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*J Immunol* 2002; 168:5667-5674; doi: 10.4049/jimmunol.168.11.5667

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IFN Regulatory Factor 4 Participates in the Human T Cell Lymphotropic Virus Type I-Mediated Activation of the IL-15 Receptor α Promoter

Jennifer M. Mariner,* Yael Mamane, † John Hiscott, † Thomas A. Waldmann,* and Nazli Azimi2**

IL-15RAα mRNA and protein levels are increased in human T cell lymphotropic virus type I (HTLV-I)-associated adult T cell leukemia. Previously, we demonstrated that IL-15RAα expression was activated by HTLV-I Tax, in part, through the action of NF-κB. However, there appeared to be additional motifs within the IL-15RAα promoter that were responsive to HTLV-I Tax. In this study, we demonstrated that IL-15RAα mRNA expression was activated in human monocytes by IFN treatment, suggesting a role for IFN regulatory factors (IRFs) in IL-15RAα transcription. In addition, an IRF element within the Tax-responsive element of the IL-15RAα promoter was necessary for maximal Tax-induced activation of this promoter. Furthermore, we demonstrated that IRF-4, a transcription factor known to be elevated in HTLV-I-infected cells, activated the IL-15RAα promoter. Inhibition of IRF-4 action lead to reduced Tax-induced activation of the IL-15RAα promoter, while inhibition of both IRF-4 and NF-κB severely inhibited the Tax-induced activation of this promoter. These findings suggest a role for both NF-κB and IRF-4 in the transcriptional regulation of IL-15RAα by HTLV-I Tax. It is possible that the HTLV-I Tax-mediated induction of IL-15RAα and IL-15 may lead to an autocrine cytokine-mediated stimulatory loop leading to the proliferation of HTLV-I infected cells. This loop of proliferation may facilitate viral propagation and play a role in HTLV-I-mediated disease progression. The Journal of Immunology, 2002, 168: 5667–5674.

Interleukin-15 is a member of the 4α-helix bundle cytokine family that shares similar properties with IL-2 (1, 2). These functional similarities can be explained in part by the use of common receptors. IL-2 and IL-15 share the IL-2Rβ and the common γ receptor subunits (1–4), yet each cytokine has its own distinct receptor, namely IL-2Rα (5) and IL-15Rα (6), respectively. IL-2 and IL-15 use the β and common γ chains to initiate similar signal transduction pathways. This common signaling pathway contributes to the shared functions of IL-2 and IL-15 in both T and NK cells.

There are expression and functional differences between IL-2 and IL-15. IL-2 mRNA is largely restricted to lymphoid tissues, yet IL-15 has a widespread mRNA expression in many cells and tissues including T cells, B cells, liver, and skeletal muscle (7). IL-15 also has activities that are not shared with IL-2. For example, addition of IL-15 to a myoblast cell line affected skeletal muscle fiber hypertrophy, suggesting that IL-15 may be an anabolic agent that increases skeletal muscle mass (8). IL-15 also plays a major role in the development, survival, and activation of NK cells (9–12). IL-2 and IL-15 also have profoundly different effects on activation-induced cell death. IL-2 activates self-reactive T cell suicide and thus, plays a role in peripheral tolerance (13–15). IL-2 is also important in the inhibition of CD8+ memory T cell maintenance (16). In contrast, IL-15 has an anti-apoptotic effect on T and B cells (17), inhibits IL-2-induced activation-induced cell death (18), and is critical for the survival of CD8+ memory cells (16, 19). IL-15 also stimulates the proliferation of mast cells (20).

The effects of IL-15 can be explained in part not only by its widespread mRNA expression, but also in part by the expression of its distinct receptor IL-15RAα. IL-15RAα is a 58–60-kDa type I transmembrane protein that does not belong to the cytokine receptor family (6). IL-15RAα-mediated functions were demonstrated in IL-15RAα null (IL-15RAα−/−) mice (21). These mice are deficient in NK cells, CD8+ lymphocytes, and TCR-yδ intraepithelial lymphocytes. In addition, IL-15RAα knockout mice exhibit marked lymphopenia due to decreased homing of lymphocytes to peripheral lymph nodes. Furthermore, IL-15 knockout mice exhibited marked reductions in the number of thymic and peripheral NK cells, CD8+ lymphocytes, and populations of intraepithelial lymphocytes (22). These findings suggest that both IL-15 and its binding receptor are necessary for the development of NK and some T cells.

IL-15 has been shown to be elevated in a number of diseases including rheumatoid arthritis, inflammatory bowel disease, human T cell lymphotropic virus type-I (HTLV-I)-associated adult T cell leukemia (ATL) (23), and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (24). In addition, IL-15RAα

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Received for publication November 16, 2001. Accepted for publication March 29, 2002.

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This work was supported in part by a grant from the National Cancer Institute of Canada (to J.H.).

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0022-1767/02/$02.00

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3 Abbreviations used in this paper: HTLV-I, human T cell lymphotrophic virus type I; ATL, adult T cell leukemia; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; IRF, IFN regulatory factor; IRF-E, IRF element; LTR, long terminal repeat; cIRF-E, consensus IRF-E.
levels are also elevated in the T cells of ATL patients (25). Increased levels of IL-15 and IL-15Rα in HTLV-I-associated diseases are induced by the HTLV-I Tax protein. Tax is expressed from the pX sequence within the HTLV-I proviral genome (26) and is responsible for the transactivation of the HTLV-I long terminal repeat (LTR) (27). In addition, HTLV-I Tax activates a variety of cellular host genes including IL-2 (28, 29), IL-2Rα (29, 30), IL-15 (23), and IL-15Rα (25). Activation of cellular genes by HTLV-I Tax is mediated by a number of cis-acting DNA elements including CAMP responsive element (26), serum responsive elements (31), and NF-κB motifs (32). Many of these genes are ILs or growth factors that may aid the virus in its propagation.

HTLV-I infection also plays a role in the expression of IFN regulatory factor (IRF)-4. IRFs are activated upon viral infection or IFN activation and act as transcription factors (33). IRF-4 was originally cloned as ICSAT, an IFN consensus binding protein that was overexpressed in ATL cell lines (34). This finding suggested that IRF-4 was activated by HTLV-I infection. Additional studies revealed that IRF-4 mRNA expression was elevated in Jurkat cells following transient expression of the HTLV-I Tax protein (34), implying that Tax was involved in the transactivation of the IRF-4 gene. Furthermore, IRF-4 is constitutively expressed in HTLV-I-infected cell lines and the IRF-4 promoter is activated by Tax gene expression (35). In addition, the peripheral blood cells of ATL patients have elevated levels of IRF-4 protein. Up-regulation of IRF-4 by HTLV-I Tax suggests that IRF-4 may be involved in the transformation of the T cells by HTLV-I (35).

We have previously demonstrated that IL-15Rα is activated by HTLV-I in part through the action of NF-κB (25). In this study, we delineate additional Tax-responsive elements within the IL-15Rα promoter. Specifically, we demonstrated that an IRF element (IRF-E) was necessary for Tax-induced activation of the IL-15Rα promoter. In addition, we showed that IRF-4 was capable of activating the IL-15Rα promoter and that inhibition of IRF-4 action reduced the Tax-induced activation of the IL-15Rα promoter. These findings suggest that HTLV-I Tax is capable of activating IL-15Rα in part through the action of IRF-4.

Materials and Methods

Cell culture
Jurkat T cell lines were cultured in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) containing 10% FCS, 2 mM l-glutamine, 0.2 M HEPES, and 100 U/ml Pen/Strep antibiotic. COS-7 cells were cultured in DMEM medium containing 10% FCS, 2 mM l-glutamine, and 100 U/ml Pen/Strep antibiotic. Cultures were incubated at 37°C in 5% CO2/95% air.

Analysis of alternative Tax-responsive elements within the IL-15Rα promoter
To delineate additional Tax-activated sites within the Tax responsive element of the IL-15Rα promoter (Del.1/pGL3pro), deletion constructs were made that included or excluded the NF-κB site (Fig. 1). Plasmid 209 contained the NF-κB site (bases −970 to −1061), while plasmid 207 contained the remaining bases in the Del.1/pGL3pro construct (bases −844 to −971), excluding the NF-κB site and all bases 5' to it. Sense and anti-sense primers of the promoter inserts were prepared with KpnI and XhoI restriction sites at the 5' and 3' ends, respectively. To anneal the primers, 10 μg of each primer was added to 10 mM NaCl and placed in a 95°C heat block. The heat block was immediately placed at room temperature and the primers were allowed to anneal gradually. Once the heat block attained room temperature, the annealed primers were ethanol precipitated. The precipitated DNA was then ligated to the pGL3 promoter vector using the XhoI and KpnI sites overhangs found on the annealed primers (see primer sequences below) and the resulting product was transformed into One Shot (Invitrogen, San Diego, CA) competent cells. The primer sequences for plasmid 207 were as follows: 5'-GACTTATCATGGAATATTATTAAGTTAATGAGAGAAATT-GAGGTTCTGGAGATTTTGAGGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
were as follows: 5′-GGTGGTATCTCCTGGTTAGA(A→G)(A→G)(A→G) AAAGATGACGTGTTGG-3′ (sense) and 5′-CCACAGGAGCAGGCGACC-3′ (antisense). The first invariant position –894 to 896 and the following primers were used in the mutagenesis of plasmid 256: 5′-TTCATTATCTTTTCTTCTC(T→C)(C→T)(C→T)TTTTCTCG AGAAGCTT-3′ (sense) and 5′-CAGATCTCAGAAAGAAA(G→A) (A→G)(A→G)AGTAGAAAAGATAA-3′ (antisense). Primers for the IL-15Rα/pGL3 construct were: 5′-CCCGATGGATGGTTCT ATATGCTTCTATC(T→C)(T→C)(T→C)TTTTTATATAGG AGACAGCACTGGC-3′ (sense) and 5′-GCAGCTTCTGTCTCTTA AATTTAAAAAGA→G)(A→G)(A→G)AGTAGAAAAGATAAATG AACCTACCATCGG-3′ (antisense). To analyze the effects of these mutations on Tax responsiveness of the promoter, cotransfection studies were performed in Jurkat cells. All transfections and luciferase procedures were conducted as described above.

Analysis of IL-15Rα activation by IRFs

Cotransfection studies were performed using 50 ng of the full-length IL-15Rα (IL-15Rα/pGL3) reporter construct and 2 μg of IRF-1/pACT (36), IRF-3 (5D)/CMVBL (37), IRF-4/pCDNA3 (38), and IRF-7/pFlag-CMV-2 (39) expression plasmids. COS-7 cells were transfected using the DEAE Dextran method in 6-well dishes. Briefly, 2.5 × 10⁵ cells were seeded per well and incubated overnight. Cells were washed with PBS and transfection mixtures (300 μl PBS containing DNA and 300 μg DEAE Dextran) were added at 37°C, gently rocking at 37°C for 5 min. Following the incubation, 1.5 ml DMEM media containing 80 μM chloroquine was added to each well and plates were incubated at 37°C for 2.5 h. Chloroquine was aspirated from the wells and 1.5 ml of DMEM containing 10% DMSO was added to each well for 2.5 min. Media was aspirated from the wells and 4 ml of DMEM was added to each well. Cells were incubated at 37°C and luciferase activity was measured in 24 h posttransfection. Assays were performed in triplicate and error bars represent the SD of the fold induction of samples over that of the negative control (pGL3 basic).

In addition, the effect of IRF-4 inhibition on Tax activation of the IL-15Rα promoter was analyzed in cotransfection studies in COS-7 cells. A total of 50 ng of the IL-15Rα/pGL3 reporter construct, 100 ng Tax/pBC72 (a kind gift from J. Brady), and 2 μg of FKBP95/pFLAG (38) and/or 2 μg superdominant IκBα/pCDNA3 were cotransfected using the DEAE Dextran method described above. Luciferase assays were performed as previously described.

Western blot analysis of IRFs

Transfected COS-7 cells were trysinized from plates and divided in half for luciferase activity or radioimmunoprecipitation buffer lysis. Thirty micrograms of each sample were run on a 4%–15% gel (Invitrogen) and transferred to polyvinylidene fluoride membrane. Membranes were blocked with Super Block (Pierce, Rockford, IL) over night and probed with anti-IRF-1, anti-IRF-3, anti-IRF-4, and anti-IRF-7 (Santa Cruz Biotechnology) was added to the cell extracts for 30 min on ice. Extracts were then mixed with the IL-15Rα IRF-E radiolabeled probe, poly(dI-dC), and BSA at room temperature for 30 min. Samples were loaded onto an acrylamide (30%)/bis-acrylamide (0.8%) gel and subjected to electrophoresis at 150 V for the initial 10 min followed by 120 V for the remainder of the run. Gels were dried and exposed to Kodak MS film (Kodak, Rochester, NY).

Results

Additional Tax-responsive elements within the IL-15Rα promoter

In previous studies, we demonstrated that the IL-15Rα promoter was induced by HTLV-I Tax expression (25). This promoter activation was mediated in part by an NF-κB motif within a 220 bp Tax-responsive element of the promoter. In addition, mutation of the NF-κB motif within the Tax-responsive region (Del.1/pGL3-pro) as well as mutation of the NF-κB motif within the full-length promoter (IL-15Rα pro/pGL3) dramatically reduced Tax activation of the IL-15Rα promoter. In this study, to further address the importance of NF-κB in the Tax-induced activation of the IL-15Rα promoter, we demonstrated that a mutant form of Tax that does not activate NF-κB (M22) also eliminates Del.1/pGL3-pro promoter activity (data not shown). In addition, a reporter construct bearing the HTLV-I LTR was activated by M22, thus demonstrating the necessity of NF-κB activation for IL-15Rα, but not the HTLV-I LTR. Previous studies showed that the HTLV-I LTR is not significantly activated by NF-κB (40). Furthermore, the level of activation of the Del.1/pGL3-pro was comparable to that of the HTLV-I LTR with wild-type Tax. This finding further demonstrates that IL-15Rα activity is mediated by NF-κB.

Cotransfection studies previously performed (25) with the Del.1/pGL3-pro, Tax, and super dominant IκBα showed that IκBα could inhibit the Tax-induced activation of the IL-15Rα promoter mediated by NF-κB. However, we did not demonstrate complete inhibition of Tax activation using this NF-κB inhibitor. This finding also suggested that additional sites within the first 200 bp of the IL-15Rα promoter were activated by Tax. To delineate these sites, we made deletion constructs within the 200 bp region and analyzed each for Tax responsiveness.

Del.1/pGL3-pro was first divided into two regions, one containing the NF-κB site (plasmid 209) and the other containing the 3′-end of this fragment (plasmid 207) (Fig. 1A). Each plasmid was analyzed for promoter activity in the presence or absence of wild-type Tax expression (Fig. 1B) in Jurkat cells. As expected, plasmid 209 was responsive to Tax expression. Interestingly, plasmid 207 was also activated by Tax expression. This finding indicated the presence of additional Tax-responsive elements within the IL-15Rα promoter.

Based on this observation, plasmid 207 was divided into four segments and each segment was cloned into the pGL3 promoter reporter construct (Fig. 1A). These plasmids (254, 255, 256, and 257) were then used in cotransfection studies in the presence or absence of Tax expression in Jurkat cells. As shown in Fig. 1B, reporter activities of plasmids 255 and 257 were not enhanced by Tax expression at the same level as plasmid 207. However, plasmids 254 and 256 were activated by Tax expression at a level greater than that of plasmid 207. This suggested that motifs located in these regions of the promoter were important for the additional Tax-induced activation of plasmid 207. Furthermore, increased Tax-induced activation of these promoter regions suggested that negative regulatory elements found in the sequence of 207 were eliminated in plasmids 254 and 256, thus increasing Tax-induced activation.

Analysis of the DNA sequence in these regions of the IL-15Rα promoter (GenBank accession no. AF283296) showed the presence of two putative IRF-1Es. This was interesting in that the IL-15 promoter was activated in part by NF-κB, IRF-1, and IRF-3 transcription factors (23, 41). Knowing that IL-15 and IL-15Rα were activated similarly by NF-κB, it was possible that IL-15Rα was also similarly regulated by IRFs.
IL-15Ra mRNA expression in human monocytes was increased upon treatment with IFNs and LPS

To examine whether IL-15Ra expression was influenced by IRFs, we first analyzed IL-15Ra mRNA expression in elutriated monocytes following treatment with IFNs. Monocytes were used because they are known to up-regulate both IL-15 and IL-15Ra upon treatment with IFNs (6, 42). IRFs are transcription factors induced upon viral infection and/or upon treatment with IFNs. If IRFs were responsible for IL-15Ra activation, treatment with IFNs would likely activate IL-15Ra mRNA expression.

Elutriated human monocytes were treated with PHA, LPS, IFN-α, IFN-β, IFN-γ, or LPS + IFN-α, -β, or -γ for 6.5 h. Elutriated monocytes were used to obtain a homogeneous population of monocytes capable of responding to IFN stimulation. Following treatment, total RNA was isolated from each condition and analyzed in a Northern blot assay for IL-15Ra, IL-15, and β-actin expression. As shown in Fig. 2, PHA had no effect on IL-15Ra expression, yet LPS induced IL-15Ra expression 12.2-fold over that of the media alone control. Furthermore, IFN-α, IFN-β, and IFN-γ increased IL-15Ra expression 27.4-, 20.2-, and 29.5-fold, respectively. The combination of LPS and IFN-α, IFN-β, or IFN-γ yielded 44.8-, 37.9-, and 44.7-fold induction of IL-15Ra mRNA expression, respectively. IL-15 induction by IFNs served as a positive control (41). These findings demonstrated that IFNs were capable of activating IL-15Ra mRNA expression. In turn, this suggested that IRFs played a role in the transcriptional regulation of the gene.

Mutations in the IRF-E within the IL-15Ra promoter lead to reduced Tax-induced activation

Mutations within the IRF-Es of the IL-15Ra promoter were made to analyze their effect on Tax-induced activation of these regions of the promoter in cotransfection studies using Jurkat cells. First, mutations were made in plasmids 254 and 256, the smallest regions of the IL-15Ra promoter that were induced by Tax expression. All mutations were made within the putative IRF-Es at sites consistent with consensus IRF-E motifs. As shown in Fig. 3, mutation of three bases within the putative IRF-E at positions −943 to −945 (TTT → CCC) using site-directed mutagenesis (plasmid 261) reduced the Tax-induced activation of this site 56%. This suggested that the putative IRF-E at this site was important for Tax-induced activation. In addition, mutations of plasmid 256 were made at positions −894 to −896 (TTT → CCC) using site-directed mutagenesis (plasmid 262). Tax-induced activation of this plasmid was inhibited 87% when compared with that of the wild-type 256 plasmid (Fig. 3). Interestingly, plasmids 254 and 256 were not activated by the NF-κB inactive mutant of Tax, M22 (data not shown). Although this result was surprising, it was shown previously by Grumont and Gerondakis (43) that IRFs could be activated by NF-κB. Therefore, in the context of plasmids 254 and 256, NF-κB might activate the IRF that acts on the IL-15Ra IRF-E sites (see Discussion). These observations indicated that the IRF-E within these isolated regions of the promoter and were essential for the Tax responsiveness of the wild-type promoter.

Although we demonstrated an effect on Tax activation by mutating the individual IRF-Es found within plasmids 254 and 256, we also wanted to examine their effect on the full-length IL-15Ra promoter in Jurkat cells to determine whether they were important
for the Tax-induced activation in the presence of the NF-κB site. This was important because previous studies showed that an NF-κB site located within the first 200 bp of the promoter was important for Tax activation (25). We also analyzed the effects of individual mutations of the NF-κB site and both of the IRF-Es on the Tax-induced activation of the full-length promoter (Fig. 4). Mutation of the first IRF-E site in plasmid 263 had no effect on the Tax-induced activation of the IL-15Rα promoter. This indicated that this IRF-E site played no essential role in the Tax activation of the full-length promoter. This also suggested that in isolation, this element could be used by Tax, yet in the context of the full-length promoter, Tax worked through alternate motifs. Mutation of the second IRF-E site in plasmid 270 showed a 41% decrease in Tax responsiveness. This decrease suggested that this region of the promoter contributed to the Tax activation of the full-length promoter. Finally, mutation of the NF-κB site (plasmid 230) again contributed to a severe inhibition (75%) of Tax activation. These findings demonstrated that the second IRF-E site and the NF-κB site were important in the Tax activation of the IL-15Rα promoter.

We also analyzed the Tax-induced activation of plasmids containing double and triple mutations. Plasmid 272 was mutated at the first IRF-E and the NF-κB site, while plasmid 271 was mutated at the second IRF-E and the NF-κB site. Both of these plasmids had a reduction in Tax responsiveness, by 56 and 78%, respectively. Mutation of the first IRF-E alone in plasmid 263 had no effect on the Tax activation of the full-length promoter; therefore, this analysis indicated that the NF-κB site appeared to be the dominant factor in the Tax activation of the promoter. In addition, a double mutation of both IRF-E sites showed a 50% decrease in Tax-induced activation similar to that of the mutation of the second IRF-E site alone (plasmid 270). Furthermore, a triple mutation of both IRF-E sites and the NF-κB site also reduced the Tax-induced activation of the promoter by 70%. In fact, the activation level of plasmids 271 and 273 in which IRF-E sites and the NF-κB motif were mutated were similar to that of plasmid 230 in which the NF-κB site alone was mutated, again indicating that the NF-κB motif was the dominant site of Tax-induced activation. Taken together, these findings suggested that the second IRF-E site was important for the Tax-induced response of the IL-15Rα promoter. Furthermore, these findings showed that activation of NF-κB is the crucial element necessary for Tax-induced activation of the IL-15Rα promoter. This suggested that the IRF-E sites within the promoter were activated by Tax; however, the NF-κB motif played a dominant role in the Tax-activation of the IL-15Rα promoter.

FIGURE 3. Mutations of IRF-E sites within plasmids 254 and 256 inhibit the Tax-induced activation of these regions of the promoter. Plasmids 254 and 256 were each mutated at 3 bp in their putative IRF-E sites and renamed plasmids 261 and 262. Mutations are highlighted using cross-hatches. A, Mutation of the IRF-E in plasmid 261 reduced Tax activation when compared with that of plasmid 254. B, Mutation of the IRF-E in plasmid 262 caused a severe reduction in Tax activation when compared with that of plasmid 256.

IRF-4 is involved in the activation of the IL-15Rα promoter

Mutational analysis of deletion constructs and the full-length promoter demonstrated that an IRF-E site was important for the transactivation of the IL-15Rα promoter (IL-15Rαpro/pGL3). We next analyzed the promoter activity of the IL-15Rα reporter construct in cotransfection assays of COS-7 cells using expression plasmids for various IRFs. We used COS-7 cells instead of Jurkat cells as shown above due to the higher transfection efficiency of COS-7 cells. Detectable levels of IRFs were seen in these cells as demonstrated by Western blot analysis (see below). In this study, cells were cotransfected with IRF-1 and IRF-3 (5D), a constitutively active form of IRF-3, both of which were previously shown to activate the IL-15 promoter (41). In the same experiment, we also cotransfected IRF-7, an IRF that is restricted to lymphoid cells, and IRF-4, an IRF expressed in HTLV-I infected cells. Although

FIGURE 4. Mutations in the IRF-E and NF-κB sites reduced the Tax responsiveness of the full-length IL-15Rα promoter. Constructs bearing the full-length IL-15Rα promoter (IL-15Rαpro/pGL3) were mutated at the IRF-E and NF-κB sites. Mutations are indicated by cross-hatches. Mutation of the second IRF-E (plasmid 270) and the NF-κB site (plasmid 230) caused significant reductions in the Tax activation of the promoter. Mutation of the first IRF-E site (plasmid 263) had no effect on the Tax-induced activation of the full-length promoter. Double mutations of both IRF-E and the NF-κB (plasmids 271 and 272) as well as a triple mutation of all elements (plasmid 273) also significantly decreased the Tax activation of the promoter.
IRF-1, IRF-3 (5D), and IRF-7 were capable of activating the IL-15Rα promoter 12, 10-, and 30-fold, respectively, IRF-4 expression demonstrated the strongest activation with an 185-fold increase over the pGL3 basic construct (Fig. 5A). Expression patterns of the various IRFs are demonstrated in Fig. 5B by Western blot analysis to ensure comparable levels were expressed to activate the IRF-E within the IL-15Rα promoter.

IRF-4 was isolated from an HTLV-I-infected ATL cell line (34). In addition, Yamagata et al. (34) showed that transient expression of Tax in Jurkat cells activated IRF-4 mRNA expression. Furthermore, Sharma et al. (35) showed that the IRF-4 promoter was reduced by Tax expression in reporter assays. We showed that IL-15Rα mRNA is also activated by Tax expression (25); therefore, IRF-4 was a good candidate for the activation of IL-15Rα by Tax through the IRF-E site defined above. As seen in Fig. 5A, coexpression of IRF-4 greatly enhanced the activity of the full-length IL-15Rα promoter construct. This finding suggested that IRF-4 in HTLV-I-infected T cells is capable of activating the IL-15Rα promoter.

An inhibitor of IRF-4 reduced the Tax-induced activation of the IL-15Rα promoter

Initial studies demonstrated that HTLV-I Tax activated the IL-15Rα promoter via an NF-κB site (25). The super dominant IκBα molecule inhibited Tax activation of the IL-15Rα promoter; however, this inhibition was not complete. As demonstrated above, an IRF-E also played a role in the Tax-induced activation of the promoter. In addition, we demonstrated that IRF-4 activated the IL-15Rα promoter in the absence of Tax expression. To examine the role of IRF-4 in the Tax-induced activation of the promoter, we performed transient coexpression assays in COS-7 cells with Tax and FKBP52, an inhibitor of IRF-4. FKBP52 inhibits IRF-4 DNA binding through its peptidyl-propyl isomerase activity (38). Furthermore, FKBP52 inhibits the action of IRF-4, but does not inhibit the action of NF-κB. As shown in Fig. 6, Tax activated the IL-15Rα promoter 83.2-fold over that of the pGL3 vector alone. The Tax-induced activation of the IL-15Rα promoter was again inhibited by a super dominant IκBα expression plasmid in a transient transfection assay. The expression of super dominant-IκBα reduced the fold activation of the IL-15Rα promoter by Tax to 2.8-fold over the pGL3 basic construct. In addition, the IRF-4 inhibitor FKBP52 also inhibited the Tax-induced activation of the promoter to 7.1-fold over that of pGL3 vector alone. These findings showed that both NF-κB and IRF-4 played roles in the Tax-activation of the IL-15Rα promoter. In addition, these findings demonstrated that both NF-κB and IRF-4 are necessary for the complete activation of the IL-15Rα promoter by HTLV-I Tax.

The functional IRF-E within the IL-15Rα promoter bound proteins in the lysates of the HTLV-I-infected T cell line, MT-2

To determine whether the IRF-E motif in the IL-15Rα promoter was capable of binding IRF-4 proteins, we performed EMSA analysis using the lysates from the HTLV-I-infected T cell line MT-2. MT-2 cells were chosen because they express high levels of IRF-4 protein. As shown in Fig. 7, the IL-15Rα IRF-E motif exhibited binding in the absence of cold competitive probes (Fig. 7, lane 1). This binding was specific because cold IL-15Rα IRF-E oligonucleotides competed out the binding of the labeled probe in a dose-dependent manner (Fig. 7, lanes 2–5). In addition, the binding of the IL-15Rα IRF-E was also competed out with the addition of a cold IRF-4 consensus probe (cIRF-E; Fig. 7, lanes 6–9). This finding suggested that IRF-4 was involved in the binding seen in Fig. 7 (lane 1). Furthermore, cold consensus NF-κB probe was used as a negative binding control. This probe did not compete for the binding of the labeled IL-15Rα IRF-E probe (Fig. 7, lanes 10–13), suggesting that the binding seen in Fig. 7 (lane 1) is specific for IRF-4. Furthermore, addition of an anti-IRF-4 Ab completely abrogated the binding of the labeled probe. This finding suggested that the interaction of IRF-4 with the IL-15Rα IRF-E site was inhibited by the addition of Abs to IRF-4. Taken together, these findings suggested that cellular proteins are capable of binding the IL-15Rα IRF-E, and that this binding is mediated by IRF-4.

FIGURE 5. IRF-4 activates the IL-15Rα promoter. A, IRF-1, IRF-3 (5D), IRF-4, and IRF-7 were used in cotransfection assays of COS-7 cells with the IL-15Rα promoter (IL-15Rαpro/pGL3) construct. IRF-4 induced the strongest activation of the IL-15Rα promoter when compared with the other expression plasmids examined. B, Lysates from cells transfected with IL-15Rαpro/pGL3 alone (lane 1), or in combination with IRF-1 (lane 2), IRF-3 (5D) (lane 3), IRF-4 (lane 4), or IRF-7 (lane 5) were examined by Western blot for protein expression. Vinculin was added to demonstrate protein loading.

FIGURE 6. FKBP52, an inhibitor of IRF-4, inhibits the Tax-induced activation of the IL-15Rα promoter. Cotransfection assays were performed in COS-7 cells using the full length IL-15Rα promoter (IL-15Rαpro/pGL3), HTLV-I Tax (Tax/pBCT2), super dominant IκBα/pCDNA3, and/or FKBP52 (FKBP52/pFLAG). Inhibition of both NF-κB and IRF-4-reduced Tax-induced activation and inhibition of both factors almost completely inhibited Tax-induced activation of the IL-15Rα promoter.
essential for maximal Tax activation; however, it is important to note that the NF-κB site is the dominant factor involved in Tax activation. For example, IRF-E sites within the IL-15Rα promoter were not activated by a Tax mutant deficient in NF-κB activation. This result suggested that NF-κB was involved in the activation of IRF-4 by HTLV-I Tax. Grumont and Gerondakis (43) previously demonstrated that IRF-4 expression was activated by NF-κB in lymphoid cells. These findings taken together suggest that NF-κB is essential for maximal Tax-induced activation of the IL-15Rα promoter.

Furthermore, we demonstrated that IRF-4 activated the IL-15Rα promoter in the absence of Tax (Fig. 5). This finding suggests that the Tax-responsive IRF-E site within the IL-15Rα promoter is responsive to IRF-4. We also demonstrated that inhibition of IRF-4 by FKBP52 severely inhibited Tax-induced activation of the IL-15Rα promoter (Fig. 6). FKBP52 exhibits peptidyl-propyl isomerase activity and interferes with the binding of IRF-4 to its DNA binding site. FKBP52 does not inhibit NF-κB activity (38); therefore, the reduced Tax activation seen in this experiment is contributed to IRF-4 inhibition. Furthermore, we demonstrated that the IRF-E within the IL-15Rα promoter was capable of binding IRF-4 proteins (Fig. 7). These findings suggested that IRF-4 activated the transcription of IL-15Rα under the influence of Tax. Taken in concert with HTLV-I Tax activation of IL-15, activation of IL-15Rα by IRF-4 and NF-κB by HTLV-I Tax (Fig. 8) could represent an activation of a host immune response to retroviral-induced proliferation of HTLV-I-infected cells.

Discussion

Previous studies showed that IL-15Rα is elevated in HTLV-I-infected T cell lines and in the T cells of patients with ATL (25). Furthermore, we demonstrated that HTLV-I Tax activated IL-15Rα expression through the action of NF-κB. Although NF-κB appeared to play a major role in the Tax-induced activity of the IL-15Rα promoter, additional elements appeared to be involved. Expression of a super dominant IκBα expression plasmid did not completely inhibit the Tax-induced activation of the promoter (25); therefore, additional transcription factors were implicated. To examine this issue, deletion constructs were made within the Tax responsive region of the promoter and tested for Tax inducible activity (Fig. 1). Interestingly, the Tax-induced activation was not limited to the constructs bearing the functional NF-κB site. In fact, Tax responsiveness also localized to two putative IRF-Es.

IFNs are cytokines that are activated in response to viral pathogens. IFN-α and IFN-β are activated in many cell types upon viral infection, while IFN-γ is produced in activated T cells and NK cells. Human monocytes treated with IFN-γ and LPS have increased levels of IL-15 mRNA (42), suggesting that effector molecules downstream of IFN-γ regulate IL-15 expression. In addition, IL-15Rα mRNA levels were increased in human monocytes following treatment with IFN-α, IFN-β, and IFN-γ (Fig. 2). Activation of genes by IFNs or viral infection is mediated by downstream transcription factors termed IRFs. These transcription factors in turn activate IFN-responsive genes. The IL-15 promoter is activated by IRF-1 and IRF-3 (41).

We asked which IRF was responsible for the Tax activation of the IL-15Rα promoter. The initial study that characterized IRF-4 demonstrated that this factor is elevated in ATL patient cells (34). In addition, HTLV-I Tax activates the IRF-4 promoter in cotransfection studies (35). These findings suggest a role for IRF-4 in HTLV-I-associated disorders; however, they do not demonstrate the downstream targets of IRF-4 activation.

In this study, we showed through mutational analysis and cotransfection studies that the IRF-E sites within the IL-15Rα promoter were necessary for its maximal Tax-induced activation (Figs. 3 and 4). The IRF-E site within the IL-15Rα promoter was transcriptionally regulated by HTLV-I Tax through the actions of NF-κB and IRF-4. HTLV-I Tax activates both NF-κB and IRF-4 in infected T cells. These transcription factors in turn activate target genes such as IL-15 and IL-15Rα. The Tax-induced activation of both cytokine and its specific binding receptor are thought to drive an autocrine loop of spontaneous T cell proliferation in HTLV-I-infected cells.
IRFs. IL-15Rs is transcriptionally regulated by these virally induced factors and in turn can participate in the spontaneous proliferation of HAM/TSP or ATL T cells. This production of cytokines and their receptors could lead to an autocrine/paracrine loop of spontaneous proliferation of T cells. Understanding the mechanisms behind the regulation of these cytokine systems could lead to a combinatorial approach directed against both IL-2 and IL-15 or their receptors for the treatment of patients with HTLV-I-associated diseases.

Acknowledgments
We thank Dr. John Brady (National Cancer Institute, National Institutes of Health, Bethesda, MD) for the kind gift of the Tax/pBC72, Tax M22/pBC72, and HTLV-I LTR/pGL3 plasmids. We also thank him for his assistance in the preparation of this manuscript.

References

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