompromised patients such as those with acquired immune deficiency syndrome or allograft recipients receiving immunosuppressive therapy evoke polyclonal B-cell proliferative disorders, frequently resulting in monoclonal malignant lymphomas.[6,7] These lymphomas are most often diffuse lymphomas of B-cell type and exhibit EBV DNA.[6,7] The suppressed immune functions in these patients are thought to lead to incomplete elimination of the cells expressing EBV latent infection genes.[8]

Malignant lymphomas frequently develop in the pleural cavity of patients with long-standing pyothorax,[9] in which a systemic immunosuppressive condition has not been noted. Thus, the term pyothorax-associated lymphoma (PAL) has been proposed for these tumors. Pyothorax, a purulent effusion of the pleural cavity, occasionally results from pulmonary tuberculosis or tuberculous pleuritis. In the chronic form, the pleural wall shows a fibrous thickening with varying degrees of calcification. Most PALs are diffuse large-cell lymphomas of B-cell type[9] and contain EBV DNA.[10,11] We recently reported the establishment of two lymphoma cell lines from biopsy specimens of PAL cases, OPL-1 and OPL-2.[12] Both lines contain a single predominant form of episomal EBV DNA, indicating clonal cellular proliferation of an EBV-infected progenitor cell.[13] Neither expresses latent membrane protein-1, and only OPL-1 expresses EBNA-2 mRNA and protein.[12] These findings indicate that lymphomas expressing the CTL target antigen EBNA-2 can develop in the nonimmunocompromised host.

It has been postulated that immunoregulatory cytokines and other factors affecting CTL induction and proliferation play an important role in the host immune reaction to EBV latent infection gene-positive cells.[2] Interleukin (IL)-10 and transforming growth factor (TGF)-β1 exert immunosuppressive effects by suppressing antigen-specific CTL induction[14,15] and by inhibiting cytokine production by helper T cells and macrophages.[14,16] Intracellular binding of TGF-β protein with latent TGF-β-binding protein (LTBP) is required for its efficient secretion and activation.[17,18] Therefore, examination of LTBP expression is necessary for the estimation of the function of TGF-β protein. In addition, there is another EBV gene, BCRF-1, that bears partial homology to the human IL-10 gene[19] and shows some of the biological activities of IL-10.[20]

In the current study, we examined the expression of these immunosuppressive factors as well as BCRF-1 in the PAL cell lines. Both OPL-1 and OPL-2 expressed TGF-β1 mRNA. However, neither expressed LTBP. The expression of IL-10 mRNA and protein was observed only in OPL-1, an EBNA-2-expressing PAL cell line.

Materials and Methods

Cells

OPL-1 and OPL-2 have been established from the biopsy specimens of PAL cases.[12] The growth characteristics and the profile of EBV latent infection gene expression are summarized in Table 1. The EBV-positive Burkitt's lymphoma cell line Raji was obtained from the Japanese Cancer Research Resources Bank. The marmoset lymphoblastoid cell line B95-8 was kindly provided by Prof. Kenzo Takada (Hokkaido University, Sapporo, Japan). All cell lines were grown in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% (Raji and B95-8) or 15% (OPL-1 and OPL-2) heat-inactivated fetal calf serum (FCS; ICN Biochemicals, Lisle, IL). Cells were collected at 3×10^5 to 5×10^5 cells per ml (greater than 95% viability) and used for mRNA extraction.

mRNA Extraction and Northern Blot

Total cellular RNA was extracted from cells by the acid guanidinium-phenol chloroform method. mRNA was purified by using oligo-dT latex (Takara, Kyoto, Japan), quantitated by measuring optical density (OD) at 260 nm, and ethanol precipitated at −80°C until use. Five micrograms of mRNA was electropho-

resed in 1% formaldehyde-agarose gels and Northern blotted as previously described.[21]

Preparation of Probes and Hybridization

The plasmids containing human TGF-β1[22] or LTBP[23] cDNA were kindly provided by Dr. Kohei Miyazono (The Cancer Institute, Tokyo, Japan). The 0.5-kb *Pst*I-*Kpn*I fragment of TGF-β1 cDNA and the 0.6-kb *Bam*HI fragment of LTBP cDNA were used as templates, and probes were labeled with α-[^{32}P]dCTP by random priming. For hybridization using cDNA probes, Northern-blotted filters were prehybridized for 4 hours at 42°C in 6X stand saline phospate-EDTA (SSPE; 0.9 mol/L NaCl, 0.06 mol/L sodium phosphate, 6 mmol/L EDTA), 5X Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 200 μg/ml denatured salmon testes DNA (Sigma Chemical Co., St. Louis, MO), 50% formamide and hybridized in a solution of the same composition containing the probe at 2×10^6 cpm/ml. Filters, hybridized for 16 hours at 42°C, were washed twice for 30 minutes at room temperature with 1X SSPE, 0.1% SDS, twice for 15 minutes at 50°C with 0.1X SSPE, 0.1% SDS, and exposed at −80°C. For detection of actin mRNA, an additional 15 minutes of washing at 60°C with 0.1X SSPE, 0.1% SDS was added.

Human IL-10 oligonucleotide probe (5′-ATGTCAAACTCACTCATGGCTTTGTAGATGCCTTT-3′)[19] was 5′-end-labeled with γ-[^{32}P]ATP by T_4 polynucleotide kinase (New England Biolabs, Beverly, MA). IL-10 oligonucleotide probe also hybridizes with the BCRF-1 gene of EBV.[24] For detection of IL-10 and BCRF-1 expression using oligonucleotide probes, filters were prehybridized for 4 hours at 42°C in 6X standard saline citrate (SSC; 0.9 mol/L NaCl, 0.09 mol/L sodium citrate), 5X Denhardt's solution, 0.5% SDS, 20 μg/ml denatured salmon testes DNA and hybridized in a solution containing the probe at 3×10^6 cpm/ml. Filters, hybridized for 16 hours at 42°C, were washed with 2X SSC, 0.1% SDS twice for 10 minutes at room temperature, twice for 15 minutes at 60°C, and exposed at −80°C.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis of Human IL-10 and BCRF-1

mRNA samples were treated with RNAse-free DNAse I (Sigma) as described[25] and ethanol precipitated. For the RT-PCR amplification of IL-10, BCRF-1, and actin mRNA, 2 μg of DNAse-treated mRNA was converted to cDNA with 400 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, MD) in the presence of 3 mmol/L $MgCl_2$, 1 mmol/L dNTP, 28 U of RNAse inhibitor (Promega, Madison, WI), 1 μg of oligo-dT primer, and 1X RT reaction buffer supplied by the manufacturer in a 40-μl reaction volume. After incubation at 37°C for 60 minutes, the RT mixture was boiled for 5 minutes and subjected to PCR amplification. A 10-μl aliquot of the cDNA sample was diluted to 50 μl containing 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 1.25 U of *Taq* polymerase (Promega), 1X *Taq* buffer, and 500 nmol/L both primers. Primer sequences were 5′-ATGCCCCAAGCTGAGAACCAAGACCCA-3′ and 5′-TCTCAAGGGGCTGGGTCAGCTATCCCA-3′ for human IL-10, amplifying the 352-bp segment of mRNA[26]; 5′-TGTGGAGGTACAGACCAATGT-3′ and 5′-CACCTGGCTTTAATTGTCATG-3′ for BCRF1, amplifying the 452-bp segment of mRNA[24]; and 5′-ATCATGTTTGAGACCTTCAA-3′ and 5′-CATCTCTTGCTCGAAGTCCA-3′ for human actin, amplifying the 318-bp segment of mRNA.[27] PCR was performed (1 cycle at 94°C for 3 minutes, 58°C for 2 minutes, and 72°C for 2 minutes; 34 cycles at 94°C for 1 minute, 58°C for 2 minutes, and 72°C for 2 minutes), and amplified products were electrophoresed in 2% agarose gels and Southern blotted as previously described.[21] Human IL-10/BCRF-1 oligonucleotide probe described above was 3′-end labeled with fluorescein-11-dUTP by using the enhanced chemiluminescence 3′-oligolabeling system (Amersham, Little Chalfont, UK). Filters were hybridized with the labeled probe, washed, and incubated with anti-fluorescein horseradish peroxidase conjugate. Signals were generated with the enhanced chemiluminescence detection reagents (Amersham) and exposed at room temperature by following the procedures suggested by the manufacturer. In the case of actin, amplified products were examined in ethidium-bromide-stained gels on an ultraviolet transilluminator.

Measurement of Human IL-10 and/or BCRF-1 Protein

The amount of human IL-10 and/or BCRF-1 protein in the culture supernatant of cell lines and in patients' sera was quantitated by enzyme-linked immunosorbent assay (ELISA) using a human IL-10 ELISA kit (Endogen, Cambridge, MA).

Recombinant IL-10 and Anti-IL-10 Antibody

Human recombinant IL-10 (rIL-10; 5×10^5 U/mg protein) was purchased from Genzyme (Cambridge, MA). Anti-human IL-10 antibodies with neutralizing activity (purified goat IgG fraction) was purchased from R&D Systems (Minneapolis, MN). These were used in the growth assay of cells described below.

Growth Assay of Cells

Growth of cell lines was measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay as described previously.[28]

Briefly, cells (2×10^4) were seeded in 96-well tissue culture plates in a 150-μl culture medium containing various factors. Cells were incubated at 37°C in 5% CO_2 for various time periods. The MTT solution (50 μl; 1:5 dilution with RPMI1640 medium of 5 mg/ml MTT (Wako Pure Chemical, Osaka, Japan) in Dulbecco's phosphate-buffered saline) was then added to each well, and the plates were incubated at 37°C for 4 hours. After the incubation, the plates were centrifuged and the supernatant was aspirated. A 150-μl volume of dimethyl sulfoxide (Wako Pure Chemical) was added to each well. The OD value of each well was read at 540 and 620 nm in a microplate spectrophotometer (Titertek, Helsinki, Finland). The results are represented as the difference between the OD at 540 nm and that at 620 nm as the background, and the mean value was calculated from the results of quadruplicate wells.[29]

Immunohistochemistry for Human IL-10 and/or BCRF-1

Immunohistochemical analysis of formalin-fixed and paraffin-embedded tissue for human IL-10 and/or BCRF-1 was performed as previously described.[30] Rat monoclonal antibodies used in this study were JES3-12G8 (Pharmingen, San Diego, CA), which reacts with both human IL-10 and BCRF-1, and JES3-6B11 (Pharmingen), which reacts with only BCRF-1.

Results

Northern Blot Analysis of Immunosuppressive Factors in OPLs

In Northern blot analysis of mRNA from cells with TGF-β1 and LTBP cDNA probes, all of the cell lines expressed TGF-β1 mRNA (Figure 1A), although

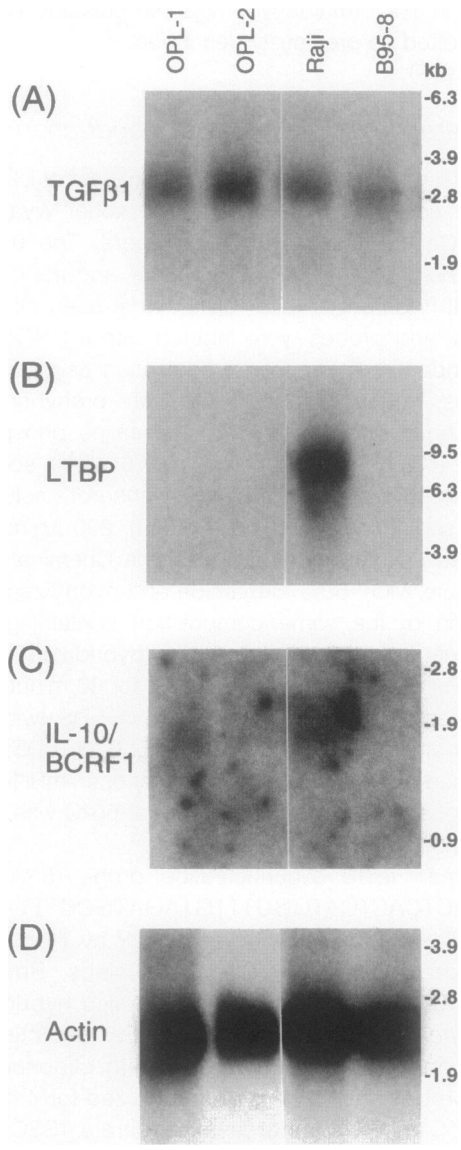

Figure 1. *Northern blot analyses of immunosuppressive factors.* **A:** *TGF-β1 mRNA.* **B:** *LTBP mRNA.* **C:** *IL-10/BCRF-1 mRNA.* **D:** *Actin mRNA. Five micrograms of mRNA was applied to each well, Northern blotted, and hybridized with ^{32}P-labeled TGF-β1, LTBP, or actin cDNA probes or with the IL-10/BCRF-1 oligonucleotide probe. RNA size markers are indicated on the right. Blots were exposed for 3* (**A, B,** *and* **D**) *or 10* (**C**) *days at −80°C.*

LTBP mRNA was expressed only in Raji and not in OPLs (Figure 1B). By Northern blot analysis with the oligonucleotide probe that can hybridize both IL-10 and BCRF-1, we observed one band in OPL-1 and Raji (Figure 1C). The size of the band was approximately 1.6 kb, which is reported as the size of human IL-10 mRNA.[19] A 0.8-kb band, the expected size of BCRF-1 mRNA,[24] was not observed in any mRNA sample from any of the cell lines.

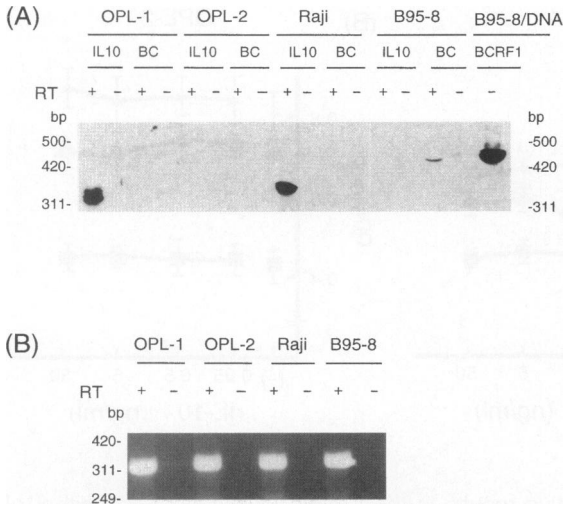

Figure 2. *RT-PCR analyses of human IL-10 and BCRF-1. The 0.5-μg samples of DNAse-treated mRNA prepared from the indicated cell lines were reverse transcribed and amplified by PCR for IL-10 (IL10) or BCRF-1 (BC;* **A**) *or for actin mRNA* (**B**). *Amplified products were electrophoresed in 2% agarose gels, Southern blotted, and hybridized with the IL-10/BCRF-1 oligonucleotide probe* (**A**) *or visualized with an ultraviolet transilluminator* (**B**). *mRNA samples without reverse transcription (RT) were also subjected to the same PCR amplification, and 0.5 μg of DNA from EBV-producing B95-8 cells was used as a positive control for PCR amplification of the BCRF-1 sequence. DNA size markers are indicated on both sides. EBV-producing B95-8 cells shows the expression of BCRF-1 mRNA. Raji and OPL-1, but not OPL-2, show the expression of human IL-10 mRNA.*

RT-PCR Analysis of Human IL-10 and BCRF-1

As both OPLs, Raji, and B95-8 contain a DNA virus, EBV, mRNA samples were treated with DNAse before RT-PCR.[25] OPL-1, but not OPL-2, expressed human IL-10 mRNA, and neither of the OPLs expressed BCRF-1 mRNA (Figure 2). Raji expressed only IL-10 mRNA, compatible with the Northern blot analysis results. By RT-PCR analysis, BCRF-1 mRNA was detected in B95-8 cells, although it was not detectable by Northern blot analysis.

Measurement of Human IL-10 and/or BCRF-1 Protein

IL-10 and/or BCRF-1 protein was detected in 10-day culture supernatant of OPL-1 (Figure 3). On the other hand, culture supernatant of OPL-2 did not contain detectable IL-10 or BCRF-1 protein. The culture supernatants of Raji or B95-8 cells contained IL-10 and/or BCRF-1 protein (Figure 3). Because of the results of the RT-PCR analysis described above, probably Raji secreted IL-10 and B95-8 secreted BCRF-1 protein into the culture media.

The sera of PAL cases 1 and 2, from which OPL-1 and OPL-2 were originated, respectively, were collected at the time of biopsy before treatment and stored at −80°C until ELISA. Case 1 showed a higher concentration of IL-10 in the serum, although case 2 contained a trace amount, a level almost equal to that of normal individuals (Figure 3). These results were compatible with the IL-10-producing activity of each cell line derived from the case.

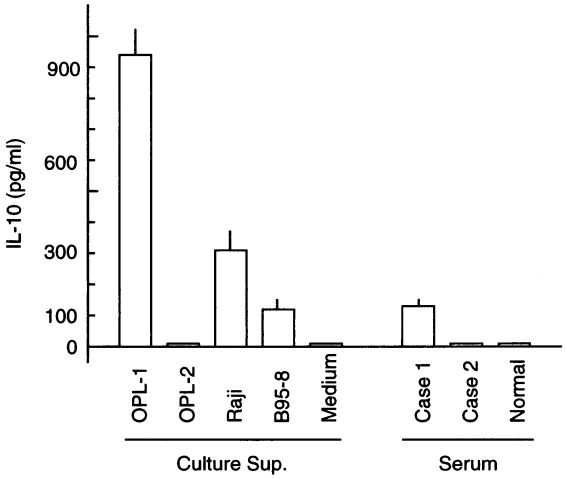

Figure 3. *Concentration of IL-10 and/or BCRF-1 protein in the culture of cell lines and in the sera of PAL patients. After the 10-day culture of cell lines, the supernatants (Sup.) were harvested by centrifugation and stored at −20°C. Serum was collected at the time of biopsy before treatment and stored at −80°C. The concentration of IL-10 and/or BCRF-1 was measured by ELISA. The bars represent the mean values of duplicates from four different supernatant preparations ± SEM in supernatant samples and the mean values of duplicates ± SEM in serum samples.*

Effects of Human rIL-10 on the Growth of OPLs

IL-10 is also reported to promote the growth of activated or neoplastic B cells.[31–33] Thus, we examined the influence of IL-10 on the growth of OPLs. Human rIL-10 did not significantly alter the growth of OPLs even at a concentration of 50 ng/ml (Figure 4), at which concentration IL-10 is reported to promote the growth of EBV-transformed B cells.[33] A decrease in the concentration of supplemented FCS to 5 or 1% did not reveal the growth effects of rIL-10 on OPLs (results not shown). Furthermore, we added anti-IL-10 antibodies to the culture of OPLs at concentrations up to 10 μg/ml. This concentration of the antibody inhibits the growth-promoting effects of 5 ng/ml human rIL-10 on the IL-10-dependent cell line MC/9. The IL-10 concentration of 5 ng/ml is approximately five times higher than that in the OPL-1 culture (Figure 3). The growth of OPLs, however, was not affected in the presence of anti-IL-10 antibodies (results not shown).

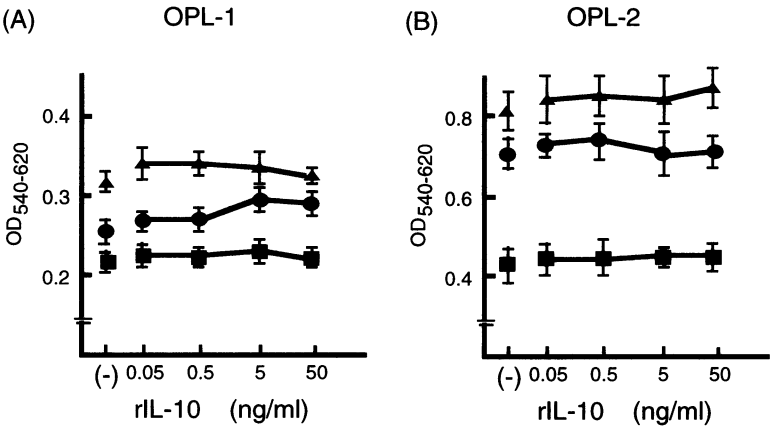

Figure 4. *Effects of human rIL-10 on the growth of OPL-1* (**A**) *and OPL-2* (**B**). *Cells* (2×10^4) *were cultured in 96-well plates in the presence of the indicated concentration of IL-10. The concentration of supplemented FCS in the culture was 15%. After incubation for the indicated time periods, the growth of the cells was estimated by the MTT method as described in Materials and Methods and presented as the value of* $OD_{540-620}$. *Each point indicated the mean ± SD of quadruplicate wells.* ■, *day 1;* ●, *day 2;* ▲, *day 3. The enhancing effects of IL-10 on the growth of OPLs were not obvious during 3 days of treatment.*

Immunohistochemistry for Human IL-10 and/or BCRF-1 Protein

After autoclaving of tissue sections, monoclonal antibody JES3-12G8 gave weak signals in some populations of lymphoma cells in case 1 but not case 2 (results not shown). On the other hand, JES3-6B11 generated higher background staining in both cases, and thus positive signals were not identified (results not shown). Therefore, some lymphoma cells in case 1 expressed IL-10 and/or BCRF-1 protein *in vivo*.

Discussion

Some lymphomas express viral antigens, which can be targeted by the host cell-mediated immune response.[2] The development and progression of such lymphomas in immunocompetent hosts may depend upon the presence of immunosuppressive cytokines. We have established two PAL cell lines, OPL-1 and OPL-2, and showed a variation in growth characteristics and expression of EBV latent infection genes.[12] OPL-1 grows slower and expresses EBNA-2, a target molecule for CTLs. Consequently, we anticipated that the slowly growing OPL-1 might express immunosuppressive cytokines to a higher level than OPL-2, which does not express EBNA-2 and shows much enhanced growth rates. In fact, OPL-1 did express IL-10 mRNA and secreted IL-10 protein into the culture medium, whereas IL-10 mRNA was not detected in OPL-2.

BCRF-1 is originally reported as an EBV gene expressed in the lytic infection cycle,[24] although Miyazaki et al[34] have recently shown that BCRF-1 mRNA is also expressed even in the latent infection state. The ELISA used in this study detects both human IL-10 and BCRF-1, and OPL-1 showed positive results in the protein level. Although neither of the OPLs expressed BCRF-1 mRNA by Northern blot analysis, we felt it important to rule out the trace amount of BCRF-1 gene expression by RT-PCR analysis using reagents specific for human IL-10 or for BCRF-1. The results confirmed those of the Northern blot analysis. IL-10 mRNA was found only in OPL-1, and neither of the OPLs expressed BCRF-1 mRNA by RT-PCR analyses. Thus, our results showed that only the slower growing OPL-1 expressed IL-10 mRNA and secreted IL-10 protein into the culture supernatant. Both OPL-1 and OPL-2 expressed TGF-β1 mRNA in the absence of LTBP mRNA. LTBP binds to TGF-β intracellularly and promotes the efficient secretion and extracellular activation of the latent form of TGF-β.[17,18] Thus, we could rule out the possibility that LTBP supplied by other cells in the *in vivo* environment functionally complements TGF-β expression by the lymphoma cells. Therefore, if the production of immunosuppressive factors contributes to the development of PALs such as OPL-1 that express CTL target antigens, IL-10 is a likely candidate molecule.

IL-10 works as an autocrine growth factor on EBV-transformed B cells in some cases.[32,33] Thus, we examined the possible growth-enhancing effects of human rIL-10 on OPLs, especially on IL-10-producing OPL-1. However, the growth-promoting effects of human rIL-10 on OPLs were not observed (Figure 4). We previously reported that IL-6 promotes the growth of OPL-1 even at a concentration of less than 1 ng/ml and that addition of anti-IL-6 antibodies to the culture of OPL-1 completely inhibits the growth.[28] Therefore, IL-6 but not IL-10 contributes to the growth in OPL-1.

The IL-10 concentration in the sera of each PAL patient reflected the IL-10-producing activity of each lymphoma cell line derived from the patient. Further-

more, immunohistochemistry of biopsy specimens for IL-10 and/or BCRF-1 protein revealed that lymphoma cells in case 1 but not case 2 were weakly positive (results not shown). Thus, IL-10 in the serum of case 1 was probably produced by lymphoma cells *in vivo*. The concentration of IL-10 in the serum of case 1 corresponds to approximately 1 U/ml and is not enough to show sufficient inhibitory activities on cytokine synthesis.[14,35] However, the IL-10 concentration in lymphoma tissue would be locally high enough to show immunosuppressive activity because the IL-10 in the OPL-1 culture reached a much higher concentration than that in the patient serum (Figure 3).

Although IL-10 production in B lymphoma cells is usually associated with the presence of EBV,[26,36] only EBNA-2-expressing OPL-1 produced IL-10 in our PAL cell lines. As OPL-1 grows more slowly than OPL-2 and as IL-10 is a B-cell growth factor, IL-10-producing lymphoma cells *in vivo* might be selected to develop into overt lymphoma through an autocrine growth loop. However, the current study revealed that IL-10 did not promote the growth of OPL-1. These findings suggest another selection process in the development of overt lymphoma in the PAL, ie, EBV antigen-positive transformed cells producing immunosuppressive factors could evade host immunological surveillance and finally develop into overt lymphoma. OPL-1 expressed HLA class I antigens detected by flow cytometry using monoclonal antibody W6/32, and OPL-2 did not (H. Kanno, S. Yamauchi, and K. Auzasd, unpublished data). Thus, lymphoma cells from which OPL-1 derived must have been eliminated by host CTLs in immunocompetent circumstances. Immunopotentiating cytokines such as IL-2 can also modify the cell-mediated immune reaction to EBV-transformed cells.[37] In this aspect, OPLs did not secrete IL-2 and interferon-γ when examined by ELISA (H. Kanno, Y. Taniguchi, and K. Auzasd, unpublished data).

Another source of immunosuppressive factors in PALs might be the inflammatory cells present in chronic pyothorax. In other words, chronic inflammatory reactions could lead to a locally immunosuppressive microenvironment and contribute to lymphomagenesis. The evaluation of expression of immunosuppressive factors *in situ* will provide a clue to cell sources of immunosuppressive factors.

In this study, we showed that the PAL cell line with a lower proliferative activity and with CTL target antigens showed IL-10 expression. The evaluation of immunosuppressive cytokines in the EBV-positive lymphomas in immunocompetent patients clarified the contribution of immunosuppressive factors to lymphomagenesis. The current results add additional evidence for the role of cytokines in the development of lymphomas.

Acknowledgments

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