Autocrine antiapoptotic stimulation of cultured adult T-cell leukemia cells by overexpression of the chemokine I-309
Tobias Ruckes, Domenica Saul, Jacques Van Snick, Olivier Hermine, and Ralph Grassmann

Adult T-cell leukemia (ATL) is an aggressive malignancy of CD4⁺ T cells caused by the human T-cell leukemia virus type 1 (HTLV-1). The viral leukemogenesis is critically dependent on its oncoprotein Tax because the protein as well as the virus can immortalize primary human lymphocytes to permanent growth. As a transcriptional transactivator, Tax can stimulate the expression of distinct cellular genes. Alterations in the expression levels of unknown growth-relevant genes may contribute to the changed growth properties of Tax-immortalized and leukemic cells. To identify genes that are linked to Tax transformation and ATL leukemogenesis, this study systematically compared the gene expression of cultured cells from patients with acute ATL with that of stimulated peripheral blood T lymphocytes. Several overexpressed RNAs that encode signal transduction functions were identified. These include a dual-specific protein phosphatase (PAC1), an interferon-inducible factor (ISG15), a basic helix-loop-helix transcription factor (DEC-1), and the secreted antiapoptotic chemokine I-309. The ATL cell culture supernatants contained an antiapoptotic activity that could be specifically inhibited by antibodies directed against I-309. Inhibition of I-309 receptor (CCR8) signaling by pertussis toxin increased the apoptosis rate of ATL cell cultures in the presence and absence of external apoptotic stimuli. Both the I-309–specific antiapoptotic activity and the proapoptotic effect of inhibitors of I-309 signaling suggest the existence of an antiapoptotic autocrine loop in ATL cells. Thus, the overexpression of this chemokine may inhibit apoptosis in ATL cells and could substantially contribute to their growth.

Introduction

Adult T-cell leukemia (ATL) is an aggressive leukemic disorder of CD4⁺ T cells caused by the human T-cell leukemia virus type 1 (HTLV-1). It develops in 1% to 3% of infected individuals after more than 2 decades of viral persistence. The contribution of the virus to the pathology of the disease is believed to involve 2 mechanisms. First, the virus directly stimulates the proliferation of the infected T cells independently of antigen. Second, the HTLV-1 infection leads to chromosomal abnormalities and an increased mutation rate in infected cells. In addition to its oncogenic properties, HTLV-1 induces a chronic neurologic disorder, termed HTLV-1–associated myelopathy or tropical spastic paraparesis (HAM/TSP), which primarily develops in patients with a specific HLA subtype.

As a complex retrovirus, HTLV-1 encodes the regulatory proteins Tax and Rex in addition to structural proteins. Rex is essential for the expression of the viral structural proteins and Tax acts as a transactivator of the viral promoter. In addition, Tax influences multiple cellular functions including gene expression and proliferation and it increases the mutation rate. Several lines of evidence indicate that the HTLV-1 regulatory protein p40tax plays a role in the leukocyte transforming features of the virus. The growth of primary human lymphocytes conditionally immortalized by Tax depends on Tax expression. The proliferation of these immortalized cells was reversibly arrested in the G₁ phase of the cell cycle by suppression of tax transcription, thus demonstrating that Tax stimulates the G₁-to-S phase transition in immortalized T lymphocytes.

The mechanism by which Tax influences G₁-to-S phase transition and the growth of transformed primary human T cells is not well understood and different Tax functions may cooperate in the stimulation of cell proliferation. This includes the property of Tax to directly interfere with the function of cell cycle–controlling proteins. For instance, Tax inhibits the transactivating functions of the tumor suppressor p53 and activates cyclin-dependent kinases CDK4 and CDK6; these CDKs are essential for the control of the G₁ phase progression. Tax has also been shown to interfere with DNA repair and in this way it possibly contributes to increase the cellular mutagenesis rate. Thus, also this Tax function may be crucial for leukemogenesis. Tax-induced mutations, which accumulate during the decades of viral persistence, might affect growth-relevant genes and as a consequence of dysregulated signaling result in overexpressed genes.

Its function as a modulator of cellular transcription is believed to play a pivotal role in the stimulation of host cell proliferation, because Tax affects the gene expression of a variety of growth-relevant genes. It activates genes encoding proto-oncogenes, the α-chain of the interleukin-2 (IL-2) receptor, cytokines, stimulators (cyclin D2), and repressors of cell cycle kinases (p21). On the other hand, the Tax protein represses the expression of DNA polymerase β, an enzyme important for DNA repair, the
CDK inhibitor p18,27 and the proapoptotic Bax protein.43 Additionally, further genes involved in signal transduction and glycoprotein synthesis have been found to be overexpressed in HTLV-1–infected T cells.28,46 Several of the mechanisms by which Tax influences the transcription of these genes are well characterized. However, for many genes that are up-regulated in HTLV-1–infected cells, including several signal transduction genes, the mechanism is not yet clear.47 The altered gene expression in HTLV-1–transformed cells is probably an important prerequisite for ATL induction. The malignant progression of the HTLV-1–infected cell into a leukemia cell is likely to affect the expression of growth-relevant genes. Hence, the identification of differentially expressed cellular genes is crucial for understanding the regulation of ATL leukemic cell proliferation.

In an attempt to identify genes that are linked to Tax transformation or ATL leukemogenesis, we systematically compared the gene expression patterns of cultured ATL cells with normal T lymphocytes using subtractive hybridization.48 By this approach multiple overexpressed messenger RNAs (mRNAs) coding for signal transduction proteins were identified, among them the antiapoptotic chemokine I-309. As reported here, the ATL cell cultures secreted an antiapoptotic activity and expressed the I-309 receptor CCR8. The Fas-mediated apoptosis rate of ATL cells was significantly increased when I-309 signaling was blocked. Taken together our data provide compelling evidence for an antiapoptotic autocrine loop in ATL cells.

Materials and methods

Cell culture and stimulation of primary human T cells

The CD4+ HTLV-1+ T-cell lines (Jurkat, HuT-78, Molt-4), the HTLV-1+ T-cell lines (C91-PL, MT-2, HuT-102), and the Tax-immortalized T-cell line (TAXI-1) were cultured as previously described.49 HTLV-1+ Mondi cells are derived from a HAM/TSP patient and were cultured in RPMI 1640 medium supplemented with 40% Panserin medium (PAN Biotech, Aidendorf, Germany), 20% fetal calf serum (FCS), 2 mM glutamine, antibiotics, and 40 U/mL IL-2 (Roche Diagnostics, Mannheim, Germany). BW5147C cells are murine thymic lymphoma cells and were cultured in Iscoves medium supplemented with 10% FCS, 1.5 mM l-glutamine, 0.24 mM L-asparagine, 0.55 mM l-arginine, and 50 μM β-mercaptoethanol (Sigma, Deisenhofen, Germany).50 The murine T-cell hybridoma cell line DO-Cep4 was kept in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, and 50 μg/mL gentamicin.51 To establish cell cultures from patients with acute ATL, peripheral blood lymphocytes from patients Champ, PaBe, StEd, and JuanaW were isolated. To study the influence of I-309–containing culture supernatants on ATL cell survival (ACS) was determined by subtraction of the background absorbance in the presence of A TL cell culture supernatant and DEX (ODDEX ) served as controls. To assess cell viability and activity under the conditions above, MT-2 (3-[4,5-dimethyl-2-thiazol-2-y]-2,5-diphenyl-tetrazolium bromide; Sigma) dye assays were carried out. After 48 hours, 250 μg/mL MTT was added and cells were incubated for further 4 hours. In viable cells MTT is converted into formasan dye granules. Cells were lysed by the addition of 0.1% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma) dye assays were carried out. After 48 hours, 250 μg/mL MTT was added and cells were incubated for further 4 hours. In viable cells MTT is converted into formasan dye granules. Cells were lysed by the addition of 180 μL 0.01 N HCl in isopropanol and the formasan absorbance was determined in a microplate reader set at 630 nm. To calculate total cell survival, absorbance in the presence of ATL cell culture supernatant and DEX (ODDEX ) was standardized to the background (absorbance in the presence of DEX only: ODDEX), the latter being arbitrarily set to 1. Additional cell survival (ACS) was determined by subtraction of the background (ODDEX).

**ACS = \[\frac{OD_{DEX+sup}}{OD_{DEX}} - OD_{DEX}\]**

To study the influence of I-309–containing culture supernatants on ATL cell growth in the presence of a proapoptotic stimulus, [3H]-thymidine...
incorporation assays were carried out. The ATL cells (3 × 10^9/well) were incubated in the presence of homologous 1:2.5 diluted supernatant, supplemented with anti-Fas antibody (10-500 ng/mL anti-human CD95; Becton Dickinson) and 12 μg/mL anti-I-309 or isotypic control antibody (monoclonal anti-IgG1; R&D Systems). In some experiments pertussis toxin (200 ng/mL) was added to abrogate intracellularly triggered I-309 signaling. After 24 hours [3H]-thymidine (Amersham Pharmacia, Freiburg, Germany) was added at a final concentration of 0.2 Ci/μmol. Cells were incubated for further 24 hours and harvested on glass fiber filters (Packard, Groningen, Netherlands). Bound radioactivity was quantified by phosphorimaging.

**Analysis of apoptosis**

One method for the detection of apoptotic cells was annexin V staining. Briefly, ATL-derived and control cells were cultured under the conditions described above in 48-well plates (4 × 10^5 cells/well in 400 μL). After 4 hours, the cells were harvested, washed in phosphate-buffered saline (PBS), and incubated with fluorescein isothiocyanate (FITC)-conjugated recombinant annexin V (Bendermedsystems Vienna, Austria) for 15 minutes. After staining, the cells were washed in PBS and measured by flow cytometry (FACScalibur; Becton Dickinson). Alternatively, cell death rate was measured using a modified propidium iodide (PI) staining assay, which allows the distinction between apoptotic, necrotic, and viable cells.53 Cells were treated with 25 μg/mL PI (Sigma) in 2% Triton-X100 and 0.05% sodium citrate and subjected to multiparameter flow cytometry analysis.

**Enzyme-linked immunosorbent assay**

The I-309 concentration in cell culture supernatants was determined by antigen capture enzyme-linked immunosorbent assay (ELISA) using monoclonal anti-I-309 capture and biotinylated anti-I-309 detection antibodies according to the manufacturer’s recommendations (R&D Systems). Briefly, 96-well microtiter plates were coated overnight with capture antibodies. The plates were incubated with serially diluted cell culture supernatants and biotinylated anti-I-309 detection antibody (R&D Systems). A standard curve was obtained using serial dilutions of recombinant human I-309 (Genentech, CA) and substrate solution (Biochrom, Berlin, Germany). Bound radioactivity was quantified by phosphorimaging.

**Results**

**Identification of differentially expressed cDNAs in ATL-derived cells**

Dysregulated gene expression frequently indicates or causes aberrant activation of growth-relevant signal transduction pathways. In ATL cells such changes may result from transactivation of cellular promoters mediated by viral proteins or mutagenesis or both. To identify genes linked to the malignant growth, the gene expression pattern of acute ATL cells was systematically compared with that of nontransformed normal postmitotic T lymphocytes using the PCR-based SSH. This approach resulted in the cloning of cDNAs derived from transcripts, which are overexpressed in the cultured HTLV-1–infected cells. After terminating the reaction with 1M H2SO4, the incubated in the presence of homologous 1:2.5 diluted supernatant, derived from transcripts, which are overexpressed in the cultured PCR-based SSH. This approach resulted in the cloning of cDNAs corresponding to HTLV-1 or genes that have already been shown to be up-regulated in HTLV-1–infected cells due to Tax transactivation. These included the IL-2 receptor α-chain and the CDK inhibitor p21WAF1/CIP1. 38,43 The genes for the transferrin receptor and thymosin β-4 have previously been found to be considerably overexpressed in ATL cells.54,55 The isolation of known HTLV-1–up-regulated genes confirmed that our experiment had resulted in cloning of differentially expressed sequences. To quantify the RNA expression, Northern blots were performed with following genes selected according to their biologic function in transcription control and cellular signaling. The basic helix-loop-helix transcription factor DEC-1 and the tumor necrosis factor-α

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HTLV indicates human T-cell leukemia virus type 1; n, number of complementary DNA isolations; TNF-α, tumor necrosis factor-α; IL-2, interleukin-2; IFN-γ, interferon γ; EST, expressed sequence tag.

*Tax-ATL-associated genes.

Table 1. Cellular genes isolated by suppressive subtractive hybridization
(TNF-α)-inducible B94 protein are putatively associated with differentiation.56,57 The dual-specific protein phosphatase PAC1 and the interferon-induced ISG15 gene were selected because of their contribution to cellular signaling.58,59 Calcylin, a calcium-binding protein, is expressed in a cell cycle–specific manner and dysregulated in various malignancies.60 The ferritin L chain and the chemokine I-309 play a role in cell proliferation and apoptosis control, respectively.50,61

These expression analyses revealed that the ATL-derived JuanaW cells contained increased mRNA levels of various transcription factor and signal transduction genes compared to PHA-stimulated T cells (PBMCPHA). To determine the fold overexpression of these genes, the specific radioactive signals were normalized to the GAPDH signals. A significant to strong overexpression could be found for the basic helix-loop-helix transcription factor DEC-1 (4-fold), the dual specific phosphatase PAC1 (7-fold), the calcium ion-binding protein calcyclin (3-fold), the ferritin L-subunit (3-fold), the TNF-inducible protein B94 (3-fold), and the interferon (IFN)-induced ISG15 gene (9-fold) as well as for the antiapoptotic chemokine I-309 (more than 40-fold) (Figures 1 and 2A). Further Northern analyses were carried out to investigate whether the up-regulation observed in JuanaW cells is specific for HTLV-1–infected or Tax-expressing T cells. The RNA samples used were extracted from HTLV-1-transformed cell lines (C91-PL, MT-2), a Tax-transformed cell line (TAXI-1), ATL cells (HuT-102, JuanaW), HTLV-1^+ CD4¹ leukemia cells (Jurkat, HuT-78), and PBMCPHA.

The mRNA levels of DEC-1, B94, and PAC1 were elevated in all HTLV-1-transformed and Tax-immortalized cell lines (Figure 1A). This expression pattern would be expected if the gene was transactivated by the viral transactivator Tax. The genes encoding the ferritin L chain, ISG15, and calcyclin were strongly overexpressed in many Tax-positive cell lines including ATL cultures (Figure 1B and data not shown). In contrast, I-309 mRNA could only be detected in the ATL-derived cell line JuanaW. These cells contained a remarkably large amount of I-309 transcripts (Figure 2A), which correlated well with the high prevalence of I-309 sequences among SSH cDNA clones (30 of 346).

**Strong overexpression of the chemokine I-309 in ATL-derived cells**

To discriminate whether the overexpression of I-309 was an individual feature of cultured JuanaW ATL cells or was due to its leukemic origin, permanently growing cultures from 3 other patients with acute ATL were established and checked for I-309 expression. Subsequent Northern blot analyses showed a huge overexpression of the I-309 gene in the ATL-derived cultures Champ, PaBe, JuanaW (Figure 2A), and StEd (data not shown). In contrast, I-309 transcripts could be neither observed in the acute lymphoblastic leukemia (ALL)-derived CD4¹ T-cell lines HuT-78 and Jurkat nor in PHA-treated PBMCs. Two specific bands corresponding to the expected mRNA sizes of approximately 550 and 2200 bases were detected. The most abundant transcript observed corresponded to the 550-base mRNA reported to code for the I-309 polypeptide.62 The larger transcript is of unknown function. To determine whether the high amount of RNA leads to the secretion of I-309 protein, cell culture supernatants were analyzed by antigen capture ELISA. As a result, I-309 was detected at concentrations ranging from about 35 ng/mL (PaBe) to more than 180 ng/mL (Champ) (Figure 2B). The same results were obtained after more than 6 months of permanent ATL cell culture. This observation indicates that I-309 overexpression is not due to uninfected activated T cells in the ATL cell preparation. In accordance with Northern blot results (data not shown), the supernatant of TAXI-1 cells, which are transformed by transduction of the Tax gene, contained only minimal amounts of I-309.

Neither HAM-derived Mondi cells nor the HTLV-1–transformed MT-2 cells produced any I-309 protein. Because both cell lines are known to express the Tax protein, this suggests that the viral
protein alone cannot account for the I-309 overexpression. Yet, the analysis of the I-309 promoter sequence with the MatInspector V2.2 (http://transfac.gbf.de/index.html) program revealed binding sites for both CREB and nuclear factor-κB (NF-κB) at upstream positions 393 bp and 272 bp, which exactly match the corresponding consensus sequences. Because Tax can stimulate the transcription via these sites, the transactivator may contribute to the activation of the I-309 gene in ATL cells. Taken together, these data suggest that the production and secretion of I-309 is a characteristic of ATL-derived cells, which is neither shared by HAM-derived nor by other HTLV-1– and Tax-expressing T cells.

Antiapoptotic activity of ATL cell supernatants is mediated by the chemokine I-309

To analyze whether the amount of I-309 secreted into the supernatant is sufficient to exert the ant apoptotic effect, ATL cell culture supernatants were titrated onto mouse thymoma BW5147C cells, which had been treated to undergo apoptosis. These cells have been shown to be highly susceptible to apoptosis induction by DEX. The addition of supernatants from 4 different ATL cultures (Champ, PaBe, JuanaW, StEd) protected the thymoma cells from DEX-induced apoptosis in a dose-dependent manner, whereas the supernatants of MT-2 and Jurkat had no ant apoptotic effect (Figure 3A). The addition of, for example, Champ supernatant increased cell survival by up to 100% compared with cell survival in the absence of ATL culture supernatant. This is consistent with ELISA results, which showed that Champ cells expressed the largest amounts of I-309 (Figure 2B). Flow cytometric analysis of the DNA content of individual nuclei confirmed that the increased cell survival is largely due to a reduced apoptosis rate (data not shown).
To confirm that the antiapoptotic activity was mediated by the I-309 receptor, CCR8, an inhibitor was added. Pertussis toxin interferes with the CCR8-associated G_i-protein signaling. As a result, the antiapoptotic effect of the ATL cell supernatant was dramatically reduced or even completely abolished in the presence of pertussis toxin (Figure 3B). The same result could be obtained with purified I-309 (Figure 3C). These observations suggested the involvement of a G_i-protein–coupled receptor like CCR8 in this pathway and corroborated the notion that I-309 is the factor in the supernatant that had prevented apoptosis. These data could be confirmed using 2 different methods (MTT and [3 H]-thymidine incorporation) in 2 murine cell lines (BW5147C, DO-Cep4) (Figure 3 and data not shown). Culturing both tester cells with ATL culture supernatant also increased cell survival by up to 45% in the presence of cisplatin, a DNA intercalating drug leading to strand breaks. Under these conditions, cell viability was considerably reduced by pertussis toxin (data not shown). To add further proof that the I-309 chemokine is the factor mediating the antiapoptotic activity, DEX-treated BW5147C cells were incubated with JuanaW supernatant and increasing amounts of monoclonal antibodies specific to I-309. As shown in Figure 3D, the antiapoptotic effect could be abolished by anti–I-309 antibodies in a dose-dependent manner, whereas the isotypic control antibody had no effect on the growth of the cells. Hence, the I-309 present in the ATL supernatant is capable of preventing apoptosis of T cells.

**Autocrine antiapoptotic activity of I-309 in an ATL cell culture contributes to cell proliferation**

The demonstration of the antiapoptotic activity in the ATL cell supernatant led us to the hypothesis that I-309 may act on the producer cells to increase their resistance to apoptosis. This, however, would require the presence of the cognate receptor (CCR8), which is highly specific for I-309, on these cells. To test for CCR8 gene expression, further Northern blot analyses were performed. As Figure 4 shows, CCR8 transcripts are present in high abundance in all ATL-derived cells tested. To obtain further proof for an antiapoptotic autocrine action of I-309, the chemokine signaling was suppressed in PaBe ATL cells that had been treated with monoclonal anti-Fas antibodies to undergo apoptosis (Figure 5). Increasing the concentration of Fas antibodies resulted in reduced [3 H]-thymidine incorporation in ATL (PaBe) and Tax-immortalized (TAXI-1) cells, which is due to an increased apoptosis rate within the culture. The level of apoptosis increased with antibody concentrations between 0 and 100 ng/mL and reached a plateau at more than 100 ng/mL, indicating a partial resistance of these cells to apoptosis. This is in good agreement with previous studies. At the highest anti-Fas concentration of 500 ng/mL, cell proliferation of both ATL and TAXI-1 cells was reduced by only 39% compared with untreated cells (Figure 5A). However, in the presence of anti–I-309 antibodies the proliferation of the ATL cells was further decreased to 55%, whereas the control (TAXI-1) was unaffected. Similar results were obtained with Champ ATL cells. In contrast, the addition of isotype-matched control antibody did not influence cell growth. The corresponding proliferation rate was
identical with the one obtained in the absence of antibody. Interestingly, even in the absence of external apoptotic signals, the anti–I-309 antibodies alone diminished cell proliferation by some 6% (Figure 5A, 0 μg/mL anti-Fas, + anti–I-309 antibody). A triple combination of anti-Fas, anti–I-309, and pertussis toxin did not substantially change the rate of cell proliferation. The moderate additional reduction in cell proliferation is probably due to pertussis toxin–mediated inhibition of I-309 signaling triggered by intracellular ligand-receptor interaction. Thus, this observation also supports the notion that interference with the I-309 receptor and no other G-protein–coupled receptor had caused the reduced cell proliferation. To specifically determine apoptosis, annexin V staining was applied, which detects a marker of early apoptosis.67 This analysis revealed that Fas-induced apoptosis in Mondi and TAXI-1 control cells remained unchanged in the presence of I-309–specific antibodies (Figure 5B and data not shown). In contrast, the anti–I-309 antibodies increased early apoptosis in ATL (PaBe) cells (Figure 5B). Together these data corroborate an autocrine antiapoptotic effect of I-309 in the presence of external apoptotic stimuli.

The high level of I-309 might counteract not only external but also internal proapoptotic stimuli. To elucidate this possibility, ATL cells (JuanaW) were incubated with increasing concentrations of pertussis toxin. As shown in Figure 6A the drug inhibited the proliferation of JuanaW cells in a dose-dependent manner; at the highest pertussis toxin concentration, cell proliferation was reduced by more than 50%. Similar results were obtained with PaBe and Champ ATL-derived cells (data not shown). In contrast, the proliferation of both CD4+ control cell lines, HuT-78 (T-ALL) and MT-2 (HTLV-1–transformed), was not affected. This indicates that pertussis toxin has no unspecific cytotoxic effect on uninfected and HTLV-1–infected T cells. To verify that the reduced proliferation was due to apoptosis, DNA contents of pertussis toxin–treated cells were determined by a modified PI staining assay that allows the simultaneous distinction of viable, apoptotic, and necrotic cells (Figure 6B).53 These analyses showed that pertussis toxin significantly increased apoptosis, whereas necrosis was only minimally affected. These results were confirmed by annexin V staining (Figure 6C). Hence, all 3 assays demonstrate that pertussis toxin induced apoptosis in ATL cells and, therefore, suggest a role for I-309 signaling in counteracting inherent apoptosis. Taken together, the autocrine antiapoptotic CCR8/I-309 signaling may stimulate the proliferation of ATL cells by rendering the leukemic cells less susceptible to internal and external apoptotic signals.

Discussion

To link lymphocyte genes to HTLV-1–induced leukemogenesis, we have identified several overexpressed mRNAs in cultured ATL cells. Many of the corresponding proteins have functions in signal...
transduction and could, thus, play a role in deregulated proliferation of the malignant cells. Up-regulated genes included the dual-specific protein phosphatase PAC1, the bHLH-transcription factor DEC-1, and the TNF-α inducible protein B94. It is conceivable that the overexpression of those genes in all tested HTLV-I–transformed and Tax-immortalized cell lines is mediated by the viral transactivator Tax. For instance, the activation of DEC-1 may be mediated by the CREB pathway. This is suggested by the observation that CREB-stimulating agents have been found to increase DEC-1 transcription and that Tax stimulates transcription via CREB. Likewise, B94 mRNA is rapidly induced by proinflammatory stimuli including TNF-α, IL-1β, and LPS, which induce the activation of NF-κB. Because Tax also activates NF-κB, Tax presumably stimulates the expression of B94.

The overexpression of the ISG15 and I-309 genes could only be found in JuanaW and other ATL-derived cell cultures. ISG15, a 15-kd protein encoded by an interferon γ (IFN-γ)-stimulated gene (ISG), is secreted from lymphocytes and induces IFN-γ production. This property possibly contributes to the increased IFN-γ mRNA levels found in JuanaW cells (Table 1) and in other ATL cells.

Four of 4 ATL patient–derived cells expressed the protein at strongly increased levels, but none of the in vitro HTLV-I–infected cell lines did so. This restricted expression pattern suggests that I-309 overexpression is a characteristic of ATL cell cultures. Long-term cultivation of one of the used lines indicated that I-309 overexpression can be maintained for a minimum of 2 years in IL-2–dependent cultures. The overexpression of the I-309 gene exclusively in ATL cells indicates that viral functions including Tax alone are not sufficient to activate its transcription. However, Tax may contribute to the I-309 overexpression. This is supported by the notion that Tax stimulates the IL-1 gene, which is required for I-309 gene expression. Furthermore, the murine homologue (TCA3) carries a NF-κB site in its promoter, which might respond to Tax-mediated NF-κB activation.

Sequence analysis of the I-309 promoter revealed potentially Tax-responsive upstream CREB and NF-κB binding sites.

The chemokine I-309 and its murine homologue TCA3 were both initially identified in T cells that had been restimulated after a history of previous activation. I-309 is a member of the family of CC chemokines, which contains 2 disulphide bonds at the N terminus. As a structural peculiarity, I-309 displays a third well-conserved disulfide bond.

In conclusion, we found that the expression of I-309 is greatly appreciated. We thank Kerstin Haller and Claudia Matteucci for helpful scientific discussions.

Acknowledgments

The technical assistance of Elisabeth Derow and Daniel Romaker is greatly appreciated. We thank Kerstin Haller and Claudia Matteucci for helpful scientific discussions.

References


Autocrine antiapoptotic stimulation of cultured adult T-cell leukemia cells by overexpression of the chemokine I-309

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