Synergistic Regulation of the Human Interleukin-12 p40 Promoter by NFκB and Ets Transcription Factors in Epstein-Barr Virus-transformed B Cells and Macrophages*

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Monocytes/macrophages produce interleukin-12 (IL-12) in response to pathogenic stimulation, whereas most Epstein-Barr virus-transformed (EBV+) B cells constitutively secrete IL-12. The molecular mechanism regulating the constitutive IL-12 gene expression in EBV+ B cells has not been addressed. In this study, using the EBV+ B cell line RPMI-8866, we localized to the human IL-12 p40 promoter two essential cis elements, the NFκB site and the Ets site. The NFκB site was shown to interact with members of the NFκB family: p50 and c-Rel. The Ets site constitutively bound a multi-component Ets-2-containing complex. While the NFκB and Ets sites appear equally critical for inducible p40 promoter activity in macrophage cell lines, NFκB plays a more dominant role in the constitutive p40 promoter activity in EBV+ B cells. Transient expression of Ets-2 and c-Rel in B, T, and monocytic cell lines synergistically activated the IL-12 p40 promoter, apparently overcoming the requirement for cell type- or stimulant-specific transcription factors. These data provide new evidence that full activation of the human IL-12 p40 promoter may result primarily from the interplay between NFκB and Ets family members.

Interleukin 12 (IL-12), a heterodimeric cytokine composed of two disulfide-linked subunits of 35 (p35) and 40 (p40) kDa, was originally identified in the supernatant fluid of Epstein-Barr virus-transformed (EBV+) human B-cell lines (BCL) (1, 2). IL-12 exerts multiple effects, including: (a) induction of cytokine production, particularly interferon-γ (IFN-γ) by T and NK cells, (b) induction of proliferation in T and NK cells and enhancement of their cytotoxic activity, and (c) induction of T helper-type 1 (Th1) responses and inhibition of Th2 responses (3). The finding that IL-12 p40 knockout mice have a severely depressed Th1 response (4) supports the role of IL-12 in Th1 cell differentiation.

IL-12 is produced by phagocytic cells and other antigen-presenting cells in response to bacteria, bacterial products, intracellular pathogens, and viruses (1, 2, 5–11). Normal B cells appear to be relatively poor producers of IL-12 (6). Most EBV+ BCL and EBV+ lymphomas produce IL-12, with the highest levels observed in EBV+ BCL derived from AIDS-associated lymphomas (9, 12). SCID mice injected with human lymphocytes from EBV-seropositive donors developed EBV+ human B cell lymphoma secreting in vivo high levels of human IL-12, suggesting that a similar production of IL-12 during initial proliferation of EBV+ B cell in patients may affect the reactivity of their immune cells against the infected cells. Although any extrapolation to the in vivo situation from data observed with established cell lines should be taken with caution, it is tempting to speculate that, in healthy individuals, IL-12-producing EBV+ cells would be easily rejected by immune response and thus only cells that have lost the ability to produce IL-12 would be able to give rise to Burkitt’s lymphomas; in immunodeficient AIDS patients, these protective mechanisms would be inefficient and their IL-12-producing EBV-transformed cells could give rise to lymphomas in a relatively high proportion of patients.

The key role of IL-12 in inflammation and in the immune response, and the importance of this cytokine in anti-tumor resistance, have raised considerable interest in the mechanisms of IL-12 gene transcription. We (13) have shown previously that, in lipopolysaccharide (LPS)- and IFN-γ-treated human monocytes, the expression of IL-12 p40 and p35 is primarily regulated at the transcriptional level. In a luciferase reporter gene construct, a 3300-bp genomic DNA fragment including the upstream sequences of the human p40 gene largely recapitulates its cell type specificity and transcriptional regulation (13). The cis elements in the p40 promoter region responsible for the inducible activation of the genes have been studied extensively in phagocytic cells, but no information is available for the elements controlling the constitutive expression of the gene in EBV+ BCL. We have previously localized a DNA sequence spanning nucleotides −292 to −196 (relative to the transcription start site) responsible for induced promoter activity (13). The core element at position −212 binds a series of IFN-γ- and LPS-induced nuclear proteins (termed the F1 complex) including Ets-2, IFN-γ-regulatory factor-1 (IRF-1), c-Rel, and Ets-related factors (14). A downstream NFκB site between bp −117 and −107 was also characterized as an LPS response element in the murine macrophage J774 cell line (15).

Recently, Plevy et al. (16) identified a third cis element located at −96 and −88 (downstream of the NFκB site) of the murine IL-12 p40 promoter, also conserved in humans, that binds members of the C/EBP family of transcription factors in activated murine macrophage cell line. The C/EBP element exhibits functional synergy with the upstream NFκB site, although no physical interactions between C/EBP and Rel proteins were observed (16). Together, these results suggest that in myeloid...
cells a complex interplay exists between multiple inducible transcription factors contributing to the regulation of IL-12 p40 gene activation.

In this study, we focused upon the role of cis-acting regulatory elements in the transcriptional activation of the human IL-12 p40 gene in EBV+ BCL. In these cells, we showed constitutive binding of multiple transcription factors to the NFκB and Ets sites of the p40 promoter. We found that cotransfection of NFκB and Ets-2 transcription factors strongly and specifically synergized in the induction of p40 promoter activity in EBV+ BCL as well as in both inducible IL-12-producing macrophage cell lines and IL-12-nonproducing EBV+ T cell lines.

EXPERIMENTAL PROCEDURES

**Cell Lines and Reagents**—Human EBV-transformed B lymphoblastoid cell line (EBV+ RPMI-8866, PA682BM-2, and AS283A (9), EBV+ Burkitt’s lymphoma cell line BJAB (a gift from E. Kieff, Harvard Medical School, Boston, MA), T cell line Jurkat, and murine macrophage cell line RAW264.7 were all grown in RPMI 1640 medium (Mediatech, Herndon, VA) supplemented with 10% fetal calf serum (Irvine Scientific, Santa Ana, CA). All tissue culture media and supplements were endotoxin-free. All polyclonal antibodies used in supershift experiments were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Recombinant murine (mIFN-γ) was a gift from Dr. G. Garotta (Human Genome Sciences, Inc., Rockville, MD). Anti-CD3 monoclonal antibody (OKT3, ascites) was produced from cells obtained from America Type Culture Collection (ATCC, Rockville, MD). LPS from *Escherichia coli* and 12-O-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma.

**Nuclear Run-on Assay**—Untreated RPMI-8866 cells, TPA-treated BJAB cells (50 ng/ml, 4 h), and TPA- and anti-CD3-treated Jurkat cells (OKT3, ascites 1:1000, 4 h) were harvested and washed with phosphate-buffered saline (BioWhittaker, Walkersville, MD). Isolation of nuclei and in vitro transcription in the presence of [32P]UTP (3000 Ci/mmol, DuPont) were performed as described (17, 18).

**Plasmids**—A 3300-bp fragment of the human IL-12 p40 promoter was cloned into the luciferase reporter construct pX2 (13) at the PstI site. All deletion mutant constructs were generated by PCR and fully sequenced for verification as described previously (13). NFκB expression vectors were a gift from Dr. K. M. Murphy (Washington University School of Medicine, St. Louis, MO). The CMV-based murine Ets-2 expression vector was a gift from Dr. R. Maki. The TNF-α promoter was obtained from a TNF cosmids library (ATCC, catalog no. 57590) by PCR amplification of a 1294-bp fragment. The sequence of the 5′ primer used is 5′-TCTCTGAAATGTGCTGTTGTTG-3′ and the 3′ primer used is 5′-CCCTTTAGGCTGCTCTG-3′. The PCR product was cloned into pX2 expression vector. All plasmids were banded twice in cesium chloride and verified by restriction mapping.

**Transient Transfection**—DNA transfection experiments were performed by electroporation as described (13). Briefly, 0.4 ml of cell suspension was mixed with 60 μg of DNA (10 μg of p40-luc/3.3 kb plus 3 μg of pCMV-β-galactosidase (transfection efficiency control) and 47 μg of BS/KSII+) and electroporated in 0.45-cm electroporation cuvettes (Gene Pulser, Bio-Rad) at 960 microfarads and 250 V for BJAB and Jurkat cells, 300 V for RPMI-8866 cells, and 350 V for RAW264.7 cells. Cells were harvested 24 h post-transfection, pelleted by centrifugation, and resuspended in 100 μl of lysis buffer (Promega, Madison, WI). Lysates were used for both luciferase and β-galactosidase assay (13).

Where indicated, RAW264.7 cells were treated with 1.2% Me2SO for 24 h and with mIFN-γ (1000 units/ml) for 8 h, stimulated with LPS (1 μg/ml) for 8 h, and harvested.

For cotransfection studies, pRSV-β-galactosidase was used instead of pCMV-β-galactosidase, and the molar ratio between the reporter plasmid (luciferase p40 promoter constructs) and expression vector was 1:2.5 for parental murine B cells and 1:1 for the Ets-2.

Luciferase activity was corrected for transfection efficiency by normalizing to the measured β-galactosidase value.

**Gel Electrophoretic Mobility Shift Assay (EMSA)**—Nuclear extracts were isolated from RPMI-8866 as described (19) and from RAW264.7 as described (20). EMSA and supershift were performed as described previously (14).

**Oligonucleotides Used for EMSA**—Oligonucleotides (292–196, −243/−196, and −222/−196 have been described (14). Oligonucleotide −135/−99 was used as a probe in the following sequence (plus strand): 5′-AAACAAAAAGAGACTTCTGGAAATTTCCCRCAAGAGG-3′ and encompasses the potential PU.1 and NFκB sites (underlined) of the hu-

![Fig. 1. IL-12 p40 and p35 gene transcription in EBV+ BCL, EBV- BCL, and T cell line.](image)

**RESULTS**

**Constitutive Transcription of the Human IL-12 p40 Gene in EBV-transformed B Cells**—Based on our previous observation that EBV+ BCL constitutively produce IL-12 protein (1, 6, 9), we tested whether the p40 and p35 genes were also constitutively transcribed in nuclear run-on assays performed with unstimulated EBV+ BCL (Fig. 1A). Nascent p40 transcripts were constitutively present in RPMI-8866 (Fig. 1A) and in all other EBV+ BCL tested (PA682BM-2 and AS283A), and expression was not further enhanced by TPA treatment (data not shown). Jurkat T cells and BJAB EBV+ BCL, which do not express p40 mRNA or protein, were used as negative controls (Fig. 1A). Transcription of the IL-12 p40 gene, unlike that of the
TNF-α gene, was not detectable in unstimulated cells or in the EBV^2 BCL or T cell lines stimulated with TPA alone or TPA in combination with α-CD3, respectively. However, TNF-α transcription was shown to be further up-regulated by TPA treatment of all cells examined. Nascent p35 transcripts were present in all cell lines and under all conditions tested. Thus, the IL-12 p40 gene appears to be constitutively transcribed in EBV^1 but not in EBV^2 BCL or T cells. We then analyzed the IL-12 p40 promoter activity in the same EBV^1, EBV^2 and T cell lines using a full-length promoter construct linked to a luciferase reporter gene (−2330/−1108) in a transient transfection assay. The promoter construct was active in EBV^1 BCL, but poorly or not active at all in EBV^2 and T cell lines (Fig. 1B), confirming that the construct contains adequate sequence information for appropriate cell type-specific expression (13).

**Important cis-Regulatory Elements Located in the −222/−1108 bp Region of the IL-12 p40 Promoter**—To delineate the cis-acting elements regulating the constitutive IL-12 p40 expression in EBV^+ B cells, we first examined the activity of the full-length promoter (−3300/−1108) and a series of nested 5' deletion constructs (Fig. 2) in EBV^+ BCL. Luciferase activity of the −3300/−1108 construct was 65-fold that of the parental, promoterless pXP2 control vector (Fig. 3). Promoter activity was progressively decreased when the full-length promoter was deleted from the 5' end at position −471 (32-fold over pXP2 control vector), −292 (38-fold), −265 (28-fold), and −243 (29-fold). Surprisingly, the activity of −222 bp plasmid was only slightly reduced compared with the full-length promoter. An additional 100-bp deletion to −122 abolished half of the full-length promoter activity. Truncation of the promoter to −28 bp (containing the TATA box) markedly diminished the constitutive promoter expression to a level only 10-fold greater than the promoterless control plasmid pXP2. These data suggest that the region between nucleotide positions −222 and −28 is most critical for constitutive promoter activity in the EBV^+ BCL.

**Functional Role of the NFκB and Ets Sites**—Since the −222/−1108 promoter region was shown to exhibit significant promoter activity, we analyzed the activity of this construct in further detail. To evaluate the functional role of the putative NFκB half-site at −117 and the Ets site at −212, we prepared a series of additional promoters with defined mutations. The −222NFκBm and −222ets-D constructs have a substitution of the core −109CC−108 with −109GG−108. The −222NFκBm construct has a substitution of the core −109CC−108 with −109GG−108. The −222ets-D construct has a 5-bp deletion that eliminates the −211TTTCC−207 sequence (13). The same mutations were made in the context of the −3300/−1108 full-length promoter and were designated respectively, −3300NFκBm and −3300ets-D. Analysis of the activity of these constructs in transiently transfected EBV^+ BCL (RPMI-8866) (Fig. 4A) revealed a dramatic decrease in the
luciferase activity of promoter constructs mutated at the NFκB site (−3300NFκBm and −222NFκBm) (80–88% less than their corresponding wild-type constructs), but only a 50% decrease for the Ets-2 deletion mutants. In contrast, analysis of these constructs in the macrophage cell line RAW264.7 (Fig. 4B) demonstrated that the mutations of both the NFκB and Ets sites almost completely abolished the inducibility of the p40 promoter by IFN-γ and LPS (reduced to 12–20% of the −3300/−108 wild-type promoter activity), the latter result being consistent with our previous finding that the Ets site plays an important role in this system (13). Together, these results suggest that the B cells may use the same cis elements and transcription factors as do macrophages, but the relative contribution of each factor may vary.

FIG. 4. Effect of NFκB and Ets-2 core mutations on constitutive and inducible promoter activity of the IL-12 p40 gene. A, the EBV⁺ BCL (RPMI-8866) was transiently transfected with the indicated luciferase plasmid. Luciferase activity was measured in the cell lysate, and values were normalized against the internal control (CMV-β-galactosidase construct) and expressed as percentage of the activity of the full-length promoter (control), −3300/−108. Results are mean (± S.E.) from four independent experiments. B, macrophage RAW264.7 cells were transfected with the indicated luciferase plasmid. At 16 h after transfection, cells were treated with 1.2% Me₂SO for 24 h and with rmIFN-γ (1000 U/ml) for 12 h before the addition of LPS (1 μg/ml, 8 h). Luciferase reporter gene activity, corrected for transfection efficiency, is expressed as percentage of the activity of the IFN-γ plus LPS-treated full-length promoter (control), −3300/+108. Results are mean (± S.E.) from six independent experiments.

FIG. 5. Binding of nuclear protein extracts from EBV⁺ cell RPMI-8866 to the −135/−99 region of the IL-12 p40 promoter. Nuclear extracts (5 μg) from EBV⁺ BCL, and RPMI-8866 were incubated with the −135/−99 probe and analyzed by EMSA. A, the indicated competitor DNAs (oligonucleotides) were mixed at 40-fold molar excess with the probe. B, before addition of the probe, nuclear extract was mixed with affinity-purified polyclonal antibodies directed against the indicated transcription factors. Arrows identify complexes detected in each panel.
Characterization of the DNA-Protein Complexes Associated with the NFκB Site—To establish the relationship between promoter activity and specific DNA-protein interactions at the NFκB site (−135/−99) in EBV BCL, EMSA were performed using a 37-mer oligonucleotide probe (−135/−99) encompassing the putative NFκB and PU.1 sites. Three DNA-protein complexes were observed via EMSA (Fig. 5A); two of them indicate specific binding to nuclear proteins since they were competed by an excess of the unlabeled oligonucleotide corresponding to the probe, but not by an unrelated (NS) oligonucleotide competitor. Competition of the slower mobility complex was observed using a 25-mer oligo (−123/−99) containing the NFκB site, but not the putative upstream PU.1 site located at −127 of the promoter. Use of an oligonucleotide with recognized binding activity for members of the NFκB/Rel family (22) as competitor resulted in complete disappearance of the top band, indicating that this DNA-protein complex likely involves members of the NFκB/Rel family. Further characterization of the NFκB protein members bound to the −135 to −99 promoter region (Fig. 5B) revealed a slowly migrating supershifted complex in the presence of NFκB anti-p50 and anti-c-Rel antibodies. The appearance of this supershifted band was accompanied by a partial disappearance of the upper complex. Thus, p50 and c-Rel-containing complexes constitutively bind to a transcriptionally active 6B site in the IL-12 p40 gene in EBV BCL. Anti-PU.1 antibody decreased the intensity of the faster-migrating band, suggesting the presence of PU.1 in this complex. No supershifted bands were detected with anti-c-Fos used as a control.

We then examined the ability of nuclear extract from IFN-γ- and LPS-treated RAW264.7 macrophages to bind the probe (−292/−99). Nuclear extracts from EBV BCL (RPMI-8866) and from IFN-γ- and LPS-stimulated macrophages (RAW264.7 γ+L) were mixed with the −292/−196 probe and analyzed by EMSA. A, competitive EMSA was performed either in the absence (none) or presence of a 50 or 100 molar excess of the indicated oligomers. B, supershift EMSA was performed with the indicated affinity-purified polyclonal antibodies. C, recognition binding activity for members of the NFκB/Rel family (22) as competitor resulted in complete disappearance of the top band, indicating that this DNA-protein complex likely involves members of the NFκB/Rel family. Further characterization of the NFκB protein members bound to the −135 to −99 promoter region (Fig. 5B) revealed a slowly migrating supershifted complex in the presence of NFκB anti-p50 and anti-c-Rel antibodies. The appearance of this supershifted band was accompanied by a partial disappearance of the upper complex. Thus, p50 and c-Rel-containing complexes constitutively bind to a transcriptionally active 6B site in the IL-12 p40 gene in EBV BCL. Anti-PU.1 antibody decreased the intensity of the faster-migrating band, suggesting the presence of PU.1 in this complex. No supershifted bands were detected with anti-c-Fos used as a control.

We then examined the ability of nuclear extract from IFN-γ- and LPS- treated RAW264.7 macrophages to bind the probe (−135/−99). In unstimulated cells, a series of three rapidly migrating complexes were observed (group III, Fig. 6A). However, only the slowest migrating complex of the three was consistently seen, suggesting that the faster migrating complexes may be proteolytic products of the upper complex. IFN-γ treatment did not yield any additional binding, in contrast to an earlier report that IFN-γ treatment of the murine macrophage...
misleading and should be corrected as follows:

**Figure 8.** Rel proteins synergize with Ets-2 in mediating activation of the p40 promoter in EBV⁺ BCL. A, EBV⁺ BCL, and RPMI-8866 were transiently cotransfected with different combinations of expression vectors for RelA (p65), c-Rel, and Ets-2 and the luciferase-based promoter (−3300/+108). B, cotransfection was performed with the Ets-2 and c-Rel expression vectors and either the wild-type or the mutated −3300/+108 constructs (−3300NFkBm and −3300ets2Δ). After 24 h, cells were assayed for luciferase expression. Luciferase counts were normalized using β-galactosidase activity determined in the same cell extract (normalized luciferase counts). The empty expression vectors for all transcription factors were tested and produced background levels of luciferase activity (data not shown). Results are mean (± S.E.) from three independent experiments.
the presence of additional noncanonical NFκB or Ets-2 sites, or a cooperativity with other endogenous transcription factors. Thus, the synergy between co-transfected NFκB and Ets-2 transcription factors in IL-12 p40 gene regulation is observed regardless of whether the endogenous gene is expressed in different cell types. This synergy is not observed in macrophage cells transfected with a luciferase construct under the control of the TNF-α promoter (Fig. 9B), also known to contain functional NFκB and Ets sites (23–25). In addition, overexpression of the two transcription factors failed to transactivate the thymidine kinase minimal promoter, lacking NFκB and Ets sites (data not shown). Taken together, these results demonstrate that the functional synergy between NFκB and Ets-2 is promoter-specific.

**DISCUSSION**

The present study was performed to delineate the molecular mechanisms by which EBV transformation of B lymphocytes results in the activation of IL-12 gene expression. We demonstrate that two regulatory elements, Ets at −212/−207 and NFκB at −117/-107, play a role in the high level expression of the human IL-12 p40 promoter in EBV+ BCL and in its inducible expression in macrophages. In EBV+ BCL, activity of a 3300-bp p40 promoter parallels transcription of the endogenous p40 gene, whereas no promoter activity was detectable in EBV− BCL or in Jurkat T cells in which the endogenous p40 gene is inactive. In EBV+ BCL, a segment of the p40 promoter containing 222 bp upstream and 108 bp downstream of the transcription start site (−222/−108) retains most of the activity of the full-length promoter, indicating that constitutive transcription from the p40 promoter is regulated by transcription factors acting on this region. This basal promoter activity is dependent mostly on the integrity of the NFκB site (−117/−107), as demonstrated by mutation analysis. A DNA probe encompassing this site binds the p50 and c-Rel NFκB family members, consistent with previous reports that NFκB proteins in mature B cell lines are constitutively present in the nucleus (26–28).

Similar experiments on activated macrophages revealed that the NFκB site, bound by p50/RelA, p50/c-Rel, and RelA/c-Rel heterodimers, is also critical for p40 promoter activity. When this site is mutated, macrophage cells completely lose their ability to activate the p40 promoter after IFN-γ and LPS stimulation. The data provided here are corroborated by the studies of Murphy et al. (15) on the murine p40 promoter in activated macrophages. Our studies indicate that NFκB transcription factors are essential for constitutive IL-12 p40 gene expression in EBV+ BCL and for p40 gene inducibility in macrophage cells. However, in both B and macrophage cells, p50, c-Rel, and RelA individually are unable to transactivate the IL-12 p40 promoter, suggesting the requirement for additional signals. Ets-2 appeared to be a likely candidate as the IL-12 p40 promoter contains a functional Ets motif, TTTCCG or AGGAAA (−212 to −207) essential for promoter activity in stimulated macrophage cells (13). Complete activation of the IL-12 p40 promoter in Ets-2 transiently transfected macrophages has been shown to require IFN-γ or LPS stimulation (13), suggesting that, although vital, Ets-2 alone is not sufficient to achieve optimal p40 promoter activation. Here, we clearly demonstrate, in both B and unstimulated macrophage cells, that Ets-2 exerts a strong enhancing effect on p40 promoter activity only in the presence of the NFκB c-Rel. Ets-2 and c-Rel are sufficient for potent transactivation of the p40 promoter even in T and EBV− BCL (data not shown), where the p40 promoter is normally inactive. The specificity of this response is demonstrated by mutation or deletion of the NFκB or Ets binding sites resulting in greatly diminished promoter activation. Cooperativity between these transcription factors seems to be specific for the IL-12 p40 promoter, since no synergistic activation was observed for the TNF-α promoter, also known to contain NFκB and Ets sites (23–25).

Physical interactions between NFκB and Ets-like protein have been reported to regulate expression of the IL-2 receptor α gene (29) and the activation of the human immunodeficiency virus enhancer (30). The Ets domain was shown to be necessary and sufficient to mediate this interaction, suggesting that the highly conserved Ets domain may act broadly to facilitate multiple protein-protein interactions. Indeed, it is now well established that Ets family proteins can activate transcription in conjunction with other proteins (31–35). Here, we have shown an association between Ets-2 and c-Rel in the F1a complex in EBV+ BCL similar to the F1 complex of activated macrophages (14). We are currently investigating whether the F1a and NFκB complexes physically interact at the p40 promoter.
The composition of the F1a complex differs from the previously characterized F1 of activated macrophages (14), in that the former contains the IRF-2 protein but not IRF-1. IRF-1-deficient mice fail to produce IL-12, resulting in a severely compromised Th1 immune response *in vivo* and *in vitro* (36, 37) strongly implicating a role for IRF-1 in IL-12 activation. IRF-1 and IRF-2 are highly homologous and bind to common DNA sequence elements (38, 39) with similar affinities. As a consequence of mutating the Ets site, we demonstrated here, in EBV+ BCL, only a partial reduction in the constitutive p40 promoter activity. In contrast, mutation of the same site in macrophages resulted in total abrogation of inducible p40 promoter activity. In other promoters, such as that of IFN-β, it has been shown that IRF-2 antagonizes the activating function of IRF-1 (40). Therefore, the possibility that IRF-2 binding to the Ets site in EBV+ BCL may function as a repressor of IRF-1 activity by preventing its binding; thus, decreasing the relative participation of this site in the constitutive activation of the p40 promoter in EBV+ BCL should be considered. However, the ability of IRF-2 to act as a suppressor is by no means absolute, as both IRF-1 and IRF-2 have been shown to up-regulate expression of two genes, the histone H4 gene F0108 and the EBNA 1 gene (41, 42).

We provided evidence of a pivotal role of the NFκB site in constitutive IL-12 p40 gene expression in EBV+ BCL and a functional cooperativity between NFκB and Ets-2 transcription factors, critical for the constitutive and inducible activation of the IL-12 p40 gene. Parallel to our findings, it was recently demonstrated that a synergy exists between C/EBP and NFκB in human and mouse p40 promoter regulation (16), but whether C/EBP plays a role in constitutive p40 gene expression in EBV+ BCL remains to be determined. Together, this information suggests that the expression of IL-12 p40 gene requires the coordinated interactions between multiple regulatory proteins. The B cell lines used in our studies expressed the EBV latent-infection membrane protein 1 (LMP1) capable of activating the NFκB transcription factors (43–46). However, transient or stable transfection of LMP1 in EBV- B cells was insufficient to transactivate the p40 promoter. Thus, this suggests that it is likely that EBV employs more elaborate mechanisms stimulating multiple transcription factors critical for the activation of the IL-12 p40 gene.

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