Growth inhibition of Tax-activated human Jurkat leukemia T cells by all-*trans* retinoic acid requires JNK-1 inhibition

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Abstract. Retinoids, including vitamin A (retinol) and its analogues, are critical for a variety of biological functions. In this study, we report that all-*trans* retinoic acid (ATRA) decreases Jun N-terminal kinase 1 (JNK-1) activity, antagonizing the effect of the Tax protein in Jurkat leukemia T cells transiently transfected for expressing the Tax protein. The Tax protein is one of the products of the human T-cell leukemia virus type 1 (HTLV-1) which is the etiologic agent of adult T-cell leukemia (ATL), an aggressive neoplasia of $CD4^+$ T cells. The decrease in JNK-1 activity was followed by a marked decrease in the expression of interleukin (IL)-2 and a weak increase in interferon (IFN)-γ in Jurkat cells treated with ATRA in a dose-dependent manner, suggesting a correlation between the expression of JNK-1 and the activity of the Tax protein. However, the expression levels of IL-4 and IL-10 were enhanced in cells transfected with Tax, compared with the levels in untransfected cells, but the expression levels were not affected following ATRA treatment. In transfection studies using a luciferase reporter construct expressing the IL-2 promoter or a tandem repeat of AP-1 or NF-κB, the inhibitory effect of ATRA on the IL-2 promoter and AP-1 construct was confirmed at the transcriptional level. However, the inhibitory effect in the NF-κB reporter construct was only marginal. In addition, our data demonstrated that JNK-1 is constitutively activated in Jurkat leukemia T cells expressing the Tax protein, suggesting that JNK-1 is required for Tax-induced proliferation of Jurkat leukemia cells.

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of adult T-cell leukemia (ATL), an aggressive neoplasia of $CD4^+$ T cells (1,2). The HTLV-1 transcriptional transactivator protein Tax transactivates the expression of several cellular genes in addition to the viral long term repeat (LTR) (3-5). The cellular genes activated by the Tax protein include several involved in cell growth which suggests that it is the expression of this protein that deregulates T-cell growth during HTLV-1 infection and that the constitutive expression of Tax is correlated with immortalization in T cells and the transformation of other cell types (6-9). Tax corresponds to a 40-kDa transforming protein (10,11) from the pathogenic retrovirus HTLV-1 that induces the expression of various family members of the transcription factor AP-1, such as c-Jun, Jun-D, c-Fos and Fra-1, at the level of RNA expression in T cells (12,13). The Jun-N-terminal kinase (JNK) is the only member of the MAP kinases to phosphorylate c-Jun, the main component of AP-1 complexes, and also has ATF-2 and Elk-1 as substrates (14,15). In mammalian cells, three mitogen-activated protein kinase (MAPK) families have been clearly characterized: namely classical MAPK (also known as ERK), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 kinase. The JNK group of MAPKs is activated in response to the treatment of cells with inflammatory cytokines and through exposure to environmental stress. JNK activation is mediated by a protein kinase cascade composed of a MAPK kinase and a MAPK kinase kinase (14-16). JNK and p38 kinases were initially proposed to mediate apoptosis in neuronal cells (17) and phosphorylation of c-Jun is necessary for neuronal cell death (18). The use of kinase inhibitors and the overexpression of dominant-negative mutant forms of MAPKs have demonstrated the involvement of JNK and/or p38 kinase in apoptosis induced in non-neuronal cells by various stimuli, including estrogen, cisplatin, UV-B radiation and singlet oxygen (19,20).

Retinoids are comprised of a group of compounds including retinoic acid (RA), vitamin A (retinol) and a series of natural and synthetic derivatives with A-like activity (21-24). Retinoids, including retinol and its analogues, are critical for a variety of biologic functions. Retinol is metabolized in retinal pigment epithelial cells to 11-cis-retinal and is required for normal ocular function (25); retinol and retroretinoids have also been implicated in maintaining and regulating immune function (26,27). Retinol is also essential for epithelial differentiation (28) and the control of embryonic development (29). The vast majority of retinoid-driven biologic effects are mediated by RAs, the active metabolites of retinol. All-*trans* retinoic acid

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(ATRA) and 9-cis-RA both play an important role in the regulation of gene transcription that ultimately results in the regulation of cell division, growth, differentiation and proliferation (30,31). RAs exert their biologic activity by binding to nuclear receptors that regulate the transcriptional activity of a variety of target genes, which are implicated in the inhibition of cell growth, the induction of cell differentiation and the induction of apoptosis in a variety of tumor cell lines (32,33).

Much of our understanding of the signaling events initiated following drug treatment has been based upon model cell systems. One of the most useful and widely studied of these T cell models has been the human Jurkat leukemia T cell line (34). Several studies have shown that Jurkat cells behave similar to normal cells (35,36). Likewise, other studies have shown a correlation between JNK and the expression of interleukin (IL)-2, and interferon (IFN)-γ in $CD4^+$ T cells (37,38). Using Jurkat cells as a model, we studied the effect of ATRA in decreasing the ability of the Tax protein to induce Jurkat cell proliferation as well as its correlation with the expression of JNK and a panel of cytokines such as IL-2, IFN-γ, IL-4 and IL-10.

Materials and methods

Reagents. ATRA was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The protease inhibitors phenylmethylsulfonyl fluoride (PMSF), leupeptin, pepstatin, aprotinin and bestatin were purchased from Roche (USA); T4 polynucleotide kinase and poly(dI-dC)2 were obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Tris-borate-EDTA buffer and acrylamide-bisacrylamide (29:1) were obtained from Bio-Rad (Richmond, CA, USA). Luciferase assay reagent, lysis buffer and the pGL-2 luciferase vector were obtained from Promega (Madison, WI, USA). TPA and ionomycin were purchased from Sigma Chemical Co. Anti-JNK, p38 and ERK antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). MBP was purchased from Stratagene (La Jolla, CA, USA).

Cell culture and ATRA treatment. Jurkat T cells and Jurkat T cells expressing the Tax protein, were grown in RPMI-1640 containing 10% heat-inactivated fetal bovine serum (FBS), 200 mM glutamine, nonessential amino acids, penicillin and streptomycin sulfate. Before ATRA treatment, cells were grown overnight (16-20 h) in medium containing 0.5% heat-inactivated FBS and subsequently stimulated in the presence of the same medium with low concentrations of serum. ATRA was diluted in a culture medium containing 0.5% FBS, with the DMSO concentration below 0.5%. Appropriate controls containing the same amount of solvent were included in each experiment. Intermittent passage in G418-containing medium was performed to ensure retention of the plasmid.

Plasmid construction and preparation of nuclear extracts. The plasmid expressing the wild-type Tax protein and the Tax inducible cell line were a gift from Dr Warner Greene (Gladstone Institute of Virology and Immunology, University of California, San Francisco, CA, USA). The human IL-2 promoter-enhancer fragment (-500 to +60) was subcloned from plasmid SV-IL-2-CAT into the luciferase vector pGL2 (35). The AP-1-luciferase reporter plasmid driven by the rat prolactin minimal promoter (-36 to +37) under the control of four copies of the human AP-1 site (36) was kindly provided by M. Rincón and R. A. Flavell (Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, USA). Plasmids containing multimers of the recognition sites for NF-κB and AP-1 were constructed and linked to the pLuc-prolactin minimal promoter plasmid (35,36). The orientation for each element was confirmed by the restriction enzyme cleavage. The tandem sequences used to construct the different plasmid multimers were as follows: i) four copies of the AP-1, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), 5'-TCGATTGAGTCAGG-GTAA3'; ii) two copies of the NF-κB-binding site of the human Ig κ light-chain enhancer 5'-GGGACTTTCC-3'; iii) IL-2 promoter construct bearing a -500 to +30 (35).

Transient transfection and luciferase assays. Transfection of cells was conducted by electroporation, using an Electro Cell Manipulator 600 (BTX, San Diego, CA, USA) using 130 V/1700 μF capacitance. Briefly, $8x10^6$ cells were transfected with 10 μg of luciferase reporter plasmid and 5 μg of each expression plasmid, and the mixture was incubated for 24 h. Transfected cells were cultured in complete medium for 24 h and stimulated with ATRA or SP600125 for another 8 h. Cells were harvested 32 h post-transfection, washed twice in phosphate-buffered saline (PBS) and treated with lysis buffer (luciferase assay; Promega) for 5-10 min on ice. Lysates were spun down for 1 min, and the total supernatants were analyzed using luciferase reagent and measured as a duplicate in a luminometer (MicroLumat LB 96 P; Berthold) for 5 sec. Background measurement was subtracted from each duplicate, and experimental values were expressed either as recorded light units of luciferase activity or as relative activity compared with extracts from unstimulated cells (35,36).

Preparation of nuclear extracts. Nuclear extracts, were prepared as previously described (34). The cells were grown at 37˚C in a humidified atmosphere of 10% CO_2. Cells ($2x10^7$), incubated with 0.1% DMSO (control) or 30 nM TPA, or 1 mM ionomycin, were collected and washed with ice-cold PBS, washed again in buffer A [10 mM HEPES (pH 7.9), 15 mM KCl, 2 mM $MgCl_2$, 6 mM DTT, 0.1 mM EDTA and 1 mM PMSF] and lysed in buffer A with 0.2% Nonidet P-40. The pelleted nuclei were re-suspended in buffer B [50 mM HEPES (pH 7.6), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF and 10% glycerol], in the presence of 0.3 M $(NH_4)_2SO_4$ (pH 7.9) and rocked for 30 min at 4˚C. The broken nuclei were centrifuged for 10 min at 100,000 x g. A 125 μl aliquot of supernatant was transferred to a second tube and additional $(NH_4)_2SO_4$ was added to a final concentration of 1.5 M followed by a second centrifugation of 50,000 x g for 5 min. The supernatant was removed and the pellet was re-suspended in 50 μl of buffer B and stored at 70˚C. Protein concentration was estimated using the Bio-Rad stain protein assay kit with bovine albumin as the standard.

Western blot analysis. Jurkat cells ($5x10^7$) were seeded onto 6-well plates. Forty-eight hours after transfection, the cells

were collected and washed twice by cold PBS, and each well was treated with 50 ml lysis buffer [2 mmol/l Tris-HCl (pH 7.4), 50 mmol/l NaCl, 25 mmol/l EDTA, 50 mmol/l NaF, 1.5 mmol/l Na_3VO_4, 1% Triton X-100 and 0.1% SDS], and supplemented with protease inhibitors (1 mmol/l phenylmethylsulfonyl fluoride, 10 mg/l pepstatin, 10 mg/l aprotinin and 5 mg/l leupeptin) (all from Sigma Chemical Co.). Protein concentrations were determined using the Bradford protein assay. Equal amounts of protein (50 mg) were separated on a 15% SDS polyacrylamide gel and transferred onto a nitrocellulose membrane (Hybond C; Amersham, Freiburg, Germany). Membranes were blocked in 5% non-fat dry milk in TBS for 1 h at room temperature and probed with rabbit anti-JNK-1 (SC-1648) antibody (dilution 1:500; Santa Cruz Biotechnology, Inc.) overnight at 4˚C. After washing 3 times with TBS containing 0.1% Tween-20, membranes were incubated with anti-rabbit IgG horseradish peroxidase (1:5,000; Santa Cruz Biotechnology, Inc.) and developed by luminal mediated chemiluminescence (Appylgen Technologies, Inc., China). To confirm equal protein loading, membranes were reprobed with a 1:1,000 dilution of an anti-actin antibody (Santa Cruz Biotechnology, Inc.). Densitometric analyses were performed using Scion Image software (39,40).

Labeling of apoptotic cells with Annexin V. Jurkat cells (500,000) were treated with RA as indicated. Cells were washed with PBS and stained with Annexin V-FITC (Pharmingen) and propidium iodide (PI) in binding buffer [10 mM HEPES (pH 7.4), 140 mM NaCl and 2.5 mM $CaCl_2$] or 15 min at room temperature in the dark. Cells were subsequently analyzed using flow cytometry (FACSCalibur) for apoptosis (FITC) and viability using PI.

Cytokine (IL-2, IL-4, IL-6 and IL-10) quantification in the culture supernatants. Jurkat leukemia T cells stably transfected with the Tax protein were cultured under a condition identical to that described for proliferation assay. ATRA was added at a final 10^{-7} M concentration 36 h after the initiation of the culture in two independent pulses (every 12 h). After 60 h of culture, supernatants were collected and the different cytokines were measured in triplicate by using an enzyme-linked immunosorbent assay (ELISA) system: IL-2, IL-4, IL-6 and IL-10 (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocols.

Results

We examined the effect of ATRA treatment on the activity of one member of the MAP kinases in Jurkat leukemia and Jurkat leukemia Tax-expressing protein.

Inhibition of JNK activity by ATRA in Jurkat-Tax expressing cells occurs in a dose-dependent manner. We examined the effect of ATRA (4 μM) treatment on JNK kinase activity in Jurkat and Jurkat Tax-expressing cells. JNK-1 and JNK-2 activity was detected in both Jurkat and Jurkat Tax-expressing cells (Fig. 1A). The strong inhibition of JNK by ATRA, in Jurkat Tax-expressing cells occurred in a dose-dependent manner, ranging from 2 to 6 μM, compared with the untreated Jurkat cells (Fig. 1B). However, the effect of ATRA in the Jurkat

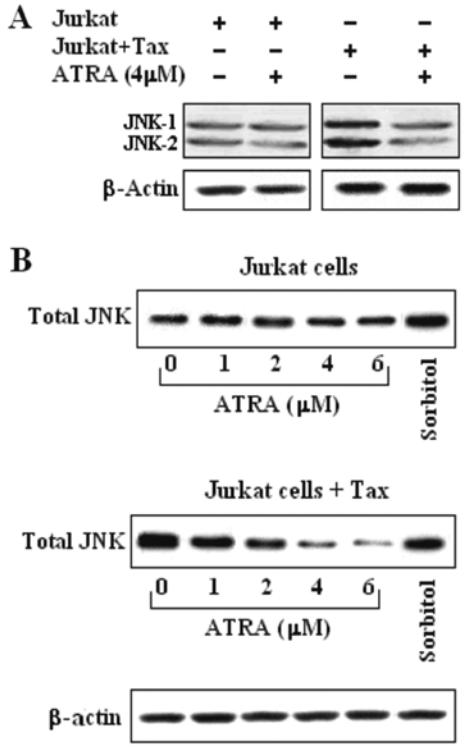

Figure 1. Effect of ATRA on the expression of JNK-1/JNK-2 protein, as assessed by western blot analysis with (A) antibodies directed to JNK-1 or/and JNK-2 or (B) antibodies to total JNK, to determine the level of the protein in Jurkat protein extracts after being separated by gel electrophoresis. Representative western blotting results are presented for Jurkat wild-type and/or Jurkat cells transfected with the Tax expression plasmid, with or without ATRA treatment. A representative blot of three is shown.

cells was lower than that observed in the untransfected Jurkat cells (Fig. 1B). The results obtained in the Jurkat and Jurkat Tax-expressing cells after 24 h of incubation are displayed in Fig. 1. Non-stimulated cells and cells treated with sorbitol were used as the control.

ATRA induces DEVDase activity Jurkat Tax-expressing cells. We investigated the effect of ATRA on the ability to induce caspase activity. Cells were pre-incubated in 10% FCS for 16 h and were later treated with 8 μM ATRA for different incubation times (Fig. 2A) and with increasing concentrations of ATRA (1-12 μM) for 4 h (Fig. 2B). Cytosol extracts were prepared at the indicated periods of time, and caspase activity was subsequently measured using DEVD-AFC as a substrate. We demonstrated that the induction of DEVDase activity was clearly dependent on the time of incubation and the concentration level of ATRA. The increase in DEVDase activity was observed with concentrations of ATRA >2 μM in the Jurkat Tax-expressing cells. The presence of Annexin V-positive (apoptotic) cells was evident after 1 h of incubation with ATRA (8 μM), and the number reached a maximum after 4 h (Fig. 2A). The apoptotic cells, dependent on ATRA concentration, as determined by Annexin V labeling, were evident following 2 μM of ATRA treatment and the number of apoptotic cells reached a maximum level following 10 μM ATRA treatment (Fig. 2B).

SP600125 and ATRA affect Tax upregulation of JNK in Jurkat Tax-expessing cells. We demonstrated that ATRA, at

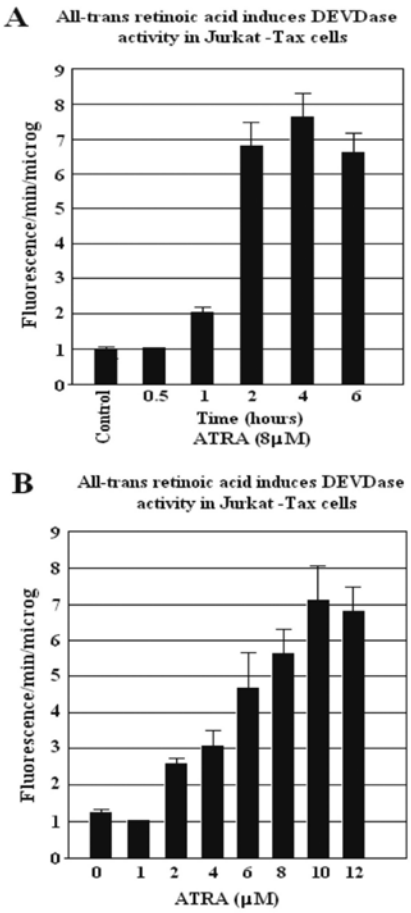

Figure 2. ATRA induces DEVDase activity in Jurkat Tax-expressing cells. Jurkat cells transfected with the Tax expression plasmid were incubated (A) with 8 μM ATRA for different periods of time and (B) in the presence of different concentrations of ATRA for 4 h and caspase activity was determined. The cells were lysed and protein extracts were prepared and subsequently analyzed for DEVDase activity. A representative experiment performed in triplicate is shown.

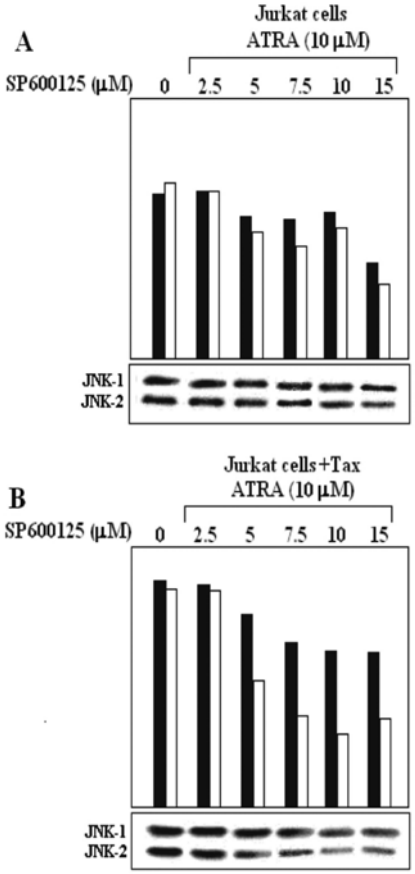

Figure 3. Effect of SP600125 in combination with ATRA, on the expression of JNK in Jurkat cells and Jurkat cells transfected with the wild-type Tax expression plasmid. (A) Jurkat and (B) Jurkat Tax-expressing cells, were treated with various concentrations of SP600125 (2.5-15 μM) and with a constant concentration of ATRA (10 μM) for 60 min. After the treatments, a western blot assay was performed as described in Materials and methods. Aliquots (50 μl) of total protein extract were subjected to SDS-PAGE followed by immunoblotting with the affinity-purified JNK antibody. A representative JNK western blotting of three separate experiments is shown.

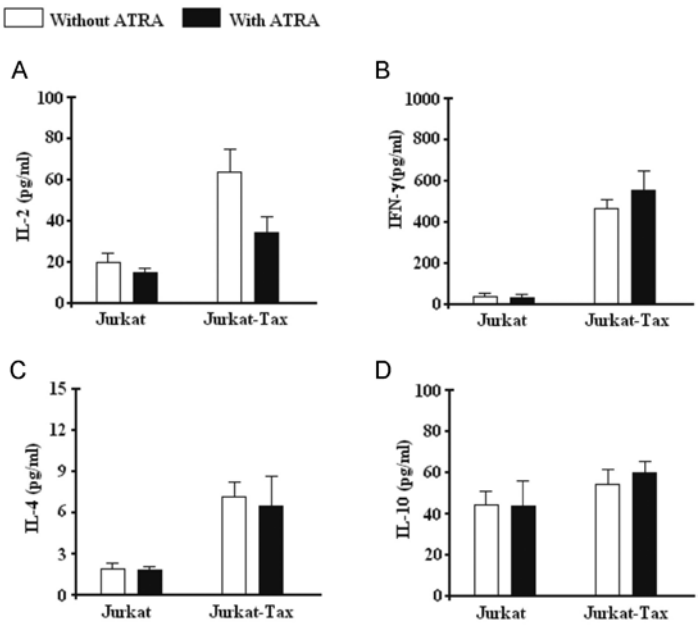

Figure 4. Effects of ATRA on the production of cytokines (A) IL-2, (B) IFN-γ, (C) IL-4 and (D) IL-10 by Jurkat and Tax-stimulated Jurkat cells. Jurkat cells and Jurkat cells transfected with the Tax expression plasmid were cultured in medium containing 10% FCS for 48 h, in the absence (open bar) or presence (solid bar) of 8 μM ATRA. Secretion of cytokines to the supernatants was measured by ELISA. Bars, SD in triplicate cultures of three similar experiments.

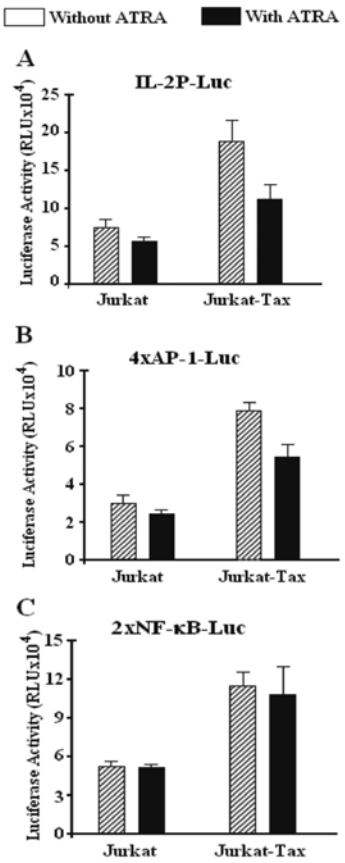

Figure 5. Transactivation of the IL-2 promoter and multimers of 4x AP-1 and 2x NF-κB luciferase reporter constructs. Jurkat cells were transiently cotransfected or not with the Tax expression plasmid and (A) either with a human IL-2 promoter (-500 to +60), or (B) a luciferase reporter construct containing four copies of the AP-1 consensus sequence (4x CGATTGAGTCAGGGTAACG) or (C) with two copies of the NF-κB reporter construct (2x NF-κB; GGGACTTTCC consensus) and cultured or not in the presence of ATRA 8 μM, 24 h post-transfection. Luciferase assay is expressed as relative luminescence units (RLU) minus background units alone. All sets of results shown are the mean values from three similar experiments. Error bars indicate the standard error of the mean.

concentration levels of 2-6 μM, was able to decrease JNK expression in Jurkat and Jurkat Tax-expressing cells (Fig. 1B). To further study the inhibitory effect of ATRA on the expression of JNK, we performed a combination assay using ATRA and SP600125 in untransfected Jurkat cells (Fig. 3A) and Jurkat cells transfected with a plasmid expressing the Tax protein (Fig. 3B). The effect of increasing concentrations of SP600125 on ATRA-mediated inhibition of JNK expression in Jurkat Tax-expressing cells was further investigated. Jurkat and Jurkat Tax-expressing cells were pre-incubated with increasing concentrations of SP600125 (2.5, 5.0, 7.5, 10 and 15 μM) for 120 min. Then, 10 μM ATRA was added and cells were incubated for an additional 180 min. Cell extracts were prepared and subsequently assayed using a western blot assay for JNK-1 and JNK-2 protein expression. We increased the ATRA concentration to 10 μM, to reach a major inhibitory effect of JNK-1 expression. The combination treatment of ATRA with SP600125 further decreased the JNK expression protein. Both ATRA and SP600125 treatments reduced the activity of JNK-1 and JNK-2 (Fig. 3A and B). ATRA-mediated inhibition of JNK-1/JNK-2 expression in untransfected Jurkat cells was lower (Fig. 3A) compared to that observed in Jurkat cells transfected with the Tax protein expression plasmid (Fig. 3B). The expression of JNK-1 was almost completely inhibited with increasing concentrations of SP600125 (15 μM) and ATRA (10 μM) (Fig. 3B).

Regulation of cytokine production by ATRA in Jurkat and Jurkat Tax-expressing cells. To further understand the effect of ATRA, cytokine induction and secretion experiments were performed in Jurkat and Jurkat Tax-expressing cells for IL-2, IL-4, IFN-γ and IL-10. A strong induction of IL-2 (Fig. 4A), IFN-γ (Fig. 4B) and IL-4 (Fig. 4C) production in Jurkat-Tax cells, compared with Jurkat cells were observed, but not for IL-10 (Fig. 4D). The mean secretion of IL-2 was statistically decreased in Jurkat Tax-expressing cells, but not for IFN-γ, IL-4 and IL-10 when ATRA was added to the Jurkat Tax-expressing stimulated cell culture.

Tax transfection strongly enhances transcriptional activity of three luciferase reporter constructs expressing the IL-2 promoter, AP-1 and NF-κB reporter construct. Jurkat and Jurkat T cells expressing the Tax protein were transiently co-transfected with luciferase plasmid reporter constructs containing the inducible region of the IL-2 enhancer/promoter (-500 to +60) (Fig. 5A) or an AP-1 (Fig. 5B), or an NF-κB (Fig. 5C) reporter construct. Tax expression was required to induce high transcription of these constructs. The effect observed after Tax expression was 3-to 4-fold higher in all three constructs, the IL-2 promoter, the AP-1- and the NF-κB-driven transcription, compared with untransfected Jurkat cells which showed a moderate transcriptional activity. However, after ATRA treatment the activity of the IL-2 promoter (Fig. 5A) and AP-1 reporter construct (Fig. 5B) was strongly affected decreasing the transcriptional activity of both the IL-2 promoter and the AP-1 reporter, but failed to affect and slightly decreased the transcriptional activity of the NF-κB reporter construct promoter (Fig. 5C). Noteworthy, NF-κB-driven transcription in the Tax-transfected and untransfected Jurkat cells was not affected by ATRA (Fig. 5C). Apparently, NF-κB was not a target for ATRA, since no activity was noted in Jurkat cells transfected or not with the Tax-expression plasmid.

Discussion

In the present study, the effect of ATRA alone or in combination with SP600125 on human Jurkat leukemia T cells, transfected or not with a Tax expression plasmid was studied. We demonstrated that ATRA in concentration levels between 4 to 10 μM, caused a significant and sustained decreasing effect on JNK expression in Jurkat Tax-expressing cells. A slight effect in all the assays performed was observed in untransfected Jurkat cells. Although the action of retinoids on human cells has been previously studied (26-29), conclusive results have not been obtained. JNK inactivation in Tax cells correlated with the induction of apoptosis, as determined by the measurement of DEVDase activity in the cells treated with ATRA. In addition, the inhibitory effect of ATRA was further increased by simultaneous stimulation with SP600125, a strong inhibitor of JNK. Several *in vitro* studies have shown that JNK signaling plays an important role in enhancing cell

viability and cell cycle progression of several cancer cell types (17,18,39). Extensive studies on several other cell lines have shown that the inhibition of JNK by SP600125 leads to G2/M cell cycle arrest (19,20,40). To further analyze the molecular mechanism of ATRA-mediated suppression of the Tax protein in Jurkat cells we studied its effects on the expression of IL-2, IFN-γ, IL-10 and IL-4, before and after ATRA treatment. The effect of retinoids in specific cytokine induction has been widely investigated in different cellular lineages with contradictory results (26-28,41). ATRA inhibition of JNK may be explained by a putative regulation of the events mediated by IL-2 and/or IFN-γ. To address this issue, IL-2, IL-4, IL-10 and IFN-γ were measured in the supernatant of Jurkat cells with or without ATRA treatment (8 μM). Tax expression strongly activated IL-2 and IFN-γ production but failed to substantially increase the activity of IL-4 and IL-10. However, ATRA treatment was able to moderately decrease the production of IL-2 and failed to decrease the production of IFN-γ. Only a slight Tax-mediated IL-4 and IL-10 increase was observed in all tested cells.

To further understand the effect of ATRA on Tax-expressing cells, we performed a luciferase assay with reporter constructs for IL-2 promoter-Luc, AP-1-Luc and NF-κB-Luc. The results showed that the addition of ATRA reduced the activity of the IL-2 promoter and AP-1 reporter construct, but failed to reduce the activity of the NF-κB reporter construct, suggesting that the activity of ATRA may occur through AP-1. Previous studies have demonstrated that the Tax response of the IL-2R α chain promoter and the HIV LTR is mediated by the activation of NF-κB (42,43). Tax has been shown to increase the expression of NF-κB proteins and to prolong its localization to the nucleus where it is transcriptionally active (44,45). Tax also interacts specifically with IκB proteins (46).

In this context, IL-2 promoters have both NF-κB and AP-1 binding sites (47) which are important for promoter activity (48,49). The AP-1 transcription factor is composed of Jun and Fos proteins that function as transcriptional regulators in a heterodimeric complex (49). The formation of heterodimers is performed by the JNK kinase, which phosphorylates cJun allowing it to bind to c-Fos and form the complex AP-1 (50). Thus, the modulation of JNK may be responsible for decreasing Tax activity observed in Jurkat cells treated with ATRA. Tax-expressing cells prior to treatment resulted in an increase of IL-2 transcription and expression. However, our results demonstrated that cells treated with ATRA demonstrated a decrease in both the transcription and expression of IL-2. Using reporter constructs for AP-1 and NF-κB, two nuclear factors presented in the IL-2 promoter, revealed that ATRA specifically downregulated AP-1, but not NF-κB response element activities, compared with the untreated cells. These results strongly correlate with the decrease of JNK-1 and JNK-2 expression, suggesting that the Tax protein may regulate the development and function of Tax-expressing cells involved in cell-mediated response, partly by inhibiting factors required for the transcription of the IL-2 gene such as AP-1, a target of JNK activity.

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