A novel function for the Epstein–Barr virus transcription factor EB1/Zta: induction of transcription of the hIL-10 gene

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INTRODUCTION

Epstein–Barr virus (EBV) is a human gammaherpesvirus that infects, with lifelong persistence, almost all the adult population. The primary infection is in general silent but might be clinically revealed by a benign disease called infectious mononucleosis. After primary infection, the viral genome persists in peripheral resting memory B cells (Thorley-Lawson, 2001). The persistence of EBV-infected cells is likely to be a major risk factor for the emergence of EBV-associated pathologies such as Burkitt’s lymphoma (BL), Hodgkin’s disease (HD), nasopharyngeal carcinoma (NPC) and post-transplant lymphoproliferative disorders (PTLD) (Rickinson & Kieff, 1996). Although the contribution of EBV to the emergence of human cancers is not understood, it is probably linked to the potent transforming activity of the virus in vivo. Indeed, the EBV latent gene products LMP (LMP-1, LMP-2A) and EBNA (EBNA-2, EBNA-3A and EBNA-3C) activate anti-apoptotic and mitogenic pathways that induce proliferation of quiescent B cells in vitro (Kieff & Rickinson, 2001). EBV infection leads to secretion of cytokines, such as TNF-α, TNF-β, hIL-8 and hIL-10, that probably act as autocrine factors and modulate the immune response (Klein et al., 1996). Induction of IL-10 is also critical for the EBV biological cycle since the viral gene BCRF1 encodes a protein, designated vIL-10, which shows extensive homology to cellular IL-10 (Moore et al., 1990). vIL-10 is expressed late in the EBV productive cycle and exhibits many properties ascribed to human (h)IL-10 (de Waal Malefyt et al., 1991; Miyazaki et al., 1993; Swaminathan et al., 1993).

hIL-10 has been described as a potent immunosuppressive cytokine since it affects cell-mediated immune responses. hIL-10 is otherwise a potent growth and differentiation factor for B cells and also protects some B cells from apoptosis. In contrast to normal non-activated B cells, EBV-positive B cells produce hIL-10, which stimulates cell growth (Moore et al., 2001). Several observations link hIL-10 production and EBV infection. Analysis of nasopharyngeal carcinoma specimens showed that the number of cytotoxic T cells was significantly lower in hIL-10-positive than in hIL-10-negative tumours (Yao et al., 1997). hIL-10 abrogates the EBV-specific memory response defined as the ability of T cells to inhibit the growth of EBV-infected B-lymphocytes (Bejarano & Masucci, 1998). In HD cases, upregulation of hIL-10 expression was linked to detection of the EBV genome in tumour cells and was associated with a low number of cytotoxic T lymphocytes surrounding the tumour (Herbst et al., 1996; Ohshima et al., 1995). Finally, a significant increase in the circulating hIL-10 level has been observed in vivo before EBV-associated post-transplant lymphomas and non-Hodgkin’s lymphoma diagnosis (Birkeland et al., 1999; Cortes & Kurzrock, 1997). All these studies suggest that hIL-10 may play a role in EBV-related neoplastic diseases.

Interleukin-10 (IL-10) plays a critical role in Epstein–Barr virus (EBV) biology. Indeed, the EBV genome contains a gene (BCRF1) with homology to the human IL-10 (hIL-10) gene. In addition to viral IL-10, which is secreted late in the productive cycle, hIL-10 production is also induced in B cells infected by EBV. The EBV protein LMP-1 and the viral small non-polyadenylated RNAs (EBERs) expressed during latency are involved in hIL-10 induction. In this study, we show that in B cells the viral transcription factor EB1, which is the main inducer of the EBV productive cycle, also activates transcription of the hIL-10 gene and secretion of the hIL-10 protein. Accordingly, EB1 bound directly to specific DNA sequences in the hIL-10 minimal promoter. Moreover, specific disruption of EB1 binding to some of these sites impaired EB1-mediated activation of transcription at the hIL-10 promoter in a transient expression assay. Therefore, an increase in IL-10 production occurs during latency and early and late during the productive cycle. This production of IL-10 might favour the survival of EBV-infected cells in vivo and/or create a microenvironment required for efficient de novo infection of B lymphocytes by EBV virions.
EBV-associated induction of hIL-10 production occurs at the transcriptional level. The viral gene products that induce hIL-10 expression have been characterized. The LMP-1 protein and the ubiquitously expressed EBV-encoded small non-polyadenylated RNAs, EBER1 and EBER2, both induce hIL-10 expression, through the p38/SAPK2 pathway and in a PKR-independent way respectively (Kitagawa et al., 2000; Nakagomi et al., 1994; Vockerodt et al., 2001). In addition, hIL-10 expression is induced on activation of the EBV lytic cycle in an EBV-carrying B-cell line by mechanisms that have still to be defined (Sairenji et al., 1998).

The productive cycle is initiated by the expression of a viral transcription factor, originally named EB1 (Chevallier-Greco et al., 1986), and later called Zta, BZLF1 or ZEBRA (Countryman et al., 1987; Lieberman et al., 1990). EB1 is a bZIP-like protein (basic Zipper) that displays sequence homology with proteins of the AP-1 family (Farrell et al., 1990), and those encoding fos (Cayrol & Flemington, 1995), TKT (Lu et al., 2000) and MMP-9 (Yoshizaki et al., 1995). EB1 activates the transcription of viral genes (Kieff & Rickinson, 2001) and Ori lyt-dependent viral DNA replication (Schepers et al., 1993) through binding to specific DNA sequences called Z-Responsive Elements (ZRE) (for references see Speck et al., 1997). EB1 also activates the transcription of cellular genes such as c-fos (Flemington & Speck, 1990), and those encoding TGF-β (Carrol & Flemington, 1995), TKT (Lu et al., 2000) and MMP-9 (Yoshizaki et al., 1999).

In this study, we show that EB1 activates endogenous hIL-10 transcription and secretion in an EBV-negative B cell line. We further demonstrate in transient transfection assays that the viral protein activates transcription at the hIL-10 promoter, linked to the luciferase reporter gene. We have localized EB1-binding sites in the hIL-10 minimal promoter by DMS interference. Mutation of these sites confirmed that EB1 directly activates transcription at the hIL-10 promoter.

**METHODS**

**Plasmid construction.** The hIL-10 promoter DNA fragment placed upstream of the luciferase gene was kindly provided by D. Kube (Göttingen, Germany). Reporter hIL-10(−315/+27) contained an hIL-10 promoter DNA fragment encompassing the putative transcriptional start site (+1) cloned into the pGL2-basic vector (Kube et al., 1995). We further introduced the following mutations in the hIL-10(−315/+27) promoter fragment: deletion mutant ΔM1 was obtained by PCR, simultaneously deleting the −216/−170 region and introducing a XhoI restriction site. Mutants M2, M3 and M4 were generated using the Quickchange Multi Site-Directed Mutagenesis Kit (Stratagene).

The EB1 and EB1 mutant expression vectors have been described elsewhere (Manet et al., 1989; Giot et al., 1991). Briefly, plasmid pSG-Z41 contains a CDNA coding for EB1 placed under the control of the SV40 early promoter-enhancer. Alkaline position 185 was replaced by a lysine residue in the EB1 mutant Z311. Amino acids between positions 25 and 42 were deleted in the EB1 mutant A25-42 (see Fig. 3A).

**Cell lines and transfection.** The EBV-negative Burkitt’s lymphoma cell line DG75 was maintained in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS) and glutamine/penicillin/streptomycin (all from Invitrogen); 24 h before transfection cells were seeded in fresh media at a cell density of 7×10⁶ cells ml⁻¹. 1×10⁶ cells were resuspended in 250 μl of PBS and transfected using electroporation (220 V and 950 μF with a Bio-Rad Gene Pulser). Cells were grown for 48 h in RPMI with 10% FCS at 37°C, collected by centrifugation and lysed using 100 μl of the reporter lysis buffer from the Luciferase Assay System (Promega).

**Detection of secreted hIL-10 by ELISA.** 1×10⁷ cells (DG75) were transfected as described above with the EB1 expression vector pSG-Z41, and resuspended in a final volume of 5 ml of complete medium. At different times following transfection, 200 μl of the culture medium was collected. The hIL-10 content of the supernatants was measured by ELISA as described elsewhere (Badou et al., 2000). The anti-hIL-10 monoclonal antibody MAB217 (R&D Systems) was used as the coating antibody and the biotinylated anti-hIL-10 polyclonal antibody BAF217 (R&D Systems) was used as the detection antibody. Absorbance was read at 490 nm. hIL-10 production was quantified from a standard curve generated by using various concentrations of recombinant human hIL-10 (R&D Systems). In our assay, the limit of detection of hIL-10 was about 30–50 pg ml⁻¹.

**Western blot analysis.** Cells were collected after transfection, washed with cold PBS and incubated with 100 μl of lysis buffer (0-05 M Tris/HCl, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0-05% bromophenol blue). Equal amounts of protein were separated by SDS-PAGE and transferred onto a nitrocellulose membrane (Amersham). The membrane was then incubated with the EB1 monoclonal antibody Z12S5 (Mikaël et al., 1993) and further incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulins (Amersham). The proteins were visualized with the ECL kit (Amersham).

**Electrophoretic mobility shift assay (EMSA).** The DNA probes used were either obtained by Ddel digestion of the (−371/+33) hIL-10 promoter fragment (see Fig. 4A) or were synthetic oligonucleotides of length 20 bp (see Fig. 5A). Double-stranded oligonucleotides were 5'-end labelled with [γ-32P]ATP (Amersham) using polynucleotide kinase (Invitrogen). Labelled probes were purified by electrophoresis in a 6% polyacrylamide gel. EB1-His-tagged was purified using Ni²⁺-NTA agarose (Qiagen) according to the manufacturer’s recommendations. 2 μl of EB1-His-tagged was incubated with 2×10⁶ c.p.m. of each labelled oligonucleotide. Incubations were for 20 min at 4°C, in a volume of 20 μl containing 20 mM Tris-HCl (pH 7-9), 1 mM MgCl₂, 0-5 mM DTT, 15% glycerol, 100 mM KCl, 0-2 μg poly(dI:dC) and 5 μg BSA. The EB1–DNA complexes were separated from the non-complexed DNA by gel electrophoresis and were visualized by autoradiography as previously described (Giot et al., 1991). In competition experiments, the non-labelled double-stranded oligonucleotide ZRE, carrying two EB1-binding sites (5’-TATGCATAGCCACAGCATTGCTAATGTAGTA-3’) was used as a specific competitor at a 100-fold molar excess (the EB1-binding sites are underlined).

**Dimethyl sulfate (DMS) interference.** Single-stranded synthetic DNA oligonucleotides of length 20 bp were 5’-end labelled with [γ-32P]ATP and annealed to the complementary unlabelled strand. Labelled probes were purified by gel electrophoresis. 8×10⁶ c.p.m. of the DNA probes was partially methylated with 1 μl of DMS (Merck) for 3 min at 18°C. The methylated probe was then incubated with 2 μl of purified EB1-His protein. After EMSA (binding conditions are described above), the retarded DNA probe (B) and the non-retarded DNA probe (F) were electroeluted and incubated in 100 μl of 1 M piperidine (Merck) for 30 min at 90°C to cleave...
the methylated guanine residues. An equal amount of the cleaved radioactive B and F probes was analysed on 20% polyacrylamide sequencing gels.

**RT-PCR**

**Total RNA.** This was extracted from $1 \times 10^7$ cells and purified by CsCl centrifugation as described (Shaw et al., 1985). Purified RNA was resuspended in RNase-free water.

**First-strand cDNA synthesis.** RNA was denatured for 10 min at 70°C and then chilled on ice. First-strand cDNA synthesis was performed from 5 µg of total RNA at 42°C for 50 min in a final volume of 20 µl: 5 µl of denatured RNA, 4:5 µl of 5× buffer, 2 µl 0:1 M DTT, 1 µl 10 mM dNTP mix (Invitrogen), 1 µl of 1 µg oligo(dT) (Biolabs) ml$^{-1}$, 1 µl of Superscript II (200 units µl$^{-1}$) (Invitrogen). Tubes were then heated for 15 min at 70°C.

**Cytokine cDNA amplification.** PCR mixture (23 µl) was added to 2 µl of first-strand cDNA. PCR mixture contained 2:5 µl of 10× buffer (100 mM Tris/HCl, 15 mM MgCl$_2$, 500 mM KCl, pH 8:3), 0:5 µl of 25 mM MgCl$_2$, 0:5 µl of 10 mM dNTP mix, 18-25 µl of sterile water, 0:5 µl of each primer (20 µM) and 0:25 µl of Taq polymerase (5 U µl$^{-1}$) (Roche). The cDNA was amplified with a Perkin-Elmer thermal cycler for 30 cycles. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. The following oligonucleotides were used: hIL-10, 5′-CTGAGAACCAAGACCCAGACATCAAGG-3′ and 5′-CAATAA-GTTTCTCAAGGGGCTGGGTC-3′; β-actin, 5′-GCTGCGTGTTGGCTCCCGAGGAG-3′ and 5′-ATCTTCATTGTGTGGTGGTGCCAG-3′.

**RESULTS**

**Induction of hIL-10 gene expression by EB1 in vitro**

Activation of the EBV productive cycle in B cells is shortly followed by activation of hIL-10 gene transcription (Sairenji et al., 1998). Since induction of the EBV productive cycle is EB1-dependent, we hypothesized that EB1 activates hIL-10 gene transcription. To test such a hypothesis, we examined the endogenous transcription of the hIL-10 gene following transfection of an EBV-negative Burkitt's lymphoma cell line, DG75, with an EB1 expression vector. As shown in Fig. 1(A), in the absence of EB1 no hIL-10 gene expression was detectable (lane 1). However, an RT-PCR-amplified cDNA was detected in DG75 cells transfected with 10, 15 and 20 µg of the EB1 expression vector respectively. In parallel, EB1 expression was evaluated by Western blotting using an EB1-specific monoclonal antibody (Fig. 1A, lower panel). hIL-10 mRNA expression was also followed at different times after transfection of the EB1 expression vector (Fig. 1B). hIL-10 mRNA was detected 5 and 8 h after transfection (Fig. 1B, lanes 3 and 5). Together, these results suggested that EB1 might directly induce transcription of the hIL-10 gene. We next determined if the EB1-induced hIL-10 transcription was followed by an increase in hIL-10 secretion. As shown in Fig. 1(C), an increase in secreted hIL-10 was clearly detected by ELISA at 10 h post-transfection. However (but as expected from the transcription analysis described above), the increased secretion of hIL-10 was only seen when DG75 cells were transfected with 10 µg of pSG-Z41. Our results strongly suggest that EB1 induces expression of the hIL-10 gene, and this is seen both at the mRNA level and at the secreted hIL-10 protein level.

![Fig. 1](http://vir.sgmjournals.org)
EB1 induces transcription at the hIL-10 promoter in transient expression assays

To further understand the EB1-mediated activation of hIL-10 gene transcription, we examined the ability of the viral protein to induce expression of a reporter gene placed under the control of different hIL-10 promoter fragments. Among the reporter plasmids tested, hIL-10(−315/+27), carrying the hIL-10 minimal promoter, was found to be sufficient for EB1-induced activation of luciferase expression (data not shown). Moreover, luciferase expression from reporter hIL-10(−315/+27) increased with increasing amounts of EB1 expression vector transfected (Fig. 2, lanes 1–5). The above results strongly suggest that EB1 directly activates transcription from the hIL-10 promoter.

Both EB1 activation and DNA-binding domains are essential for EB1 activation of transcription at the hIL-10 promoter

To investigate further the EB1-induced activation of hIL-10 gene expression, various EB1 mutants were compared for their ability to activate transcription at the hIL-10 gene promoter in reporter assays. Three domains [activation (AD), DNA-binding (DBD) and dimerization (Di)] have been identified in the EB1 transcription factor (Fig. 3A). Mutant Δ25–42 carries a deletion of 17 amino acids inactivating the transcriptional activation function. Mutant Z311 has a single amino-acid change (mutation A185K) in the EB1 basic domain and does not bind detectably to DNA in vitro (Giot et al., 1991). As shown in Fig. 3(B), both the activation domain (lane 2) and the DNA-binding domain (lane 3) were essential for efficient EB1 activation of transcription at the hIL-10 minimum promoter. The expression level of each mutant was comparable as determined by Western blotting (data not shown).

EB1 binds to the hIL-10 promoter in vitro

In order to locate EB1-binding sites, several DNA fragments of the hIL-10 minimal promoter were used in EMSAs together with a purified EB1-His-tagged protein (Fig. 4A). As shown in Fig. 4(B), EB1 bound only to DNA fragments III and VI (lanes 8 and 17). On these two fragments more than one retarded complex was visualized, suggesting that these fragments contained more than one EB1-binding site. A 100-fold molar excess of an oligonucleotide harbouring

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Fig. 2. EB1-induced activation of transcription at the hIL-10 minimal promoter. A luciferase reporter plasmid carrying the hIL-10(−315/+27) promoter fragment was transfected into DG75 cells along with increasing amounts of the EB1 expression vector pSG-Z41 (1, 5, 10, 15 and 20 μg). Luciferase values are relative to the maximum activation induced by EB1, arbitrarily considered as 100 (a 12-fold activation of luciferase production at 20 μg of EB1 expression plasmid as compared to transfection of the empty vector was observed), and are the means of several independent transfections. Error bars indicate standard deviations.

Fig. 3. EB1-mediated activation of hIL-10 transcription in DG75 requires both the EB1-activation and the DNA-binding domains. (A) Schematic representation of the EB1 domains carrying the transcription activation function (AD), the DNA-binding function (DBD) and the homodimerization function (Di). Δ25–42 is a mutant from which amino acids 25 to 42 were deleted. Z311 is a single amino acid substitution mutant in the DBD (underlined). (B) DG75 cells were cotransfected with the luciferase reporter plasmid hIL-10(−315/+27) and the wtEB1 or the EB1 mutant expression plasmids. Luciferase values are relative to the activation induced by wild-type EB1, arbitrarily considered as 100 (a 10-fold activation in luciferase activity by wtEB1 was observed), and are the means of several independent transfections. Error bars indicate standard deviations.

Fig. 4. EB1 binding to the hIL-10 promoter. (A) EMSA using the hIL-10 promoter fragments III and VI and a purified EB1-His-tagged protein. (B) EMSA showing the binding of EB1 to the hIL-10 promoter fragments III and VI.
two functional EB1-specific binding sites (ZRE) (Fig. 4B, lanes 19, 20 and 21), efficiently competed with EB1 binding on probe III and VI (Fig. 4B, lanes 9 and 18). To further locate the EB1-binding sites on hIL-10 promoter regions III and VI, synthetic oligonucleotides covering these regions were used in an EMSA (Fig. 5A). As shown in Fig. 5(B), EB1 interacted with probe E (lane 1) but very weakly with probe A (lane 2). Accordingly, EB1 interacted with probes B and C (lanes 4 and 6), but weakly with probe D (lane 8), suggesting that at least one EB1-binding site was present in region III. As shown in Fig. 5(C), among the oligonucleotides tested encompassing region VI, oligos J, L and G interacted with purified EB1 (lanes 14, 16 and 18 respectively), whereas a very weak binding of EB1 was seen on oligos F and M (lanes 10 and 24 respectively). These results suggested that there are several binding sites for EB1 in the hIL-10 minimal promoter.

**EB1 binds to at least five sites in the hIL-10 minimum promoter**

Since the purified EB1-His protein bound to oligonucleotides B, C, D, G, J and L in vitro, we characterized precisely the EB1-binding sites in these oligonucleotides by DMS interference (Fig. 6A, B). For oligos B, C and G (Fig. 6A and 6B respectively), methylation of the guanine residues only partly impaired binding of EB1, indicating that probably more than one EB1-binding site was present on the probes. For oligos J and L (Fig. 6B), methylation of one or two guanine residues completely impaired binding of EB1, indicating that the residues were important for the *in vitro* interaction. The results of the DMS interference experiments are summarized in Fig. 6(C) and the putative EB1-binding sites in the hIL-10 minimum promoter are listed and aligned together with several known functional EB1-binding sites. Methylation interference did not allow to define the EB1–DNA contacts on oligos D, F, K and M (data not shown), probably due to very weak binding of EB1 to these DNA probes. Indeed, in oligonucleotides F, M and K, the EB1-binding sites are at the extremity of the double stranded oligonucleotides, and in oligonucleotides F and K, the EB1-binding site is also partially deleted (Fig. 6C). Taken together these data showed that at least five EB1-binding sites are present in the hIL-10 minimum promoter.
Fig. 5. Localization of EB1-binding sites in the hIL-10 minimal promoter. (A) Schematic representation of DNA probes used in the EMSA. Numbers delineate the position of the ends of the DNA probes relative to the putative transcription start site labelled +1. (B, C) The 32P-labelled probes, indicated over the panels, were incubated with (+) or without (−) purified EB1-His protein. The EB1–DNA complexes (B) and the non-complexed DNA (F) are indicated.
Some, but not all, characterized EB1-binding sites are essential for EB1-mediated hIL-10 gene activation of transcription

To estimate the contribution of the various identified EB1-binding sites in EB1-mediated induction of luciferase expression from the hIL-10 promoter, deletions or mutations of the EB1 sites were tested (Fig. 7A). Deletion of EB1-binding sites located between positions −216 and −170 (mutant AM1; Fig. 7A) was silent (data not shown). Among the other mutations tested, those affecting individually site 3 (mutant M3) or site 4 (mutant M4) significantly reduced EB1-induced activation of transcription (Fig. 7B, lanes 2 and 3). Simultaneous mutations of sites 2 and 3 (mutant M2 + M3, lane 4) did not increase the effect of mutation M3 alone, confirming that site 2 was not functional in vivo, even though EB1 bound to site 2 in vitro (Fig. 5C, lane 18). Combined mutations M2 + M3 + M4 further reduced transcriptional activation by EB1 (Fig. 7B, lane 5), demonstrating that the EB1-induced transcription from the hIL-10 minimal promoter was mediated by proximal EB1-binding sites.

**DISCUSSION**

In this report, we have shown that the viral transcription factor EB1, encoded by the BZLF1 gene, is an activator of...
hIL-10 gene transcription and of hIL-10 secretion in EBV-negative Burkitt’s lymphoma cells, suggesting that no other EBV gene product was required. The essential hIL-10 gene promoter region active in EBV-infected B cells was found to be located in the 227 bp upstream of the transcription start site (Kube et al., 1995). Interestingly, we found that nearly the same hIL-10 promoter region contained at least five EB1-binding sites. No consensus AP-1 sites have been localized in this region (Eskdale et al., 1997), suggesting that the EB1-responsive sites we mapped are specific for EB1. After extensive mutagenesis, we found that the sites distal to the hIL-10 gene putative transcription start site were dispensable for EB1-induced activation of transcription in transient expression assays. However, according to previous reports on EBV gene promoters (Lieberman et al., 1990; Urier et al., 1989), EB1-induced activation was mediated essentially through the sites proximal to the putative transcription start site.

Our findings extend the notion that hIL-10 production is activated at different stages of and essential to the EBV infectious cycle. Indeed, hIL-10 is upregulated in latently infected B cells and the viral gene products responsible for this induction are the transmembrane protein LMP-1 (Nakagomi et al., 1994; Vockerodt et al., 2001) and the EBV-encoded RNAs (EBERs) (Kitagawa et al., 2000). vIL-10, a viral homologue of hIL-10 encoded by the BCRF1 gene, is also expressed late in the productive cycle (Moore et al., 1990) and shares a subset of hIL-10 biological activities (Moore et al., 2001). In addition, we show here that hIL-10 expression was activated by the inducer of the viral productive cycle, EB1, extending considerably previous results demonstrating that hIL-10 production was detected on induction of EBV reactivation (Sairenji et al., 1998).

On induction of the EBV productive cycle in latently infected B cells, viral gene products are expressed as a result of the transcriptional activity of the EB1 and R proteins. The viral gene EBERs are transcribed during productive infection and the BNLF-1 gene, encoding the LMP-1 protein, is also still expressed (Kieff & Rickinson, 2001). It has been reported that the BNLF-1 gene encodes a truncated form of LMP-1, called lytic LMP-1 (Hudson et al., 1985), and it has been demonstrated that latent LMP-1 signalling is impaired by lyLMP-1 protein (Erickson & Martin, 2000). EB1 also interferes with latent LMP-1 signalling since it was

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**Fig. 7.** EB1-induced activation of transcription at the hIL-10 minimal promoter is mediated by three EB1-binding sites. (A) EB1-binding sites characterized in the hIL-10 minimal promoter are underlined. ΔM1 (open rectangle under the sequence) is a deletion mutant. M2, M3 and M4 DNA sequences were mutated as indicated under the sequence. (B) Reporter plasmids were cotransfected into DG75 cells with an EB1 expression vector. Luciferase values are relative to EB1-induced activation of wild-type hIL-10 minimal promoter, arbitrarily considered as 100 (a 8-fold activation of luciferase activity was observed), and are the means of several independent transfections. Error bars indicate standard deviations.
recently shown that EB1 completely inhibits the upregulation of MHC class I expression mediated by LMP1 (Keating et al., 2002). As there is no evidence suggesting that LMP-1 and EBERs are still inducing hIL-10 expression during the productive cycle, one may speculate that EB1-induced hIL-10 expression reflects a selection pressure that imposed the persistence of hIL-10 expression.

We also hypothesize that, at the time of virus reactivation, hIL-10 expression might create a favourable microenvironment for de novo infection of B cells through three different mechanisms: (i) When the productive cycle is initiated, many EBV lytic gene products allow CTL recognition and lysis of the infected cell (Rickinson & Moss, 1997). As described above, EB1 has been associated with a strong decrease of MHC class I molecules at the B cell surface (Keating et al., 2002). We suggest that EB1-induced hIL-10 confers a broader effect on MHC molecules and thereby on cell types, strongly impairing the cell-mediated immune responses. Indeed, hIL-10 is known to inhibit expression of MHC class II on monocytes (de Waal Malefyt et al., 1991) and to downregulate MHC class I presentation of antigens (Zeidler et al., 1997). In this way, hIL-10 suppresses T lymphocyte activities through downregulation of antigen presentation and through direct inhibition of T cell proliferation (de Waal Malefyt et al., 1991, 1993). (ii) hIL-10 is also known to downregulate IFN-γ production by blood mononuclear cells, by acting on T-helper 1 lymphocytes, monocytes and presumably on dendritic cells in response to stimulation by virus (Moore et al., 2001; Payvandi et al., 1998). This biological activity of hIL-10 could be crucial at the time of EBV lytic gene activation of transcription, since it inhibits the host antiviral response and thus protects the infected cell from lysis induced by specific cytotoxic T lymphocytes. (iii) Finally, as hIL-10 is a potent growth factor for B cells (Rousset et al., 1992), it is reasonable to hypothesize that it might increase the pool of new target cells in vivo through a paracrine pathway.

There is increasing evidence suggesting that EB1 interferes with cellular signalling pathways. Indeed, EB1 is thought to modulate local immune responses as it induces production of the potent immunosuppressive cytokine TGF-β (Cayrol & Flemington, 1995) and inhibits the IFN-γ signalling pathway (Morrison et al., 2001). EB1 also causes dispersal of nuclear PML bodies, which are presumably involved in the regulation of MHC class I antigen presentation (Adamson & Kenney, 2001). In addition to these previous reports, we have documented a novel function of the viral EB1 protein, induction of hIL-10 gene transcription. Our results, together with those published by others, confirm that IL-10 plays a central role in EBV biology. However, the operation of this role in vivo is not yet clearly understood.

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