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Differential Expression of Viral and Human Interleukin-10 (IL-10) by Primary B Cell Tumors and B Cell Lines

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Human and viral interleukin-10 (IL-10) possess growth factor activity for human B cells and may act as autocrine growth factors in B cell malignancies. To study this possibility we have measured viral (v) and human (h) IL-10 expression in EBV-positive and negative B lineage tumors and tumor cell lines. Previous studies demonstrated IL-10 expression in cell lines and now we describe the pattern of IL-10 expression in primary Burkitt's lymphoma (BL) tumor biopsies and in BL lines of defined phenotypes. vIL-10 was expressed during the lytic (productive) phase of EBV infection, but not during virus latency. Although hIL-10 was expressed in the majority of B cell lines, it was not expressed in two BL biopsy specimens. Expression of hIL-10 did not correlate with the presence of EBV, but was associated with the differentiation state of the B cell line. Thus, vIL-10 may enhance the persistence of B cells infected at sites of virus replication, and while hIL-10 may be a factor in the growth both *in vivo* and *in vitro* of some BLs and EBV-transformed B cells, it is not an absolute requirement. © 1994 Academic Press, Inc.

INTRODUCTION

The Epstein–Barr virus (EBV) is a herpesvirus which can establish a nonproductive or latent infection in human B lymphocytes both *in vivo* and *in vitro*. The virus is associated with several B cell tumors including Burkitt's lymphoma (BL; Epstein *et al.*, 1964) and polyclonal lymphoproliferations in immunosuppressed individuals (Cleary *et al.*, 1986). Permanent tumor lines have been established from many BL biopsies, the majority of which contain EBV. In addition to its association with tumors, EBV also possesses the ability to transform normal, resting B cells *in vitro* into permanent lines, termed lymphoblastoid cell lines (LCLs).

EBV can transform normal B cells at a number of different stages in B cell differentiation. However, the LCLs which grow out have a phenotype which is representative of B-blasts (Nilsson and Klein, 1982; Katamine *et al.*, 1984; Ernberg *et al.*, 1987; Gregory *et al.*, 1987; 1988a). In contrast, Burkitt's lymphoma (BL) tumor cells and early-passage BL lines, whether they contain EBV or not, are representative of an earlier stage in B cell differentiation than LCLs (Rooney *et al.*, 1986; Gregory *et al.*, 1988a,b).

EBV gene expression differs between cell types and in BL lines has been found to alter upon growth *in vitro*. In BL tumor biopsies and certain BL-derived lines, EBV displays a minimal, type I latency where only EBNA 1 is expressed. Upon passage, EBV gene expression normally progresses toward type III latency, which is that seen in LCLs. In this state the virus expresses an additional five EBNAs and three membrane proteins (Rowe *et al.*, 1987; Gregory *et al.*, 1990). An alteration in cellular gene expression and hence surface phenotype is concomitant with the progression in EBV gene expression. Thus BL biopsy cells and certain lines retain a biopsy-like, group I phenotype, whereas upon passage the majority of lines progress toward a more activated, LCL-like group III phenotype (Rowe *et al.*, 1985; Gregory *et al.*, 1990).

Interleukin-10 (IL-10) is a polypeptide growth factor which has profound effects on cells involved in the immune response (for review see Spits and de Waal Malefyt, 1992). Analysis of the IL-10 coding sequence revealed that it was highly homologous to the EBV open reading frame BCRF1 (Moore *et al.*, 1990; Vieira *et al.*, 1991). Expression of this gene showed that the EBV-encoded product had the same biological activities as hIL-10 and hence it was termed viral IL-10 (vIL-10).

IL-10 affects the functions of many cell types, notably macrophages and B cells. Macrophages are essentially deactivated by IL-10 due to downregulation of MHC class II expression and cytokine synthesis (de Waal Malefyt *et al.*, 1991 a,b; Fiorentino *et al.*, 1991 a,b). In contrast, IL-10 acts as a stimulatory factor for B cells. Thus, it enhances the viability of resting B cells

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(Go *et al.*, 1990) and enhances the proliferation and immunoglobulin (Ig) secretion of activated B cells (Rousset *et al.*, 1992; Burdin *et al.*, 1993).

hIL-10 is produced by a range of cell types including activated T cells of all subsets (Spits and de Waal Malefyt, 1992), activated monocytes (de Waal Malefyt *et al.*, 1991a), and B cells. Thus some, but not all, LCLs, BL lines, and AIDS-associated B lymphomas have been shown to produce hIL-10 (Benjamin *et al.*, 1992; Emilie *et al.*, 1992; Burdin *et al.*, 1993). In contrast, vIL-10, at least in the prototypic B95-8 strain, is a product of the EBV lytic cycle and thus is not normally expressed by B cells since they harbor the virus in a latent state (Hudson *et al.*, 1985; Stewart and Rooney, 1992).

The aim of this work was to study the expression of vIL-10 and hIL-10 by B cells which are a natural host of EBV and hence assess their potential role in EBV persistence and tumorigenesis. We studied expression in BL biopsy specimens which had not been grown in tissue culture as well as BL lines which were of a defined biopsy-like phenotype (gI) and LCLs. In addition, we studied the relationship between hIL-10 expression and the differentiation state of these lines.

MATERIALS AND METHODS

Cells

All cell lines were maintained at exponential growth in RPMI 1640 (GIBCO/BRL, Paisley, Scotland) containing 10% fetal calf serum (Globepharm, Esher).

The lines Akata (Takada, 1984), Eli (Rooney *et al.*, 1986), Mutu gI (Gregory *et al.*, 1990), and Odhi (Rowe *et al.*, 1992) are all EBV-positive BL-derived lines with a clearly defined gI phenotype. Mutu gIII (Gregory *et al.*, 1990) was derived from the same biopsy as Mutu gI but has a defined gIII phenotype. Raji (Pulvertaft, 1964) and AG876 (Pizzo *et al.*, 1978) are EBV-positive BL lines. The lines IARC-BL2, BL30, BL41 (Lenoir *et al.*, 1985), and Ramos (Klein *et al.*, 1975) are EBV-negative BL-derived lines. The Namalwa sublines CSN/70, IPN/45, PNT, and KN2 were all derived from the same EBV-positive BL line (Guy *et al.*, 1990). B95-8 (Miller *et al.*, 1972) is a marmoset LCL. The lines JS/B95, MM/B95, NG/B95, and Kloek/B95 are LCLs obtained by *in vitro* transformation of donor lymphocytes with EBV (strain B95-8). EG/LCL and MT4/L are EBV-positive LCLs which grew out spontaneously in culture. Akata, Eli, Mutu, and Odhi were obtained from Professor A. B. Rickinson (University of Birmingham, UK). BL2 and BL30 were available within the laboratory but were additionally obtained from Dr. L. Young (University of Birmingham, UK). Kloek/B95 was obtained from Dr. J. Reittie (Royal Free Hospital, London). MT4/L was obtained from Dr. S. Hopkins (Hope Hospital, Salford, UK). The four variant Namalwa sublines were obtained from the German Collection of Microorganisms and cell cultures.

Two BL biopsies were obtained from patients within St. Jude Children's Research Hospital and were termed Jude2 and Jude3. Both were histologically diagnosed as BL and carried chromosomal translocations associated with BL, either t(8:22) or t(8:14) for Jude2 and Jude3, respectively. Jude2 was found to be positive and Jude3 negative when tested for the presence of EBV by Southern blotting using the EBV *Bam*HI W fragment as probe.

HLA typing was performed using standard serological techniques by Dr. E. V. Turner (St. Jude Children's Research Hospital).

Productive EBV replication was induced in cells by the addition of 20 ng/ml 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA; Sigma) for 3d.

Generation of probes for IL-10

The probe used for vIL-10 was a 935-bp DNA fragment (EBV coordinates 9659–10,594) which contains the complete BCRF 1 open reading frame. This was excised from a plasmid containing cloned EBV DNA (Arrand *et al.*, 1981) with the enzyme *Stu*I using standard techniques (Sambrook *et al.*, 1989).

The hIL-10 probe was generated by RT-PCR using RNA prepared from the line JS/B95 and the primers 5'-GAAGGCATGCACAGCTCAGC-3' (sense strand) and 5'-GTCCTAGAGTCTATAGAGTCG-3' (anti-sense strand).

The probe used for the EBV early gene product BZLF 1 was a 1.8-kbp cDNA fragment which was excised from pUC18 using the enzymes *Bam*HI and *Eco*RI.

The probe for β-actin was a 2-kbp fragment comprising the chicken β-actin cDNA which was obtained from Dr. J. Cleveland (St. Jude Children's Research Hospital, Memphis, TN) and was excised from pBR322 using the enzyme *Pst*I.

Double-stranded DNAs (25 ng) were radiolabeled using [α-^{32}P]dATP (NEN) and a random-primed DNA labeling kit (Boehringer) to a specific activity of $>1 \times 10^9$ cpm/μg.

Northern blotting

Cytoplasmic RNA was prepared by acid phenol extraction exactly as described by Rooney *et al.*, (1991). Total RNA (10 μg/track) was electrophoresed through 1.2% agarose gels containing formaldehyde and blotted onto nylon membranes (MagnaGraph, MSI) by capillary transfer exactly as described (Sambrook *et al.*, 1989). Blots were hybridized and washed according to the manufacturers instructions. Molecular weight determinations were made using an RNA ladder (GIBCO/BRL).

Southern blotting

This was performed exactly as described by Sambrook *et al.*, (1989). Genomic DNA (10 μg/track) was digested with the appropriate restriction enzyme and electrophoresed through 0.7% agarose gels. Molecular weight determinations were made using a 1-kb ladder (GIBCO/BRL).

FACS analysis

Cells (1×10^6) were first washed with PBSAA (PBS containing 0.2% BSA and 0.2% azide) before being resuspended in 100 μl PBSAA. FITC-conjugated antibodies (10 μl) were then added and the mixture incubated for 10 min in the dark. The FITC conjugates used were monoclonal mouse anti-CD3 (Dakopatts) or monoclonal mouse anti-CD38 (Serotec). Excess antibody was removed by washing three times with PBSAA and the cells resuspended finally in 500 μl PBS containing 0.5% paraformaldehyde. The fluorescence was then analyzed using a FACS IV (Becton Dickinson). The results were expressed as the mean fluorescence of 30,000 events on a linear scale and at equivalent gain or the percentage of fluorescence-positive cells relative to the negative control (anti-CD3). Experiments were repeated on two separate occasions with comparable results.

ELISA for secreted immunoglobulin

Cells were seeded at a density of 1×10^5/ml and were grown for 4 days after which time the medium was harvested. The amount of Ig in supernatants was quantitated using a double-antibody sandwich ELISA as described by Lems-Van Kan *et al.*, (1983). The sensitivity of both assays was <0.1 ng/ml. Assays were performed in quadruplicate and the results expressed as the mean. The coefficient of variation was <10% in all cases.

RESULTS

Specificity of probes for viral and human IL-10

Expression of vIL-10 and hIL-10 was assessed by Northern blotting. Since the sequences encoding vIL-10 and hIL-10 share 71% identity (Vieira *et al.*, 1991), we first probed RNA derived from cell lines which expressed either one or the other species of IL-10 and determined that both probes were specific (results not shown).

Expression of viral IL-10 by human B cells

It had been shown previously in B95-8, a marmoset LCL displaying type III latency, that vIL-10 mRNA was produced only during EBV lytic cycle gene expression and not during latency (Hudson *et al.*, 1985). We wished to investigate whether this pattern held for other types of EBV-positive B cells. Expression of vIL-10 was therefore assessed in BL tumor biopsy specimens as well as lines displaying both type III and type I latency. Selected lines were tested before and after treatment with TPA which induces EBV lytic cycle gene expression. The degree of induction was assessed by reprobing the same blot for BZLF1 mRNA which is a sensitive marker of EBV productive cycle gene expression (Rooney *et al.*, 1988).

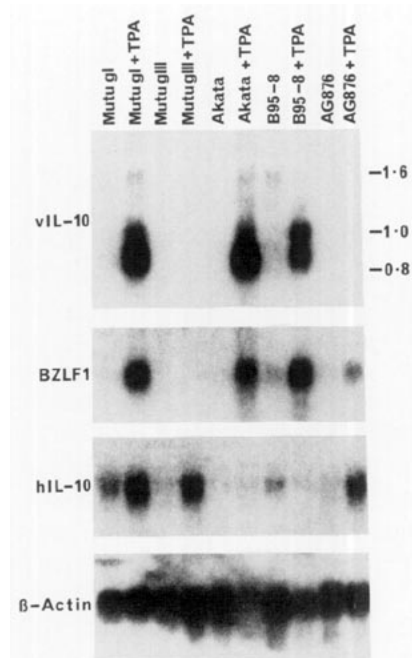

FIG. 1. Expression of vIL-10 and hIL-10 with relation to productive EBV replication. RNA was extracted from the lines indicated either before or after treatment with TPA and subjected to Northern blot analysis. The blot was probed sequentially with sequences encoding vIL-10, BZLF1, hIL-10, and β-actin. The sizes of the vIL-10 mRNAs in kb are indicated at the right.

The results (Figs. 1, 2 and Table 1) showed that BL biopsies and the untreated B cell lines, with the exception of B95-8, did not express detectable amounts of vIL-10 mRNA whether they expressed type I or type III latency. Figure 1 shows that again, with the exception of B95-8, the expression of BZLF1 mRNA and hence lytic cycle gene expression was not seen untreated B cell lines. After addition of TPA, the expression of BZLF1 mRNA in all but Mutu gIII cells and the expression of vIL-10 mRNAs followed this pattern exactly. Three vIL-10 mRNAs of sizes 0.8, 1.0, and 1.6 kb were observed in Mutu gI, Akata (gI) and B95-8 lines with the 0.8-kb species being predominant. This pattern is exactly as described previously for B95-8 cells (Hudson *et al.*, 1985). vIL-10 mRNA was induced in TPA-treated AG876 cells but the levels were extremely low and the signal was lost upon photographic reproduction.

Thus, vIL-10 was not expressed in the majority of B cells tested, including freshly isolated BL biopsies and

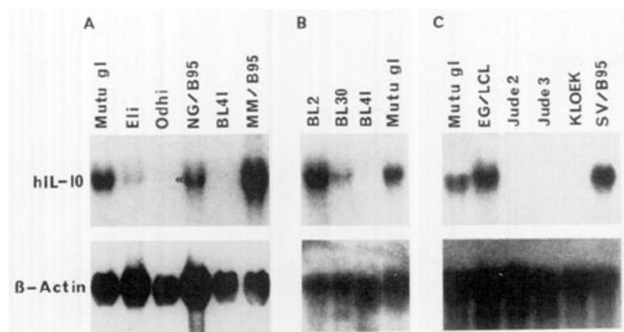

FIG. 2. Expression of hIL-10 by B cells. RNA was extracted from the B cell lines and BL biopsies indicated and subjected to Northern blot analysis. The results of three individual blots (A, B, and C) are shown. RNA from the Mutu gI line was included in each blot as an internal positive control for hIL-10. Each blot was probed sequentially with hIL-10 and then β-actin. The double arrowheads show the position of the hIL-10 mRNA in Odhi cells which could be seen on the autoradiograph but which was lost upon reproduction.

gI BL lines and as these cells harbor EBV in a predominantly latent state; vIL-10 expression was observed only during EBV lytic cycle gene expression.

Expression of human IL-10 by B cells

The same B cells were analyzed for expression of hIL-10. As can be seen in Fig. 2c, no hIL-10 expression was observed in the two BL tumor biopsy specimens. However, expression of a 1.8-kb hIL-10 mRNA was observed in the majority of B cell lines tested whether they had a gI or gIII phenotype (Figs. 1 and 2). One of seven LCLs (Kloek/B95) and two of four EBV-negative BL lines (BL41 and Ramos) did not express detectable hIL-10 mRNA.

The levels of hIL-10 mRNA relative to β-actin varied considerably between the cell lines in which it was expressed. In general, as can be seen in Fig. 2, LCLs (e.g., MM/B95, EG/LCL, and SV/B95) expressed higher levels of hIL-10 than the BL lines (e.g., Mutu gI, Eli, Odhi, and BL30). The levels of expression between BL lines appeared to be highly variable from BL2 which expressed as much mRNA as some LCLs to Odhi in which the signal was barely detectable. It was also found that the gI subclone of Mutu expressed more hIL-10 mRNA than Mutu gIII cells (Fig. 1).

Since it had been reported by other workers that BL2 and BL30 did not express hIL-10 (Vieira *et al.*, 1991; Burdin *et al.*, 1993) and our data showed the opposite (Fig. 2b), we checked the authenticity of the lines by HLA-typing. The HLA types of the lines were determined using standard techniques and were found to be identical to those reported previously (A. B. Rickinson, personal communication). In addition, we obtained BL2 and BL30 from another laboratory and these cells showed an identical pattern of hIL-10 expression. It is therefore likely that the differences between the two sets of results are due to the divergence of cellular phenotype upon passage as has been reported for other BL lines (Guy *et al.*, 1990).

The expression of hIL-10 was also influenced by TPA (Fig. 1). TPA markedly increased the levels of hIL-10 in Mutu gI, Mutu gIII, and AG876, had no effect in Akata, and decreased its levels in B95-8 cells. Thus, TPA had differential effects upon the levels of hIL-10 mRNA depending on the cell line, which was independent of its influence on vIL-10 expression.

To determine whether the lack of basal expression of hIL-10 by Kloek/B95, BL41, and Ramos was due to chromosomal abnormality, genomic DNAs from these lines, as well as JS/B95 as a control, were analyzed by Southern blotting using the hIL-10 coding sequence as a probe. The result of using DNA digested with the enzymes *Bgl*II (Fig. 3), *Taq*I, or *Hin*dIII (not shown) revealed no restriction fragment-length polymorphisms in this locus between the lines. Thus, there was no evidence of loss or gross rearrangement of the hIL-10 coding region. We next determined whether hIL-10 expression could be induced. After addition of TPA,

TABLE 1

EXPRESSION OF vIL-10 AND hIL-10 BY HUMAN B CELL LINES

A. Lymphoblastoid cell lines

Cell Line	vIL-10 mRNA	hIL-10 mRNA
B95-8	± (+)[a]	+[b]
EG/LCL	−	+
JS/B95	−	+
KLOEK/B95	−	−
MM/B95	−	+
MT4/L	−	+
NG/B95	−	+
SV/B95	−	+

B. Burkitt's lymphoma cells

Cell	EBV Status	vIL-10 mRNA	hIL-10 mRNA
BL2	−	−	+
BL30	−	−	+
BL41	−	−	−
Ramos	−	−	−
AG876	+	− (+)[a]	+
Akata[c]	+	− (+)	+
Eli[c]	+	−	+
Mutu gI[c]	+	− (+)	+
Mutu gIII	+	− (−)	+
Odhi[c]	+	−	+
Raji	+	−	+
Jude 2[d]	+	−	−
Jude 3[d]	−	−	−

[a] + denotes signal on northern blot; − denotes no signal; brackets denote signal after treatment with TPA.

[b] Marmoset cell line thus signal to cross-reactivity with marmoset IL-10.

[c] BL lines of a defined biopsy-like (gI) phenotype.

[d] BL biopsy material.

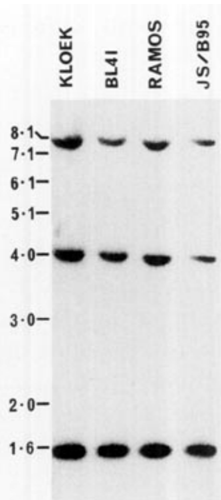

FIG. 3. Integrity of the hIL-10 locus. High-molecular-weight DNA was extracted from the cell lines indicated, cut with the restriction enzyme *BglII*, and analyzed by Southern blotting using the hIL-10 coding sequence as a probe. The position of the molecular weight standards in kbp are indicated at the left.

Kloek/B95 cells produced hIL-10 mRNA, whereas BL41 and Ramos still remained negative. Thus, at least in Kloek/B95, the lack of detectable hIL-10 mRNA expression in untreated cells could not be attributed to a genetic defect.

Differential expression of hIL-10 in variant Namalwa subclones

To assess whether there was a correlation between hIL-10 expression and B cell differentiation we first analyzed variant sublines of the Burkitt's lymphoma line Namalwa. The four sublines used are all derived from one original BL line but have diverged upon passage and display the phenotype of B cells arrested at different stages in B cell development (Guy *et al.*, 1990). The results (Fig. 4) show that the three less differentiated sublines (CSN/70, IPN/45 and PNT) did not express detectable amounts of hIL-10 mRNA, whereas the most differentiated subline (KN2) did. Thus, the differentiation/activation state of the B cell is associated with the expression of hIL-10 in these lines.

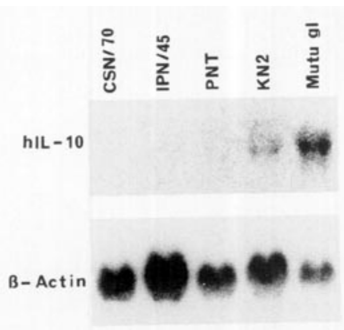

FIG. 4. Expression of hIL-10 by Namalwa sublines. RNA was extracted from four Namalwa sublines (CSN/70, IPN/45, PNT, and KN2) and Mutu gI as a control and subjected to Northern blotting. The same blot was probed sequentially with hIL-10 and β-actin.

TABLE 2

CORRELATION OF hIL-10 EXPRESSION WITH IMMUNOGLOBULIN EXPRESSION

A. Lymphoblastoid cell lines

Cell line	hIL-10 mRNA	Ig isotype	Secreted immunoglobulin[a]/ng ml^{-1}
NG/B95	+	G	11136
KLOEK/B95	−	G	9856
JS/B95	+	G	661
MM/B95	+	M	601
MT4/L	+	M	128

B. Burkitt's lymphoma cell lines

Cell line	EBV status	hIL-10 mRNA	Ig isotype	Secreted immunoglobulin[a]/ng ml^{-1}
Akata	+	+	G	277
BL41	−	−	M	39
Ramos	−	−	M	22
Mutu gIII	+	+	M	18
Eli	+	+	M	1.1
Mutu gI	+	+	M	0.8
BL2	−	+	M	0.7
BL30	−	+	M	0.5
Raji	+	+	M	0.2
Odhi	+	+	M	<0.1

[a] Cell lines placed in order of Ig secretion.

Relationship between B cell differentiation and hIL-10 expression in B cell lines

We next determined whether hIL-10 expression was related to differentiation state in other B cell lines. Immunoglobulin (Ig) and CD38 antigen expression both vary during B cell development and can be used to analyze B cell differentiation (Nilsson and Klein, 1982; Bhan *et al.*, 1981; Tedder *et al.*, 1984). The levels of Ig secretion increase with differentiation. In contrast, the levels of CD38 antigen decrease from early B cells which are high expressors to mature B cells (e.g., LCLs) which are low expressors and then levels are again elevated on terminally differentiated plasma cells (Tedder *et al.*, 1984; Guy *et al.*, 1990; Rochford *et al.*, 1993). We therefore used a combination of Ig isotype, the level of Ig secretion and the level of CD38 antigen expression to assess the relative differentiation state of a selection of B cell lines and correlated this with hIL-10 expression. Ig secretion was measured by a capture ELISA and the level of CD38 antigen expression by FACS analysis. The results are shown in Tables 2 and 3 and are summarized diagrammatically in Fig. 5.

TABLE 3

CORRELATION OF hIL-10 EXPRESSION WITH CD38 ANTIGEN EXPRESSION

Cell line	hIL-10 mRNA	CD38[a] MFL[b]	CD38[a] % Positive
A. Lymphoblastoid cell lines			
KLOEK/B95	−	29	19
JS/B95	+	45	41
NG/B95	+	66	54
MT4/L	+	94	81
B. Burkitt's lymphoma cell lines			
RAJI	+	135	99
ODHI	+	135	99
RAMOS	−	225	99
BL41	−	340	99

[a] Values determined by FACS analysis relative to CD3 as a negative control.

[b] Mean fluorescence on a linear scale at equivalent gain.

Table 2 shows that LCLs secreted over 100 ng/ml Ig, whereas BL lines, with the exception of Akata, secreted less than 40 ng/ml. This was consistent with BL lines representing an earlier stage of B cell differentiation than LCLs (Nilsson and Klein, 1982). Although the most differentiated LCLs secreted a significant amount of Ig, the levels were still much lower than those expected from plasmacytoid/plasma cells and thus these lines are mature, activated B cells. The results of Ig secretion were consistent with CD38 antigen expression (Table 3) which was high on BL lines and low on LCLs.

On the basis of Ig secretion (Table 2A), the one LCL which did not express detectable hIL-10 mRNA (Kloek/B95) was well differentiated as compared with other LCLs and on a par with NG/B95. The difference between the levels Ig secretion from Kloek/B95 and NG/B95 was within the limits of variation of the experiment which was 10%. However, analysis of CD38 (Table 3A) showed that both the number of positive cells and the level of expression was less in Kloek/B95 than the other LCLs. These results are consistent with Kloek/B95 cells being more differentiated than the other LCLs analyzed.

BL41 and Ramos, which did not express detectable hIL-10, were relatively well differentiated on the basis of Ig secretion compared with the majority of the other BL lines (Table 2B). However, based on CD38 antigen expression (Table 3B) these two lines were the least differentiated of the cells tested. One possible reason for this discrepancy could be the presence of a small proportion of cells secreting a large amount of Ig: such cells would not affect the CD38 antigen analysis. Our results therefore suggest that a minor subpopulation of BL41 and Ramos have undergone differentiation *in vitro*; however, the majority of the cells are poorly differentiated. Thus, like the Namalwa sublines, the differentiation state of other BL lines is associated with the expression of hIL-10.

In conclusion, as is represented in Fig. 5, hIL-10 was expressed by B cells within a discrete period of B cell

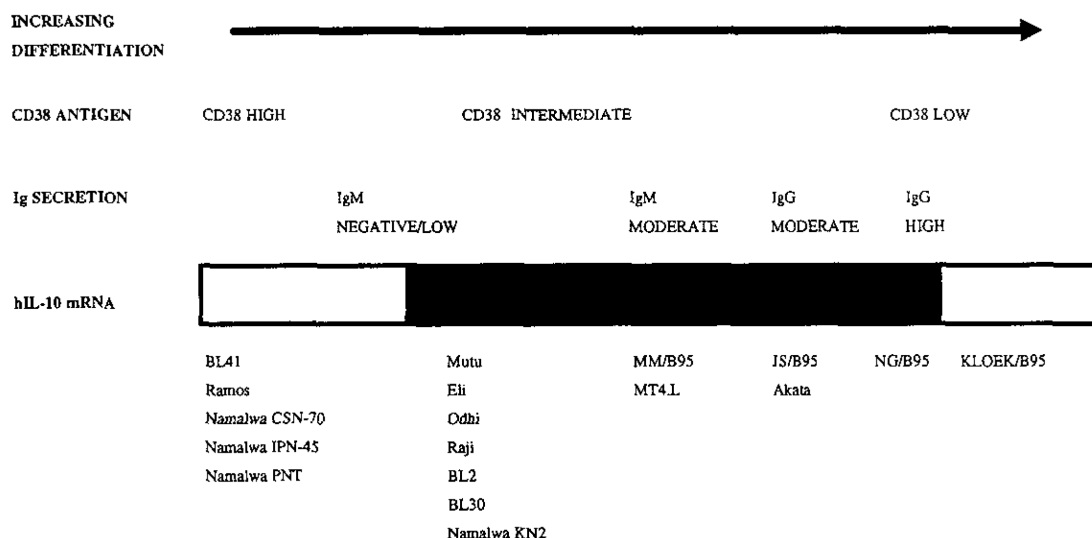

FIG. 5. Diagrammatic representation of the differentiation state of B cell lines and its relationship to hIL-10 expression. The lines are grouped according to the differentiation state represented starting with the least differentiated at the left and the most differentiated at the right. Above this are shown the expression of hIL-10, relative level of Ig secretion, and relative level of CD38 expression. hIL-10 expression is shown by a shaded box and lack of expression by an open box. It should be noted that although the relative level of Ig secreted by NG/B95 and Kloek/B95 is described as high, the amount of Ig secreted is relatively low as compared with a plasmacytoid/plasma cell and thus these lines are not plasmacytoid.

differentiation which was represented by most of the B cells tested. B cell lines which were either more or less differentiated did not express detectable hIL-10 mRNA.

DISCUSSION

This work describes the expression of vIL-10 and hIL-10 mRNA in human B cells which are associated with EBV infection. The cell types analyzed were EBV-transformed B cells (LCLs) and BL cells which were either EBV-positive or EBV-negative. The BL samples included freshly isolated BL tumor biopsy specimens and BL lines which were of a biopsy-like, group I phenotype and expressed type I EBV latency. We have also studied the relationship between the expression of hIL-10 and the stage of B cell differentiation displayed by cell lines. Our results showed that vIL-10 mRNA was detected only during productive EBV replication and not during latency (either type I or type III). Neither of the two BL tumor biopsy specimens expressed hIL-10, including the EBV-positive biopsy Jude2. In contrast, 7/8 LCLs and 9/11 BL lines tested, including gl lines expressed hIL-10. The expression of hIL-10 correlated with differentiation state. Hence, hIL-10 was expressed by B cells within a discrete period of B cell differentiation which was represented by most of the B cells tested. B cell lines which were either more or less differentiated did not express detectable hIL-10 mRNA.

Northern blotting was used to analyze IL-10 expression for several reasons. First, it afforded a rapid and sensitive method of assessing expression not only in cell lines but also in freshly isolated biopsy material. Second, although it is not as sensitive as polymerase chain reaction-based methods, Northern blotting is semiquantitative and is not prone to the same false negative and false positive problems. Finally, Northern analysis obviates low signal or false negative problems associated with ELISA assays which are caused by autocrine or juxtacrine use of the secreted product or sequestration by soluble receptor.

The vIL-10 gene had been shown to be expressed only during the productive phase of EBV replication in B95-8 cells which display type III latency (Hudson *et al.*, 1985; Stewart and Rooney, 1992). The comparison of mRNA from gl BL cells before and after induction showed that the vIL-10 gene is regulated in a similar manner in cells which display type I latency. It would be predicted, therefore, that vIL-10 is not involved during latency *in vivo* but only during periods of viral replication and spread.

Expression of hIL-10 by LCLs and BL lines was reported by other groups while this work was in progress (Vieira *et al.*, 1991; Benjamin *et al.*, 1992; Burdin *et al.*, 1993). Our results confirmed these data and extended the observations to include BL biopsy samples as well as BL lines which were of a defined gl phenotype and are thus thought not to have differentiated *in vitro* (e.g., Akata, Mutu gl, Eli, and Odhi). The gl lines are thought to represent BL lines growing *in vivo*. However, all the gl lines expressed hIL-10, whereas neither BL biopsy did. These results are in agreement with Emilie *et al.*, (1993) who showed, although their probe did not clearly differentiate between genes, that neither vIL-10 nor hIL-10 were transcribed in non-AIDS-associated BL biopsy specimens. This suggests that even gl lines, upon outgrowth *in vitro,* may have undergone an activation or differentiation event which activated hIL-10 transcription. Since hIL-10 is a B cell growth factor (Rousset *et al.*, 1992), it is likely that cells expressing hIL-10 would have a selective advantage for growth *in vitro.* The numbers of BL biopsies studied was too small to draw any general conclusions from; however, our results do indicate that hIL-10 is not a requirement for the development of all cases of BL *in vivo,* but may be important for their outgrowth *in vitro.*

What factors influence the transcription of hIL-10 in B cells? A number of EBV-positive cell types (Kloek/B95, three Namalwa sublines and Jude2 BL biopsy) did not express detectable levels of hIL-10 mRNA and two EBV-negative lines (BL2 and BL30) were hIL-10 positive. Thus, hIL-10 expression was not dependent upon the presence of EBV in the lines tested as had been suggested previously (Burdin *et al.*, 1993). In addition, the comparison of separate clones of Mutu BL did not reveal any upregulation of hIL-10 expression in type III latency as compared with type I latency. Thus, the regulation of this gene is unlike that of some B cell activation antigens, such as CD23, whose expression is specifically increased by EBV proteins (e.g., EBNA2 and LMP) in type III latency (Wang *et al.*, 1987, 1988, 1990; Gregory *et al.*, 1990). Further, the lack of detectable hIL-10 expression by BL41, Ramos and Kloek/B95 could not be explained by deletion of the locus. The expression of hIL-10 did, however, correlate with the differentiation state of B cell lines. The least differentiated lines (Namalwa CSN/70, IPN/45, PNT, BL41, and Ramos) and the most differentiated line (Kloek/B95) examined did not express hIL-10, whereas the intermediate lines did. Our results are in agreement with Burdin *et al.*, (1993), who found that, in contrast to the majority of mature B cell lines (e.g., BL and LCLs), early B cell lines and terminally differentiated B cell (plasmacytoid) lines did not express hIL-10. These observations, taken together with ours, suggest that hIL-10 is expressed during a discrete period in B cell differentiation corresponding to mature and/or activated B cells. The functional significance of this observation is, however, hard to assess and awaits the results of further experiments, in particular with normal B cells.

After initial infection and replication, EBV becomes latent in B cells and becomes clinically apparent only in tumors such as BL and lymphomas in immunosuppressed individuals. Both hIL-10 and vIL-10 have potent effects on the survival, growth, and differentiation of human B cells (Defrance *et al.,* 1992; Rousset *et al.,* 1992; Burdin *et al.,* 1993). What influence do these cytokines have on EBV persistence and pathogenesis? The pattern of expression of vIL-10 suggests that it might aid the dissemination of the virus in the lymphoid compartment after initial replication at the oropharyngeal epithelium by enhancing the survival and growth of infected B cells. Also, while vIL-10 may be involved in virus replication and spread prior to BL tumor initiation it is not necessary for the continued growth of tumor cells either *in vivo* or *in vitro.* Both our expression data and that of Emille *et al.* (1993) would suggest that hIL-10, on the other hand, may be involved in the proliferation of at least a proportion of EBV-associated tumors and the outgrowth of both tumor cells and EBV-transformed B cells *in vitro.* Both hIL-10 and vIL-10 might therefore have important roles the EBV life cycle and their continued study may provide valuable insight into the mechanisms of EBV persistence and tumorigenesis.

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