Estradiol Represses Human T-cell Leukemia Virus Type 1 Tax Activation of Tumor Necrosis Factor-α Gene Transcription*

Christina Tzagarakis-Foster‡, Romas Geleziunas§, Abderrahim Lomri‡, Jinping An‡, and Dale C. Leitman‡

From the Department of Obstetrics, Gynecology and Reproductive Sciences, Center for Reproductive Sciences, University of California, San Francisco, San Francisco, California 94143 and Merck Research Laboratories, West Point, Pennsylvania 19486

Adult T-cell leukemia is caused by human T-cell leukemia virus type I (HTLV-I). HTLV-I Tax protein is essential for clinical manifestations because it activates viral and cellular gene transcription. Tax enhances production of tumor necrosis factor-α (TNF-α), which may lead to bone and joint destruction. Because estrogens might prevent osteoporosis by repressing TNF-α gene transcription, we investigated whether estrogens inhibit the transcriptional effects of Tax on the TNF-α promoter. Tax activated the −1044, −163, and −125 TNF-α promoters by 9–25-fold but not the −82 promoter, demonstrating that Tax activation requires the −125 to −82 region, known as the TNF response element (TNF-RE). Three copies of the TNF-RE upstream of the minimal thymidine kinase promoter conferred a similar magnitude of activation by Tax. We demonstrated that c-Jun, NFκB, p50, and p65 interact with and activate the TNF-RE by using mutational analysis of the TNF-RE, Tax mutants that selectively activate NFκB or the cAMP-response element binding protein/activating transcription factor pathway, and gel shift assays with nuclear extracts. Estradiol markedly repressed Tax-activated transcription of the TNF-α gene with estrogen receptor (ER) α or β. Nuclear extracts from U2OS cells stably transfected with ERα demonstrated that ERs interact with the TNF-RE. Our studies provide evidence that ERs repress Tax-activated TNF-α transcription by interacting with a c-Jun and NFκB platform on the TNF-RE. Estrogens may ameliorate bone and inflammatory joint diseases in patients infected with HTLV-I by repressing transcription of the TNF-α gene.

* This work was supported by a National Institutes of Health postdoctoral training grant and a Bank of America Giannini postdoctoral fellowship (to C. T.-F.) and grants from the Paul B. Komen Foundation (to D. C. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Hop Lariboisiere, INSERM, U349, F-75475 Paris, France.

§ To whom correspondence should be addressed: University of California, San Francisco, Center for Reproductive Sciences, HSE 1619 P.O. Box 0556, San Francisco, CA 94143-0556. Tel.: 415-502-5261; Fax: 415-753-3271; E-mail: leitman@obgyn.ucsf.edu.

The abbreviations used are: HTLV-I, human T-cell leukemia virus type 1; ATL, adult T-cell leukemia; TNF, tumor necrosis factor; TNF-RE, TNF-response element; NFκB, nuclear factor κB; NFATp, nuclear T-lymphoproliferative disorder (1, 2), and a chronic progressive disease of the central nervous system termed HTLV-1-associated myelopathy/tropical spastic paraparesis (3). HTLV-1 infection is also associated with several autoimmune disorders such as Sjogren’s syndrome and arthropathy, which is a chronic inflammatory disorder of joints similar to idiopathic rheumatoid arthritis (4, 5). In addition to genes such as gag, pol, and env encoding retroviral structural proteins, HTLV-I also encodes for regulatory genes such as Tax (6, 7). Tax is a 40-kDa zinc finger protein that is involved in the etiology of ATL and its associated diseases by stimulating viral and cellular gene expression (8). Tax regulates gene expression mainly by activating a variety of transcription factors that interact with promoters of target genes (9–16). Tax likely participates in the pathogenesis of diseases associated with HTLV-I infection by inducing multiple cytokine genes including interleukin-2, interleukin-2Rα, granulocyte-macrophage colony stimulating factor, interleukin-6, and tumor necrosis factor-α (TNF-α) (17–24). Transgenic mice expressing Tax display thymic aplasia, neurofibromas, and skeletal alterations such as an increased number of osteoclasts (25–28). Tax-expressing mice also exhibit a bone phenotype similar to that observed in HTLV-1-infected humans. Tax promotes bone diseases and hypercalcemia by increasing the expression of several cytokines. For example, T-cells infected with HTLV-I express constitutively high levels of TNF-α (29), leading to increased serum levels of TNF-α (30). Because TNF-α acts as an inhibitory factor for the proliferation of osteoblasts and promotes the differentiation of precursor cells to mature osteoclasts (31), excessive TNF-α production leads to bone resorption and hypercalcemia (32), two characteristic features of ATL.

Albrecht et al. (33) reported that Tax could activate the TNF-α promoter in a region that binds NFκB. We previously identified a region in the TNF-α promoter, the TNF-response element (TNF-RE), that is activated by TNF-α (34). We also found that estradiol (E2) represses TNF-α activation of the TNF-α promoter in the presence of estrogen receptor α (ERα) or β (ERβ) (35). Intriguingly, repression by ERs does not require direct DNA binding, because deletion of the DNA binding domain of ER does not prevent E2 from inhibiting TNF-α activation of the TNF-α gene (35). This observation suggests that repression by ERs is not mediated through DNA binding but most likely through protein-protein interactions with other transcription factors at the TNF-α promoter.

Several studies (36, 37) indicate that HTLV-I infection exhibits gender-specific differences in clinical outcomes, which...
are thought to result from different levels of sex hormones. Women infected with HTLV-1 have a lower level of Tax expression and viral load as well as a decreased incidence of ATL compared with men (38). Furthermore, Hisada et al. (37) reported that the mortality from ATL is 4-fold higher in males relative to females. These observations suggest that higher levels of estrogens in women may attenuate the clinical effects of Tax. By investigating the action of Tax, we investigated whether estrogens repress Tax-mediated stimulation of TNF-α expression. The results shown above demonstrate that ERα represses Tax activation of TNF-α gene transcription in the presence of E2 by interacting with c-Jun and NFκB. E2 repression of Tax-induced TNF-α gene expression suggests that estrogens may provide a potential therapeutic approach to ameliorate HTLV-1-associated bone and inflammatory joint diseases.

**MATERIALS AND METHODS**

**Cell Culture, Transient Transfection, and Luciferase Assays—**U937 (human monocytic leukemia cells) and U2OS (human osteosarcoma cells) were maintained as described previously (38). For transient transfection assays, cells were collected, transfected to a cuvette, and electroporated using a Bio-Rad Gene Pulser as described previously (38, 39). Following electroporation, cells were resuspended in phenol red-free Dulbecco’s modified Eagle’s medium/F-12 media containing 5% fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin and plated in 12-well tissue culture dishes. Cells were transfected with 17β-estradiol for 24 h following transfection and then collected and lysed in 200 μl of 1× lysis buffer (Promega, Madison, WI). Luciferase assays were performed according to the manufacturer’s instructions (Promega) using a Monolight luminometer. All experiments presented in the Figs. 1–7 legends were performed at least three times, and the data were similar between experiments. U2OS cells stably transfected with a plasmid that expresses a tetracycline repressor were purchased from Invitrogen. These cells were then stably transfected with full-length ERα cloned downstream of a cytomegalovirus promoter that contains two tetracycline-responsive elements (pcDNA TO vector). The U2OS-ERα cells were selected with glycyrin and zeocin. Individual clones were screened for the presence of Erα by reverse transcription PCR and Western blotting.

**Electrophoretic Mobility Shift Assays—**Binding reactions were performed using purified NFκB p50 or c-Jun (Promega). 1 μl of undiluted c-Jun or 1 μl of diluted NFκB p50 (diluted 1:25 in buffer containing 20 mM HEPES (pH 7.9), 400 mM KCl, 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0), 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol) was added to 15 μl of total binding buffer (10 mM HEPES (pH 7.9), 50 mM KCl, 0.2 mM EDTA (pH 8.0), 10% glycerol, 2.5 mM dithiothreitol, 200 μg bovine serum albumin, and 2 μg of poly(dI-dC)). Binding reactions were incubated for 15 min at 4 °C. Following binding, anti-c-Jun (Cell Signaling Technologies) or anti-NFκB p50 (gift from Dr. Warner Greene, Gladstone Institute of Virology and Immunology, San Francisco, CA) were added to the reactions, which were incubated for an additional 15 min at 4 °C. Radiolabeled wild-type TNF-RE (containing the −125 to −82 region of the TNF-α promoter) probe was then added (40,000 cpm per reaction), and binding reactions were allowed to incubate for an additional 15 min at room temperature. The resulting complexes were electrophoresed through a 5% nondenaturing polyacrylamide gel with 1× TBE running buffer (200 mM Tris, 200 mM boric acid, and 25 mM EDTA) at 100 V for 30 min. Following electrophoresis, gels were dried and exposed to film or examined using a Storm PhosphorImager and analysis software (Amersham Biosciences).

**Quantitative Real Time PCR—**U2OS-vector control or U2OS-ERα stable cells were transiently transfected with 0.5 μg of Tax expression plasmid (a gift from Dr. Warner Greene) and treated with E2 or ethanol for 24 h. Following, total RNA was isolated using TriRe (Invitrogen). Reverse transcription reactions were performed using 500 ng of total RNA, 250 ng of random primers (Invitrogen), 200 units of Moloney reverse transcriptase (Invitrogen), 1× reverse transcriptase buffer, 1 μM dNTP mix, 7.5 mM MgCl₂, and 40 units of RNAse inhibitor (Roche Molecular Biochemicals). Reactions were incubated at 25 °C for 10 min, 42 °C for 40 min, and 95 °C for 5 min. Real-time PCR detection of TNF-α expression was performed using the pre-developed TaqMan assay reagents target kit for TNF-α (Applied Biosystems, Foster City, CA) and the ABI PRISM® 7700 (Applied Biosystems). Control reactions were performed using primers and a probe to detect β-glucuronidase activity and Immunology, San Francisco, CA) were added to the reactions, which were incubated for an additional 15 min at 4 °C. Radiolabeled wild-type TNF-RE (containing the −125 to −82 region of the TNF-α promoter) probe was then added (40,000 cpm per reaction), and binding reactions were allowed to incubate for an additional 15 min at room temperature. The resulting complexes were electrophoresed through a 5% nondenaturing polyacrylamide gel with 1× TBE running buffer (200 mM Tris, 200 mM boric acid, and 25 mM EDTA) at 100 V for 30 min. Following electrophoresis, gels were dried and exposed to film or examined using a Storm PhosphorImager and analysis software (Amersham Biosciences).

**RESULTS**

**Tax Activation and ERα and ERβ Repression of the TNF-α Promoter in U937 Cells—**We used the human monocytic leukemia cell line U937 to identify regions within the TNF-α promoter that may be responsive to HTLV-1 Tax. This cell line is known to express the TNF-α gene in response to cytokines (34). U937 cells were co-transfected with the luciferase reporter containing several deletions of the TNF-α promoter (−1044 to −82) and a Tax expression vector. Tax activated the −1044, −163, and −125 TNF-α promoter constructs by about 9–25-fold (Fig. 1). Deleting the TNF-α promoter from −125 to −82 abolished Tax activation. The −125 to −82 region of the TNF-α promoter was previously termed the TNF-response element, because it is activated by Tax (34). Tax activated three copies of the TNF-RE upstream of the minimal thymidine kinase promoter by a similar magnitude to the −1044 TNF-α promoter (Fig. 2A). This finding demonstrates that the TNF-RE contains elements that confer responsiveness to Tax. Expression of ERα or ERβ resulted in a marked repression (88 and 73%, respectively) of Tax-stimulated TNF-RE activity in response to E2. The repression by E2 was dose-dependent with maximal effect observed at 1 nM (Fig. 2B). E2 also repressed Tax activation of the TNF-RE in an adult human osteoblastic (AHTO) cell line (40) that is immortalized by the SV-40 large T oncoprotein (Fig. 2C), demonstrating that the effect of E2 also occurs in bone cells.

**ERα and ERβ Repress Endogenous TNF-α Expression in U2OS Cells—**To investigate whether the repression by E2 is physiologically relevant, we performed quantitative real time PCR analysis to determine whether E2 also represses Tax-mediated activation of the endogenous TNF-α gene. For these studies, we used U2OS-ERα cells, which are human osteosarcoma cells stably transfected with a tetracycline-inducible cytomegalovirus promoter that drives the expression of the ERα cDNA. The U2OS-ERα cells or a vector control cell line (U2OS-vector) were induced with doxycycline, transfected with the Tax expression plasmid, and treated with E2 for 12 h. Quantitative PCR analysis demonstrates that Tax activates the endogenous TNF-α gene by 3-fold compared with the
increasing concentrations (10^5 U937 cells were transfected as described above and then treated with repression of ER mediated repression of Tax activation of the TNF-RE. 

CRE are apparently the most critical elements that regulate nated the ETS binding site as being central to Tax-activated transcription of the TNF-RE in human (AHTO) osteo-

harvested, and luciferase activity was assayed. 

TNF gene expression in an E 2-dependent manner. As shown in previous experiments, Tax activates the wild type TNF-RE — 10-fold (Fig. 4), whereas mutations in the c-Jun/CRE or NFATp/NFκB binding site severely diminished Tax activation. As expected, mutations in both c-Jun/CRE and NFATp/NFκB binding sites also dramatically impaired Tax activation. No differences were observed between the levels of Tax activation with the single mutation reporters compared with the double mutation reporter constructs. Therefore, maximal Tax activation of the TNF-α promoter requires both c-Jun/CRE and NFATp/NFκB elements.

NFκB Activity Is Necessary for Tax-mediated Activation of the TNF-RE—To further dissect the pathways involved in Tax activation of the TNF-α promoter, we utilized two previously characterized Tax mutants, Tax M22 and Tax M47. The Tax M22 mutant is unable to activate NFκB while maintaining its ability to activate transcription factors of the CREB/ATF family (45–47), whereas the Tax M47 mutant activates the NFκB pathway but not the CREB/ATF pathway (48). As shown in Fig. 5, wild type and Tax M47 activated the TNF-RE, which was inhibited by E2. In contrast, Tax M22 was ineffective at activating the TNF-RE, and no repression by E2 was observed. These results demonstrate that Tax has to activate NFκB but not CREB/ATF to stimulate the TNF-α promoter.

NFκB Binding p50 and c-Jun Bind to the TNF-RE—We investigated the ability of c-Jun and NFκB to bind the TNF-RE by electrophoretic mobility shift assays. Binding reactions were done with purified p50 and c-Jun, the DNA binding components of NFκB and AP-1, respectively. As shown in Fig. 6, both purified NFκB p50 and c-Jun bind to the TNF-RE probe (lanes 1 and 2, respectively). When reactions containing both NFκB p50 and c-Jun were incubated for 15 or 60 min prior to binding to the TNF-RE probe, there were shifted complexes that correspond to the individual proteins (compare lanes 3 and 4 with lanes 1 and 2). By using selective antibodies, it is clear that the slower migrating complex (marked by the arrow) contains both NFκB p50 and c-Jun. (lanes 5 and 6). These results indicate that NFκB p50 and c-Jun bind simultaneously to the TNF-RE.

ERα Binds to a c-Jun/NFκB Complex in the TNF-RE—To begin to probe the mechanism whereby E2 represses Tax-mediated activation of the TNF-α promoter, we investigated our hypothesis that ERα bind to the TNF-α promoter indirectly via interactions with transcription factors bound to the TNF-RE, because we reported previously that ERs do not bind directly to the TNF-RE (35). For these studies, we used U2OS

E2 Repression of the TNF-α Gene

Fig. 2. A, ERα and ERβ repress Tax-activated transcription of the TNF-RE in U937 cells. U937 cells were transfected with the TNF-RE-tk-luc plasmid (3 μg), a Tax expression vector (500 ng), and an expression vector for ERα (1 μg) or ERβ (1 μg). Cells were treated with 10 nM E2 for 24 h, and luciferase activity was measured. B, dose-dependent repression of ER mediated repression of Tax activation of the TNF-RE. U937 cells were transfected as described above and then treated with increasing concentrations (10^-11–10^-6 μM) of E2. After 24 h cells were harvested, and luciferase activity was assayed. C, ERα and ERβ repress Tax-activated transcription of the TNF-RE in human (AHTO) osteoblasts. AHTO cells were transfected with the TNF-RE-tk-luc plasmid (3 μg), a Tax expression vector (500 ng), and an expression vector for ERα (1 μg) or ERβ (1 μg). Cells were treated with 10 nM E2 for 24 h, and then luciferase activity was measured. Each data point is the average of triplicate determinations. The S.E. was <10%.

U2OS-vector control cells without Tax (Fig. 3). E2 produces a 50% reduction in Tax-induced TNF-α mRNA levels in the U2OS-ERα cells. These results demonstrate that, similarly to transient transfection assays, ERα represses Tax activation of the endogenous TNF-α gene expression in an E2-dependent manner.

c-Jun/CRE and NFATp/NFκB Elements Are Required for Tax Activation of the TNF-RE—We next sought to explore the mechanism whereby Tax activates the TNF-α promoter. Several studies showed that ETS-, ATF-2-, NFATp-, NFκB-, and c-Jun/CREB-related transactivation factors bind to the TNF-RE in the TNF-α promoter (34, 41–43). NFATp/NFκB and c-Jun/CRE are apparently the most critical elements that regulate the TNF-α promoter function, because previous studies eliminated the ETS binding site as being central to TNF-α promoter activity (44). To investigate the role of NFATp/NFκB (5'-GGGGTTCCTC-3') and c-Jun/CRE (5'-TGAGCTCA-3') elements in Tax activation of the TNF-α promoter, transfection assays were performed using luciferase reporters containing the wild type TNF-RE or mutations of the c-Jun/CRE or NFATp/NFκB binding sites upstream of the thymidine kinase promoter. As observed in previous experiments, Tax activates
cells stably transfected with ERα. Following treatment with doxycycline to induce ERα expression, the cells were transfected with the Tax expression plasmid and treated with E2 for 12 h. Binding reactions were performed with U2OS-ERα nuclear extracts and specific antibodies to c-Jun, NFκB p50, NFκB p65, ATF-2, and ERα to identify candidate proteins that bind the TNF-RE (Fig. 6, lanes 2–6 respectively). A predominant single shifted band is observed with the nuclear extract (Fig. 7). This band is supershifted with antibodies to c-Jun, NFκB p50, and NFκB p65, but not with an antibody to ATF-2. This pattern demonstrates that a p50 and p65 heterodimer binds to the NFκB site in the TNF-RE. Furthermore, c-Jun binds to the TNF-RE but not ATF-2, suggesting that the complex that binds to the c-Jun/CRE element is a homodimer of c-Jun or, more likely, a heterodimer with another transcription factor. There is also a strong supershift with the ERα antibody, indicating that it is also present in the complex bound to the TNF-RE. Taken together, these data suggest that the complex that binds to the TNF-RE contains c-Jun and NFκB p50, and c-Jun interacts on the TNF-RE.

**DISCUSSION**

Our studies demonstrate that ERs repress Tax activation of the TNF-α promoter in transient transfection assays. The results from quantitative PCR analysis showed that the repression by E2 is not an artifact of reporter plasmids, because E2 produced a similar magnitude of repression of the endogenous TNF-α gene. Deletion studies showed that the −125 to −82 region of the TNF-α promoter is necessary for Tax activation. This region contains binding sites for ETS, c-Jun, ATF-2, NFATp, and NFκB transcription factors that might mediate
the Tax activation of the TNF-α promoter. Tsai et al. (42) reported that a c-Jun/ATF-2 heterodimer binds to the c-Jun/CRE in the TNF-RE. The CREB/ATF-defective M47 Tax mutant activated the TNF-RE more than wild-type Tax, suggesting that CREB and ATF-2 are not the factors activated by Tax in these cells. Carter et al. (49) showed that M47 Tax is more stable than wild type Tax, which can explain the finding that M47 Tax produced a greater activation of the TNF-RE compared with wild type Tax. Activation of the NFκB element by Tax is essential for maximum activation of the TNF-α promoter, because the M22 Tax mutant deficient in activating the NFκB pathway is unable to stimulate the TNF-RE reporter. Our in vitro DNA binding studies using nuclear extracts from Tax-stimulated human osteosarcoma cells showed that NFκB p50, NFκB p65, c-Jun, and ERα but not ATF-2 interact with the TNF-RE element. These results indicate that the activation of the TNF-α promoter by Tax requires c-Jun and NFκB but not ATF-2. Furthermore, c-Jun and NFκB must bind simultaneously to the TNF-RE to activate the TNF-α promoter, because a mutation in either site abrogates activation by Tax. Other studies reported that related factors such as c-Fos do not interact with the TNF-RE (34).

Our results suggest the following model whereby ER represses the activation by Tax at the TNF-RE. After the activation of NFκB by Tax, the NFκB p50/p65 complex binds next to c-Jun on the TNF-RE, leading to the activation of the TNF-α promoter. The ER is then recruited to the promoter by binding to a platform comprised of c-Jun and NFκB. Support for this model of ER action is provided by evidence that ERα or ERβ do not directly bind to the TNF-α promoter (35) and that NFκB p65 and ERα can interact (50–53). Furthermore, ERα and c-Jun proteins interact directly (54) in glutathione S-transferase pull-down and mammalian two-hybrid assays (55). Alternatively, ER could be recruited to the TNF-α promoter through a bridging protein in direct contact with the c-Jun/NFκB complex.

Examination of the sequence within the TNF-RE region of the TNF-α promoter reveals that there is only one nucleotide separating the c-Jun/CRE and NFATp/NFκB elements. Previous experiments have found that nucleotide insertions resulting in either 0.5, 1, or 1.5 extra turns of the DNA helix abolishes TNF-α activation (44). Therefore, it is likely that c-Jun and NFκB on the TNF-α promoter are critical for providing the initial platform for ER binding to the TNF-α promoter, and disruption of the platform prevents the assembly of other factors necessary for activation of the TNF-α gene by Tax.

One key question that remains is what are the additional proteins in the complex that mediate repression. There is evidence suggesting that coactivators such as glucocorticoid receptor-interacting protein-1 (GRIP1) are involved in steroid receptor repression of gene transcription. GRIP1 potentiates ER-mediated repression of the TNF-α gene (35) and glucocorticoid receptor-mediated repression of the collagenase gene (56). Another protein that may be part of the repression complex is p300/CBP, because it is involved in Tax-mediated activation of HTLV-1 transcription in vitro (57). Whether these proteins are an integral part of the repression complex at the TNF-α promoter or whether other proteins participate in repression remains to be determined.

Individuals infected with HTLV-1 suffer from various bone and joint diseases most likely due to an increased expression of several cytokines, including TNF-α. Hypercalcemia, which results from enhanced bone resorption, is a prevalent complication observed in patients with ATL and has been linked to early death (58). The cause of hypercalcemia in ATL patients is unknown, but it has been suggested that TNF-α plays a role in this disease because it is associated with increased serum levels of TNF-α (30). Tax is also strongly expressed in human synovial cells, which leads to joint destruction. In a transgenic mouse model of HTLV-I infection, mice expressing Tax exhibit skeletal alterations resembling Paget’s disease, a chronic disorder that results in enlarged and deformed bones and have chronic inflammatory polyarthritis (8, 59). Analysis of the joints in these transgenic mice demonstrated enhanced expression of several cytokines, including TNF-α (59).

Intriguingly, similarly to HTLV-I infected patients, many postmenopausal women develop bone and joint diseases that might occur from excessive TNF-α production. A prominent role for TNF-α in the pathogenesis of osteoporosis is supported by animal studies. For example, overexpression of TNF-α in mice produces profound hypercalcemia from enhanced bone resorption (60). Furthermore, the loss of bone mineral density observed in mice after an oophorectomy can be prevented with TNF-binding proteins (61) or a soluble TNF receptor that prevents the action of TNF-α (62). TNF-α might cause osteoporosis by inducing several proteins responsible for differentiating precursor monocyctic cells into bone resorbing osteoclasts (63, 64).

Estrogens are used extensively in postmenopausal women to prevent osteoporosis (65, 66). Several studies indicate that the bone-sparing effect of estrogens is at least in part due to its ability to repress TNF-α gene transcription and down-regulate TNF-α levels (67, 68). Other studies indicate that TNF-α is involved in the destruction of the articular cartilage that is observed in osteoarthritis (69). Osteoarthritis is a prevalent condition that is exacerbated by estrogen deficiency in postmenopausal women and shows some improvement with estrogen replacement (70), possibly by decreasing TNF-α levels. Whereas estrogens are useful in postmenopausal bone and joint diseases, our study suggests that they also might be a potential therapeutic approach for HTLV-1-associated bone and inflammatory joint diseases by directly targeting TNF-α gene expression. Estrogens are known to exhibit anti-inflammatory properties (71), but it is not known if this effect occurs through ERα, ERβ, or both of these ERs. Our results demonstrating that ERβ is more effective than ERα at inhibiting TNF-α and Tax-activation of TNF-α gene transcription suggest that ERβ may be the predominant receptor that mediates the anti-inflammatory effects of estrogens. The finding that E2 is a potent repressor of Tax activation of the TNF-α gene may also account for the observation that females infected with HTLV-1 exhibit less severe clinical manifestations and lower mortality compared with males (37).

Acknowledgments—We thank P. Chambon, J.-A. Gustafsson, and W. Greene for providing plasmids.

REFERENCES

1. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7415–7419
2. Yoshida, M., Miyoshi, I., and Hinuma, Y. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2031–2035
3. Gessain, A., Barin, F., Vernant, J. C., Gout, O., Mauris, L., Calender, A., and de The, G. (1985) Lancet 2, 407–410
4. Terada, K., Kato, H., De Kvd, E. F., Dervan, P. B., and Nyborg, J. K. (1999) J. Mol. Biol. 291, 731–744
5. Johnson, J. M., Harrold, R., and Franchini, G. (2001) Int. J. Exp. Pathol. 82, 155–147
6. Ruddle, N. H., Li, C. B., Horne, W. C., Santiago, P., Troiano, N., Jay, G., Horowitz, M., and Baron, R. (1993) Virology 197, 196–204
7. Zhao, J. L., and Giam, C. Z. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11445–11449
8. Fujii, M., Takao, H., Shinohara, T., Akazawa, T., and Seiki, M. (1992) Genes Dev. 6, 2066–2076
9. Hirai, H., Suzuki, T., Fujisawa, J., Inoue, J., and Yoshida, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3584–3588
10. Franklin, A. A., Kubik, M. F., Uittenbogaard, M. N., Brauwasser, A.,
Estradiol Represses Human T-cell Leukemia Virus Type 1 Tax Activation of Tumor Necrosis Factor-α Gene Transcription
Christina Tzagarakis-Foster, Romas Geleziunas, Abderrahim Lomri, Jinping An and Dale C. Leitman

J. Biol. Chem. 2002, 277:44772-44777.
doi: 10.1074/jbc.M205355200 originally published online September 16, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205355200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 71 references, 38 of which can be accessed free at
http://www.jbc.org/content/277/47/44772.full.html#ref-list-1