Regulation of the Human Interleukin-5 Promoter by Ets Transcription Factors

ETS1 AND ETS2, BUT NOT ELF-1, COOPERATE WITH GATA3 AND HTLV-I TAX1

(Received for publication, October 6, 1998, and in revised form, February 18, 1999)

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The cytokine interleukin-5 (IL-5) activates eosinophiles (1, 2) and basophiles (3, 4) and seems to be responsible for the development of eosinophilia associated with a number of diseases, including asthma and HTLV-I transformed adult T-cell leukemia (5–9). Primarily, IL-5 is synthesized by Th2 cells, a subset of T helper cells, following stimulation with antigens or mitogens (10, 11). Regulation of IL-5 expression is likely to involve calcium and protein kinase C-dependent signaling pathways, as a calcium ionophore and phorbol ester synergistically increase IL-5 expression and IL-5 promoter activity (12, 13). Activation of Th2 cells is accompanied by increased activities of phorbol ester responsive AP1 and calcium responsive NFAT as well as of GATA3 (14). All three transcription factors have been found to be able to activate the IL-5 promoter (15–18), suggesting that they may play an important role in controlling IL-5 expression. In retrovirally transformed adult T-cell leukemia cells, the viral protein Tax, may contribute to the production of IL-5 (17). Tax, has been shown to deregulate a variety of cellular promoters (for a review, see Ref. 19) by interacting with various transcription factors, such as CREB, NF-κB, SRF, NF-Y, and Ets1 (20–29), and with basal factors, such as TBP, TFIIA, and TAF1B (28–30).

Ets proteins are transcription factors that share a unique DNA-binding domain, the Ets domain, allowing these proteins to interact specifically with GGA(A/T)-based recognition sites (for a review, see Ref. 33). Ets transcription factors have been found to play a crucial role in controlling transcription of a variety of genes involved in important cellular processes, such as proliferation or differentiation. They have also been shown to contribute to the development of certain human diseases (for review, see Ref. 34). Some Ets proteins, such as Ets1, Elf-1, and Fli-1, are primarily expressed in T-cells where they fulfill important functions, e.g. Ets1 has been reported to play a crucial role in T-cell survival (35, 36).

The IL-5 promoter shares with other cytokine promoters, such as the granulocyte macrophage-colony stimulating factor promoter, the so-called conserved lymphokine element (CLE0) (37). It is a composite AP1/Ets element, which supports activation of the granulocyte macrophage-colony stimulating factor promoter by Ets1 in an AP1/NF-κB dependent manner (37–39). This raises the question of whether Ets proteins may also be able to regulate the IL-5 promoter. In order to address this possibility, we performed transient transfection studies with Jurkat T-cells and the myeloid Kasumi cell line. We found that Ets1 and Ets2, but not Elf-1, were able to activate the IL-5 promoter in Jurkat cells in the presence of either PMA plus ionomycin or PMA plus Tax1. Using Kasumi cells, we could show that Ets1 and Ets2 needed to cooperate with GATA3 for their stimulatory activities on the IL-5 promoter. This raises the question of whether Ets proteins may also be able to regulate the IL-5 promoter.

Materials and Methods

Cell Lines and Plasmids—Jurkat T-cells and myeloid Kasumi cells were maintained in RPMI medium supplemented with 10% fetal calf serum in the absence of any antibiotic.

Plasmids pKCR3-c-ets1, ΔEtsBeta1 ΔΔ243–330, pCTax, pCMS-Tax, and pCM7-Tax for expression of p54NF-κB, p46NF-κB, WT-Tax1, M5-Tax1, or M7-Tax1, respectively, are described elsewhere (40–43). The plasmids ΔEβets2 and ΔEβelf1 for expression of Ets2 or Elf-1, respectively, were
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Fig. 1. Ets1 is able to trans-activate the human IL-5 promoter in PMA/ionomycin or Tax-, treated Jurkat cells. A-C, Jurkat cells were transiently transfected with 5 µg of the −494/+44 IL-5 promoter/luciferase plasmid in the presence or absence of 6 µg of pKCR3-c-ets1 and/or 4 µg of pCTax and treated with PMA and/or ionomycin (or left untreated, control), as indicated. Seven hours after transfection, cell extracts were assayed for luciferase activity (RLU, relative light units) as described under “Materials and Methods.” Bars represent the average luciferase activity calculated from four independent experiments. In panel C, WT, M5, and M7 denote wild-type, D22A/C23S, and C29A/P30S mutant Tax, proteins, respectively. D-G, Western blot analyses of extracts from Jurkat cells treated as indicated. Blots were probed with an antibody either directed to Ets1 (D and E) or Tax (E-G). In panel E, I, II, and III denote bands, which likely correspond to degradation products of Ets1. The asterisk indicates a nonspecific band.

generous gifts from Jacques Ghysdael. The plasmid RSVhG3 for expression of human GATA3 was kindly provided by James D. Engel. For construction of the −494/+44 IL-5 promoter/luciferase plasmid (pIL5P.luc), the IL-5 promoter fragment was first amplified by polymerase chain reaction using the primers as described previously (13), followed by cloning this fragment into a luciferase-containing vector (pKCR3-c-ets1). As shown in Fig. 1, Jurkat cells were transiently transfected with the luciferase reporter plasmid (pIL5P.luc) and incubation on ice for 15 min, cells were lysed by addition of 120 µl of Nonidet P-40 followed by vortexing for 10 s. The nuclei were pelleted by centrifugation at 13,000 rpm for 30 s at room temperature and extracted by addition of buffer C (20 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The nuclear lysate was cleared by centrifugation at 13,000 rpm for 5 min at 4 °C and stored at −80 °C.

Western Blot Analysis—Western blot analysis of Jurkat cell lysates was carried out as described previously (29). Rabbit anti-Ets1 (C-20), anti-Ets1/2 (C-275), or anti-Elf-1 (C-20) serum, all provided by Santa Cruz Biotechnology, or mouse anti-Tax, was diluted to 1:7500, 1:1000, 1:4000, or 1:1300, respectively, prior to use. Anti-IgG horseradish peroxidase and ECL reagents were obtained from Amersham Corp.

EMSA—Ets1 baculovirus extract (47) was mixed with 400 pg of Klenow α-32P-labeled IL-5 WT (sense strand: 5'-GGTTAGAAATTACTTTCTCAGACG-3') bearing the IL-5 specific sequence from position −106 to −82. For creating the G to A mutation at position −44 of the antisense strand, we polymerase chain reaction amplified an approximately 500-base pair long fragment containing the IL-5 promoter fragment from −81 to +44 and part of the luciferase gene by using the following primers: 5'-GGGACTCCCTCCTTGATTGTTAGAAATTATTCCAG-3' and 5'-AATGGAGAGAATTTTCATTCTCAGACG-3' (the mutated nucleotide is underlined). The fragment was cloned into the polymerase chain reaction 2.1 vector (Invitrogen) and, after cutting with XbaI and BamHI, inserted into the −106/+44 IL-5 luciferase construct creating the −106/+44 EM IL-5 promoter luciferase plasmid.

Transient Transfection and Luciferase Assay—Jurkat and Kasumi cells were transiently transfected with 5 µg of an IL-5 luciferase construct by electroporation using a Bio-Rad gene pulser under conditions, as described previously (45). For expression of Ets1, Ets2, or Elf-1, 6 µg of the corresponding expression plasmid was added to the transfection mixture, while for expression of Tax, 4 µg of pCTax or 2 µg of RSVhG3, respectively, was used. One hour after transfection either PMA (final concentration: 10 ng/ml) and/or ionomycin (final concentration: 2 µM), both dissolved in dimethyl sulfoxide, or dimethyl sulfoxide (control) was added to the cells. After another 6 h cells were harvested. Cell lysates were assayed for luciferase activity according to Ref. 46.

Preparation of Nuclear Extracts—Nuclear extracts from Jurkat cells were prepared essentially as described (28). Briefly, cells were harvested and washed in phosphate-buffered saline. After resuspension in 1 ml of buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) and incubation on ice for 15 min, cells were lysed by addition of 120 µl of Nonidet P-40 followed by vortexing for 10 s. The nuclei were pelleted by centrifugation at 13,000 rpm for 30 s at room temperature and extracted by addition of buffer C (20 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The nuclear lysate was cleared by centrifugation at 13,000 rpm for 5 min at 4 °C and stored at −80 °C.

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EMSA—Ets1 baculovirus extract (47) was mixed with 400 pg of Klenow α-32P-labeled IL-5 WT (sense strand: 5'-GGTTAGAAATTACTTTCTCAGACG-3'), corresponding to the IL-5 promoter sequence from nucleotides at position −63 to −37, in the presence of 2.5% CHAPS (48), 1 mM Tris, pH 7.5, 3 mM Hepes, pH 7.9, 70 mM NaCl, 0.25 mM EDTA, 0.15 mM EGTA, and 0.01% bovine serum albumin, and incubated for 5 min at room temperature. For competition experiments, an IL-5 specific oligonucleotide carrying a C to A mutation at −44 (IL-5 EM) or unlabeled IL-5 WT was used in a 100-fold molar excess over the probe. Electrophoresis was carried out as described (49).

RESULTS

Ets1 Transactivates the Human IL-5 Promoter in Jurkat T-cells in the Presence of PMA Plus Ionomycin or PMA Plus Tax—We first studied the ability of Ets1 to regulate the activity of the human IL-5 promoter. Jurkat T-cells were transiently transfected with the luciferase reporter plasmid pIL5P.luc containing a −494/+44 fragment of the IL-5 promoter either in the presence or absence of an expression plasmid coding for p54Ets (pKCR3-c-ets1). As shown in Fig. 1A, Ets1 was able to increase IL-5 promoter activity approximately
3-fold, when cells were simultaneously treated with PMA and ionomycin. Withdrawal of either one or both of these agents prevented activation of the IL-5 promoter by Ets1. In addition to ionomycin, HTLV-I Tax, was able to act in concert with PMA to stimulate Ets1 activity (Fig. 1B). For this stimulatory effect on Ets1, Tax, had to be active, as two inactive Tax, mutants, M5 and M7 Tax, (43), failed to support trans-activation of the IL-5 promoter by Ets1 (Fig. 1C). At the same time, wild-type and mutant Tax, were expressed at the same level (Fig. 1F).

Importantly, p54ets2 expression was not altered by any of these treatments (Fig. 1, D and E). Note, however, that ionomycin affected the production of three smaller proteins (I-III) that could be recognized by the Ets1 antibody (Fig. 1E). These proteins probably resulted from an exon VII domain-dependent calcium-triggered degradation of p54ets2 (50–53), a process that Tax, apparently could partially prevent (Fig. 1E, compare lanes 2 and 4 with 3 and 5). When we repeated the experiments with the natural ΔVII Ets1 mutant protein p46ets2, we found that exon VII indeed was required for the production of proteins I-III, but was dispensable for Ets1 activation of the IL-5 promoter (data not shown). This demonstrates that proteins I-III were not important for the Ets1 effect on the IL-5 promoter. Collectively, these data show that certain conditions were required to allow Ets1 activation of the IL-5 promoter in Jurkat cells.

Tax, was able to increase IL-5 promoter activity 20-fold, when cells were treated with either PMA alone or PMA plus ionomycin (Fig. 1B). In the presence of PMA alone, a cooperative effect of Ets1 with Tax, was observed. Western blot analysis showed that PMA had a strong up-regulating effect on cytomegalovirus-promoter driven Tax, expression (Fig. 1G) suggesting that PMA was mainly required to obtain Tax, protein levels sufficient for trans-activation.

The GGAA Motif within the Cle0 Element Is Required for Activation of the Human IL-5 Promoter by Ets1 and Tax,—The −494/+4 IL-5 promoter fragment contains eight GGA(A/T) core motifs (E), all of which could potentially interact with Ets1 (Fig. 2A). To analyze the importance of the Cle0 GGAA motif for Ets1-mediated activation, we first removed the sequence between −494 and −106 (Fig. 2A). This deletion altered neither Ets1- nor Tax, dependent activation (Fig. 2C). This suggests that the seven GGA(A/T) motifs upstream of nucleotide position −106 were dispensable for stimulation of promoter activity by these proteins. However, compared with the −494/+4 IL-5 promoter, the −106/+4 promoter fragment had a reduced ability to respond to PMA plus ionomycin. This is likely due to the fact that the ionomycin-responsive NFAT site at −116 is missing in the smaller promoter construct (Fig. 2A).

On the other hand, removal of the NFAT site did not impair the ability of the IL-5 promoter to respond to Ets1 in a PMA/ionomycin-dependent manner. This suggests that ionomycin had two different effects on the IL-5 promoter: One was required for activation of the promoter by Ets1, while the other was likely to be mediated by activation of NFAT.

We next mutated the GGAA motif of the Cle0 element to AGAA (Fig. 2B), thereby, creating the Ets-binding mutant −106/+4 EM IL-5 promoter (Fig. 2A). As shown in Fig. 2D, this mutation completely abrogated the ability of Ets1 to trans-activate the IL-5 promoter.

To demonstrate that Ets1 can specifically interact with the
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Nuclear extracts from Jurkat cells were analyzed for expression of Ets1, Ets2, or Elf-1 by the Western blot technique. A comparison of the Ets1 levels in the nuclei of Jurkat cells treated either with PMA (P), ionomycin (I), PMA/ionomycin (P/I), or with dimethyl sulfoxide (Co) for 5 h. B, in addition to Ets1, significant levels of Ets2 and Elf-1 proteins were also detectable in Jurkat cell nuclear extracts. The Western blot was subsequently probed with an anti-Ets1, anti-Ets1/2, and anti-Elf1 antibody. Note that the antibody (α-Ets1/2) used for detection of Ets2 also recognized Ets1.

GGAA motif of the IL-5 Cle0 element, EMSAs were performed using a 32P-labeled oligonucleotide that corresponded to the IL-5 promoter sequence between -63 and -37. By using a p54gne-enriched baculovirus extract, we could show that Ets1 was able to bind to this probe (Fig. 2E, lane 2). The formation of the Ets1 complex could be prevented by the addition of unlabeled wild type -63/-37 oligonucleotide (IL-5 WT) (lane 3), but not by adding an Ets mutant version of this oligonucleotide (IL-5 EM) that contained a mutation at the first G of the GGAA motif (lane 4). These results show that Ets1 interacts with the IL-5 Cle0 element by contacting its GGAA motif. These data suggest that responsiveness of the IL-5 promoter to Ets1 relied on the Ets-binding site within the Cle0 element.

Ets2, but Not Elf-1, Is Able to Substitute for Ets1 in Trans-activating the IL-5 Promoter in the Presence of PMA/Ionomycin or PMA/Tax1—The G to A mutation of the Cle0 Ets-binding site not only inhibited the activation of the IL-5 promoter by Ets1, but also interfered with the ability of Tax1 to stimulate IL-5 promoter activity (Fig. 2D). This suggests that the stimulatory effect of Tax1 on the IL-5 promoter required an Ets protein already present in Jurkat T-cells. Full-length Ets1 and ΔVII Ets1 are endogenously expressed in Jurkat T-cells (Fig. 3A). Treatment with PMA or PMA plus ionomycin, which allowed Tax1 to trans-activate the IL-5 promoter, did not significantly change the expression levels of these Ets1 proteins relative to control conditions (Fig. 3A). Therefore, it is possible that Tax1 cooperated with Jurkat cell-derived Ets1, when no Ets1 had been added exogenously. Alternatively, a different Ets transcription factor, such as Ets2 or Elf-1, which are also expressed by Jurkat cells (Fig. 3B), could instead have mediated the effect of Tax1 on the IL-5 promoter. To study this possibility we tested the effects of ectopically expressed Ets2 and Elf-1 on the IL-5 promoter. We found that Ets2 was twice as potent as Ets1 in its ability to activate the IL-5 promoter in Jurkat cells, causing a 6–10-fold increase in promoter activity in the presence of PMA plus ionomycin (Fig. 4A) or PMA plus Tax1 (Fig. 4B), respectively. Interestingly, the cooperative effect of Ets2 with Tax1 was stronger than that of Ets1 with Tax1 (Fig. 4B). Ets2 also had a significant stimulatory effect on the promoter in the presence of PMA alone (Fig. 4A).

Elf-1, although able to activate the IL-5 promoter in cells treated with PMA, failed to stimulate IL-5 promoter activity in the presence of PMA plus ionomycin or PMA plus Tax1 (Fig. 4A). Interestingly, the PMA/Tax1-mediated activation was even inhibited by Elf-1 (Fig. 4B). These data show that, in addition to Ets1, Ets2 is able to activate the IL-5 promoter, while Elf-1 lacks this ability and rather seems to have the potential to repress Tax1-induced IL-5 promoter activity.

GATA3 Is Important for Ets1/Ets2-mediated Activation of the IL-5 Promoter—In the myeloid Kasumi cell line, Ets1 and Tax1 were found to be unable to activate the IL-5 promoter under the conditions that allowed these proteins to stimulate promoter activity in Jurkat cells (Fig. 5). This may suggest that a T-cell specific factor may have played a role in Tax1- and Ets1-mediated activation of the IL-5 promoter in Jurkat cells. A potential candidate is the T-cell specific factor GATA3, which is highly expressed in Jurkat T-cells (17, 54) and is able to activate the IL-5 promoter through a proximal GATA site (Fig. 2A), shown to be critical for IL-5 promoter activity (15). By Western blot analyses of unfractionated and fast protein liquid chromatography fractionated nuclear extracts, we confirmed that GATA3 is endogenously expressed in Jurkat cells (data not shown). To test the ability of GATA3 to rescue Ets1/2 and Tax1 stimulatory activity we analyzed the effects of Ets1/2, Elf-1, and Tax1 on the -494/+44 IL-5 promoter in myeloid Kasumi cells, when GATA3 was overexpressed. As shown in Fig. 5, overexpression of GATA3 had no effect on the IL-5 promoter (Fig. 5). However, when combined with Ets1 in the presence of PMA a 3-fold increase in promoter activity was observed. This suggests that Ets1 was able to cooperate with GATA3 to trans-activate the IL-5 promoter. Further addition of Tax1 or ionomycin strongly increased this effect resulting in a 14- or 11-fold induction of promoter activity, respectively. Neither Ets1 nor GATA3 alone was able to efficiently activate the IL-5 promoter under these conditions. This shows that Tax1 and ionomycin could only exert their effects on Ets1 and GATA3, when both of these transcription factors were present. It suggests that Tax1 and ionomycin strengthened the functional interaction between Ets1 and GATA3.
Similar to Ets1, Ets2 was unable to activate the IL-5 promoter in Kasumi cells, unless GATA3 was added (Fig. 5). However, the cooperative effect of Ets2 with GATA3 was stronger relative to that of Ets1 leading to an 11-fold induction of IL-5 promoter activity in the presence of PMA, as compared with a 3-fold stimulation by Ets1 under the same conditions. Addition of Tax1 or ionomycin further increased promoter activity 4- or 2-fold, respectively. As found with Jurkat cells, under optimum conditions, Ets2 was twice as effective as Ets1 in activating the IL-5 promoter. Interestingly, compared with Ets1, Ets2 also seemed to be the better partner for Tax1, as Ets2 could synergize with Tax1 even in the absence of GATA3. These data suggest that the interactions of Ets2 with GATA3 and Tax1 may be stronger than those of Ets1 with these proteins.

In the presence of PMA alone or PMA plus ionomycin, Elf-1 induced IL-5 promoter activity by approximately 2-fold, while it activated the promoter 3-fold in Kasumi cells treated with PMA plus Tax1. However, under any of these conditions, coexpression with GATA3 did not further increase promoter activity, suggesting that Elf-1 is unable to act in synergy with GATA3.

Similar data were obtained, when the experiments were repeated with the −106/+44 IL-5 promoter fragment (data not shown). However, when the Ets mutant 106/+44 EM promoter fragment was used instead, Ets1 and Ets2 failed to synergize with GATA3 and/or Tax1 (data not shown). These results show that the Cle0 Ets-binding site is essential for cooperative effects of Ets1 or Ets2 with GATA3 and/or Tax1.

**DISCUSSION**

We show here for the first time that in the presence of PMA, Ets1 and Ets2, but not Elf-1, are able to cooperate with GATA3 to synergistically activate the IL-5 promoter. Our results further suggest that Tax1 or ionomycin can enhance this Ets/GATA3 synergy. Based on these data, we propose that IL-5 expression is regulated by the concerted action of at least three transcription factors, Ets1/2, AP1, and GATA3, whose combined activities can be modulated by Tax1, or by a putative ionomycin-regulated cellular factor (Fig. 6). In this model, we suggest PMA to be needed for recruitment of AP1 to the Cle0 element. Both Ets1 and Ets2 are able to cooperate with AP1 to activate a variety of promoters and to mediate PMA-dependent activation (15, 39, 55–59). In Jurkat cells, PMA rapidly increases c-jun and c-fos steady-state mRNA levels, which is followed by an accumulation of c-Fos and c-Jun proteins reaching maximum levels after 6 h (60, 61). In addition, coexpression of c-Fos and c-Jun was shown to eliminate the requirement for PMA for Cle0-dependent Ets1 trans-activation of the granulocyte macrophage-colony stimulating factor promoter in Jurkat cells (39) suggesting again that PMA stimulates AP1 activity in Jurkat cells. Furthermore, in the presence of both Tax1 and PMA, c-Jun was demonstrated to be a strong activator of the human IL-5 promoter (17). Like PMA, Tax1 may have also activated AP1, as it can up-regulate c-fos and c-jun expression (61–64), activate the c-Jun N-terminal kinase-1 (65), and increase AP1 binding activity (66–68). In addition, Tax1 may have directly affected Ets1 activity by binding to Ets1 (29) and also by inhibiting exon VII-dependent degradation of Ets1 (Fig. 1E). Furthermore, Tax1 may have promoted Ets-AP1 and/or Ets-GATA3 interactions by forming ternary complexes with these proteins, as previously shown for Ets1 and Sp1 (29). Finally, Tax1 may have facilitated the interaction of Ets with CBP and p300 (69) as was shown for the binding of CREB to these co-factors (25, 70, 71).

Ionomycin may have mimicked Tax1 action by recruiting a factor (proposed factor X in Fig. 6) with a similar function as Tax1. Alternatively, ionomycin may have stimulated Ets-dependent activation of the IL-5 promoter by activating Ras (72, 73). Ras is known to super-activate Ets1 and Ets2 and to induce transcription through AP1/Ets composite elements (74–76). Activation of calcium has also been reported to activate CBP (77) and may, as proposed for Tax1, have promoted CBP-Ets interaction.

It is unlikely, however, that Tax1 and ionomycin exerted their stimulatory effects on Ets1 or Ets2 by activating NFAT (78–85), which can bind upstream of GATA3 (Fig. 2B). This is suggested by the fact that removal of the NFAT site at −116
(Fig. 2B) did not influence the Ets1 or Ets2 response of the IL-5 promoter. Nor did PMA, ionomycin or Tax1 seem to have an effect on the binding of Ets1 and Ets2 to the C10 element as suggested from EMSA data obtained with nuclear extracts from Jurkat cells (data not shown).

Although able to form a complex with AP1 on the C10 element (37), Elf-1 failed to cooperate with GATA3 in Kasumi cells and to activate the IL-5 promoter in Jurkat cells. Interestingly, Elf-1 (but not Ets1) was reported not to be able to activate the C10-regulated granulocyte-macrophage-colony stimulating factor promoter, whose activity is dependent upon NF-kB instead of a GATA factor, either (38, 39). It might suggest that Elf-1 may act as a repressor on C10-dependent transcription by competing with stimulatory Ets factors, such as Ets1 or Ets2, for binding to the C10 element. This notion is supported by the finding that Elf-1 inhibited activation of the IL-5 promoter by Tax1, which depended on the C10 Ets-binding site (Fig. 4).

Interestingly, as previously reported for Ets1 (29, 45), Ets2 seems to be even stronger than that of Ets1 with Tax1. Although adult T-cell leukemia cells have been reported to express Ets1 (86), the Ets1 expression level has been found to vary among HTLV-I transformed cell lines (87). For Tax1-dependent IL-5 expression in HTLV-I transformed cells and to activate the IL-5 promoter in Jurkat cells. Interestingly, as previously reported for Ets1 (29, 45), Ets2 may render Tax 1 less dependent upon the expression of a particular Ets protein regarding its ability to trans-activate Ets-regulated promoters.

Collectively, these data suggest an important role for Ets proteins, such as Ets1 and Ets2, in the regulation of IL-5 expression. Ets2 may be of particular importance in activated Th2 cells, as its expression is up-regulated upon antigenic stimulation of T-cells, while, at the same time, the Ets1 level declines (87). For Tax1-dependent IL-5 expression in HTLV-I transformed leukemic cells, Ets1 and Ets2 may be equally important.

Acknowledgment—We thank Jacques Ghysdael for providing plasmids ΔEBets2 and ΔEBetl-1 and Douglas Engel for RSV/hG3.

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