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Cytokine Induction in HTLV-I Associated Myelopathy and Adult T-Cell Leukemia: Alternate Molecular Mechanisms Underlying Retroviral Pathogenesis

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Abstract The human T-cell lymphotropic virus type I (HTLV-I) is capable of inducing a variety of host cellular genes including many of the cytokines responsible for immune regulation and osteoclast activation. This derangement in cytokine expression may contribute to the panoply of disease states associated with HTLV-I infection such as the adult T-cell leukemia (ATL) and HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). We wished to determine if there was a correlation between the expression of an array of cytokines and the diverse clinical manifestations of ATL and HAM/TSP. Utilizing the techniques of specific mRNA amplification by the polymerase chain reaction (PCR) as well as Northern blotting, we analyzed the ex vivo mRNA expression of γ-interferon (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and transforming growth factor-β_1 (TGF-β_1) in the peripheral blood of HAM/TSP and ATL patients as well as asymptomatic seropositive carriers. IFN-γ, TNF-α, and IL-1β transcripts were up-regulated in patients with HAM/TSP and seropositive carriers when compared to their levels in ATL and normal controls. In contrast, the ATL patients constitutively expressed higher levels of TGF-β_1 mRNA than HAM/TSP and seropositive carriers. In addition, TNF-α and IL-1β serum levels were elevated in HAM/TSP, but not in ATL patients nor seropositive carriers. However, the circulating leukemic cells from ATL patients secreted increased levels of TGF-β_1 protein into the culture medium than T-cells derived from HAM/TSP patients. Collectively these results suggest that induction of IFN-γ, TNF-α, and IL-1β in HAM/TSP may initiate an inflammatory cascade with subsequent events leading to immune mediated destruction of the central nervous system in these patients. Expression of osteoclast activators such as TNF-α and IL-1β is not associated with hypercalcemia in ATL. Finally, impaired cellular and humoral immune responses present in ATL, but not in HAM/TSP, may be related to elevated levels of TGF-β_1 produced by the leukemic cells. These differences in retroviral-induced host cytokine expression in ATL and HAM/TSP suggest alternate roles in disease pathogenesis.

Key words: γ-interferon, tumor necrosis factor-α, interleukin-1β, transforming growth factor-β_1, immunosuppression

Cytokines play an important role in many biological processes including the proliferation and differentiation of the cellular elements within the immune system. Dysregulation of their tightly controlled expression may result in a variety of autoimmune and neoplastic disorders [1–4]. The human T-cell lymphotropic virus type I (HTLV-I), via its transcriptional activator Tax, is capable of inducing an array of cellular genes in vitro, especially those that encode for the products of activated lymphocytes [5–8]. Previous studies have reported that HTLV-I infected cell lines abnormally express interleukins 2, 3, 5, and 6 as well as tumor necrosis factor-α (TNF-α), lymphotoxin, granulocyte/macrophage colony stimulating factor, and γ-interferon (IFN-γ) [9–12]. Aberrant regulation of host cytokines by the retroviral Tax protein may contribute to the pathogenesis and

Abbreviations: ATL, adult T-cell leukemia; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; HTLV-I, human T-cell lymphotropic virus type I; IFN-γ, gamma-interferon; IL-1β, interleukin-1-beta; PCR, polymerase chain reaction; TGF-β_1, transforming growth factor-beta-1; TNF-α, tumor necrosis factor-alpha.

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clinical diversity of the two disease states associated with HTLV-I infection known as the adult T-cell leukemia (ATL) [13] and HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) [14,15]. ATL is an aggressive malignancy of mature T-cells frequently associated with infiltrative skin lesions, hepatosplenomegaly, lymphadenopathy, hypercalcemia, and an increased incidence of opportunistic infections [16]. The paraneoplastic features such as immunosuppression, hepatic dysfunction, and hypercalcemia may reflect an altered regulation of specific cytokine genes by HTLV-I transactivation. Recently, freshly isolated ATL cells were shown to express increased mRNA levels of both the osteoclast activator interleukin-1β (IL-1β) and the potent immunosuppressive factor transforming growth factor-β_1 (TGF-β_1) [17,18]. However, neither study compared this up-regulation of IL-1β and TGF-β_1 in ATL with respective levels of expression in HAM/TSP to better define their clinical relevance to retroviral pathogenesis. HAM/TSP is a chronic, slowly progressive, neurological condition characterized by spasticity and motor weakness along with bladder and bowel dysfunction [19]. In contrast to ATL, patients with HAM/TSP are rarely immunosuppressed, but rather display an ongoing state of lymphocyte activation mediated by Tax induction of interleukin-2 and its receptor [20]. Although ATL and HAM/TSP are associated with the same retrovirus, differences in both functional and clinical characteristics suggest an altered host immune responsiveness. In this study we have examined the expression of IFN-γ, TNF-α, IL-1β, and TGF-β_1 in ATL and HAM/TSP patients, as well as asymptomatic seropositive carriers, to determine which cytokines are potential mediators of the diverse pathophysiological characteristics observed in these HTLV-I related disorders.

MATERIALS AND METHODS

Patient Populations

Eight patients with ATL, 8 with HAM/TSP, 8 asymptomatic seropositive carriers, and 6 seronegative individuals were studied. The ATL group consisted of 4 females and 4 males with a mean age of 43 years (range, 24–64). Five were of Caribbean descent and three were American-born. All of the ATL patients had impaired delayed-type hypersensitivity manifested by cutaneous anergy and three were hypercalcemic (serum calcium > 5.3 meq/L). A diagnosis of ATL was established by the following criteria: presence of a circulating pool of abnormal lymphocytes with a predominant $CD4^+$, $CD8^-$, $CD25^+$ T-cell phenotype, demonstration of a clonal integration of the HTLV-I genome as well as a clonal rearrangement of the T-cell receptor β-chain gene in the circulating lymphocytes, and presence of HTLV-I antibodies in the serum. The HAM/TSP population was comprised of 3 females and 5 males with a mean age of 54 years (range, 36–67) and all but two were of Caribbean descent. None of the 8 HAM/TSP patients had any clinical evidence of immunosuppression and formal skin testing in 3 revealed a normal delayed-type hypersensitivity response. A diagnosis of HAM/TSP met the criteria established by the World Health Organization [21]. The asymptomatic seropositive carriers were all from the Caribbean Basin and six healthy NIH blood donors served as normal controls.

Preparation of Cells and RNA Isolation

Peripheral blood mononuclear cells (PBMC) from the patient populations were separated from heparinized or EGTA-treated venous blood by centrifugation over lymphocyte separation medium (LSM, Organon Technika) at 600*g* for 30 minutes. Interface cells were washed twice and then lysed in 4M guanidinium thiocyanate or RNAzol (Cinna/Biotecx). Total cellular RNA was isolated by the method of Chirgwin et al. [22] or Chomczynski and Sacchi [23], quantified by spectrophotometry, and stored at −70°C. In a subset of patients, the PBMC were further purified to obtain a highly enriched $CD4^+$ T-cell population. The isolated PBMC underwent density gradient centrifugation through 46.5% Percoll (Pharmacia) at 800*g* for 20 minutes. Percoll-dense cells were then incubated with a combination of the following antibodies: OKT8 (anti-CD8) at 5 μg/mL, THB5 (anti-CD21) at 1 μg/mL, B4 (anti-CD19) at a 1:100 dilution, 3G8 (anti-CD16) at 5 μg/mL, and 63D3 (anti-monocyte antigen) at 5 μg/mL, for 30 minutes on ice. For lymphocyte suspensions from ATL patients, 3A1 (anti-CD7) at 5 μg/mL was included in the preceding cocktail of antibodies to remove the non-leukemic T-cells. The antibody coated cells then underwent magnetic bead depletion with goat anti-mouse IgG beads (Collaborative Research; 70 particles per target cell). Following incubation with magnetic beads on ice for 20 minutes, the cell suspension was placed on a magnetic separator and residual non-magne-

tized cells were collected and retreated with above antibodies for 30 minutes. Antibody coated cells were then mixed with goat anti-mouse magnetic Dynabeads (Bioproducts for Science; 5 particles per target cell) on a rotator in the cold for 1 hour. This cell suspension was exposed to a magnetic field for 5 minutes and free, non-magnetized cells were collected. An aliquot of each purified cell fraction (via magnetic bead depletion) was analyzed by flow cytometry verifying the presence of a highly purified $CD4^+$ population with < 1% $CD8^+$, $CD20^+$, $CD14^+$, or $CD16^+$ contaminating cells.

Analysis of IFN-γ and TNF-α mRNA Expression

Oligonucleotide primers and probes (Table I) were synthesized on an automated DNA synthesizer (Applied Biosystems) by the phosphoramidite method [24]. Base sequences for IFN-γ, TNF-α, and actin amplifications were derived from published sequences by Gray et al. [25], Pennica et al. [26], and Ponte et al. [27], respectively. Briefly, IFN-γ and TNF-α cDNA synthesis was performed on 1 μg of PBMC RNA from the same six patients in each study population and from the HTLV-I infected cell line HUT 102 as previously described [20], using the IFN-γ/2 or TNF-α/2 primers to respectively direct specific first strand synthesis. Negative controls included the uninduced Jurkat T-cell line as well as a reagent control without template RNA. Reverse transcription products were diluted to obtain a final PCR mixture containing 25 mM Tris-HCL (pH 8.3), 50 mM KCI, 1.5 mM $MgCl_2$ all four dNTPs at 0.2 mM each, 0.3 μM IFN-γ/1 or TNF-α/1 primer, 0.3 μM IFN-γ/2 or TNF-α/2 primer, and 0.01% gelatin in 100 μL. This mixture was heated to 94°C for 5 minutes, quenched on ice, and supplemented with 5 U of Thermus aquaticus polymerase (Perkin-Elmer/Cetus). Samples were subjected to 30 cycles of amplification consisting of denaturation for 1 minute at 94°C, primer annealing for 1 minute at 53°C, and polymerization for 2 minutes at 72°C. An actin-targeted sequence served as an internal test control and the amplified 636 base-pair actin signal was detected by an internal 40-base probe (see Table I). PCR products following IFN-γ or TNF-α mRNA amplification were analyzed by Southern blot hybridization as follows: 20 μL of each PCR reaction were electrophoresed through a 1% agarose gel, and then blot-transferred on to nitrocellulose filters (BAS NC, Schleicher and Schuell). For TNF-α the filter was prehybridized in a previously described buffer [28], and hybridized overnight at 60°C with 4.5 mL of buffer containing a ^{32}P nick-translated (1×10^7 dpm/mL) cDNA probe for human TNF-α (pAW 711, obtained from the American Type Culture Collection). Filter was washed [28] and autoradiographed for 4 hours at −70°C. IFN-γ filter was hybridized with a ^{32}P-end-labelled internal 48-base IFN-γ sequence (see Table I) using standard conditions for hybridization of oligonucleotide probes [29]. The blot was washed twice in 2X SSC and 0.1% SDS at 55°C for 30 minutes. Autoradiography was performed for 24–48 hours at −70°C with an intensifying screen.

Analysis of IL-1β and TGF-$β_1$ mRNA Expression

Equal amounts of RNA (10 μg) from patient PBMC, HUT 102, and Jurkat cell lines, as well as from resting and PHA-activated T-cells, were

TABLE I. Oligonucleotide Primers and Probes for Detection of IFN-γ, TNF-α, and Actin mRNAs

Oligomer	Function[a]	Location[b]	Sequence (5′—3′)
IFN-γ/1	Primer (+)	170–187	TGTTACTGCCAGGACCCA
IFN-γ/2	Primer (−)	499–482	GCGTTGGACATTCAAGTC
IFN-γ/P	Probe (−)	409–362	CATGTCTTCCTTGATGGTCTCCAC-ACTCTTTTGGATGCTCTGGTCATC
TNF-α/1	Primer (+)	375–392	CAGGCAGTCAGATCATCT
TNF-α/2	Primer (−)	803–786	ATAGTCGGGCCGATTGAT
Actin/1	Primer (+)	309–328	TTCTACAATGAGCTGCGTGT
Actin/2	Primer (−)	944–925	GCCAGACAGCACTGTGTTGG
Actin/P	Probe (+)	601–640	ACTACCTCATGAAGATCCTCA-CCGAGCGCGGCTACAGCTT

[a](+) and (−) designates complementarity of oligomer.
[b]Oligonucleotide location corresponds to the published numbered cDNA sequences for IFN-γ[25], TNF-α[26], and Actin[27], respectively.

subjected to electrophoresis through 1% agarose gels containing 0.66 M formaldehyde. Gels were photographed and then blot-transferred on to nitrocellulose. Blots were hybridized in succession with the following ^{32}P-random-primed cDNA probes according to the method of Church and Gilbert [30]: a 1.3 kB PstI cDNA clone of human IL-1β (pβIL-1; provided by S. Clark, Genetics Institute), a 218-bp single-stranded anti-sense TGF-β_1 probe [31], and a 1.9 kB BamHI cDNA clone for human β-actin (provided by W. Tsang, National Institutes of Health).

Serum Assays for IFN-γ, TNF-α, and IL-1β

Clear, non-hemolyzed serum was isolated from four individuals in each patient population and stored frozen at −20°C. All patient samples were assayed in duplicate for IFN-γ, TNF-α, and IL-1β with the following commercial kits: Gamma Interferon Radioimmunoassay (Centocor), Biokine TNF Test Kit (T cell Sciences), and Quantikine IL-1β Immunoassay (R & D Systems). A standard curve for the recombinant cytokine of interest, containing the same percentage of serum as the test samples, was included in each assay. The minimum detectable levels for the respective cytokines was determined to be 0.1 units/mL for IFN-γ, 10 pg/mL for TNF-α, and 4.5 pg/mL for IL-1β. The mean (± SEM) IFN-γ, TNF-α, and IL-1β concentrations were calculated for each patient population. The distribution of values for the respective cytokines among the patient populations was compared using the Wilcoxon rank-sum statistic.

Quantification of TGF-β_1 Secreted by Circulating Cells From HTLV-I Infected Patient Populations

Tissue culture flasks were coated with 0.5% human serum albumin in phosphate buffered saline for 30 minutes and then washed thoroughly with phosphate buffered saline. Purified CD4$^+$ T-cell populations from 2 ATL and 2 HAM/TSP patients as well as from 1 asymptomatic seropositive carrier and 2 normal controls were resuspended in serum-free Nutridoma-HU-supplemented (1:75) RPMI 1640 at 3×10^6 cells/mL, and cultured for 72 hours at 37°C. PBMC from two additional ATL patients [18] were cultured in identical fashion. Culture supernatants were harvested and spun again at 10,000*g* for 10 minutes to remove residual cellular debris. TGF-β levels in the conditioned media were measured by the specific sandwich ELISA for TGF-β_1 as previously described [32].

RESULTS

Expression of IFN-γ and TNF-α mRNA in Patients With ATL Versus HAM/TSP

Since previous studies had demonstrated an induction of the genes that encode for both IFN-γ and TNF-α in HTLV-I infected cell lines, we wished to further examine the regulation and potential physiological roles of these cytokines in patients with HTLV-I-associated diseases. We used the PCR technique to selectively amplify IFN-γ and TNF-α mRNA sequences from the same patient samples and quantitatively determine their respective levels of expression in the PBMC of ATL and HAM/TSP patients as well as seropositive carriers and normal controls. A 330-bp sequence representing IFN-γ mRNA was strongly amplified from all six HAM/TSP patients and six seropositive carriers studied, but in the majority of ATL patients and normal controls IFN-γ expression could not be detected. A single representative Southern blot including four patients in each diagnostic category is displayed in Figure 1. Furthermore, IFN-γ was highly expressed in the HTLV-I-infected cell line HUT 102, but not in Jurkat, an HTLV-I-negative T-cell line (data not shown). TNF-α transcription, assessed by amplification of a specific 429-bp sequence, was markedly up-regulated in HAM/TSP and seropositive carriers as compared to the constitutive low level of expression in ATL and normal controls (Fig. 2). Despite increased mRNA expression of TNF-α in HUT 102, no representative sequence could be amplified from the Jurkat T-cell line (data not shown). Thus the genes encoding the inflammatory-promoting cytokines IFN-γ and TNF-α were transactivated in both HAM/TSP and seropositive carriers, but not in ATL.

Expression of IL-1β and TGF-β_1 mRNA in Patients With ATL Versus HAM/TSP

Northern blot analysis was performed on total RNA from ATL and HAM/TSP patients, as well as seropositive carriers, to compare the levels of IL-1β and TGF-β_1 cellular gene expression in these HTLV-I infected patient populations. The 1.8 kB IL-1β mRNA was expressed to some degree in four out of four HAM/TSP patients and seropositive carriers, but could only be detected in two out of four patients with ATL and one normal control (Fig. 3). Furthermore, IL-1β transcription was observed in only one of the three ATL patients who were hypercalcemic

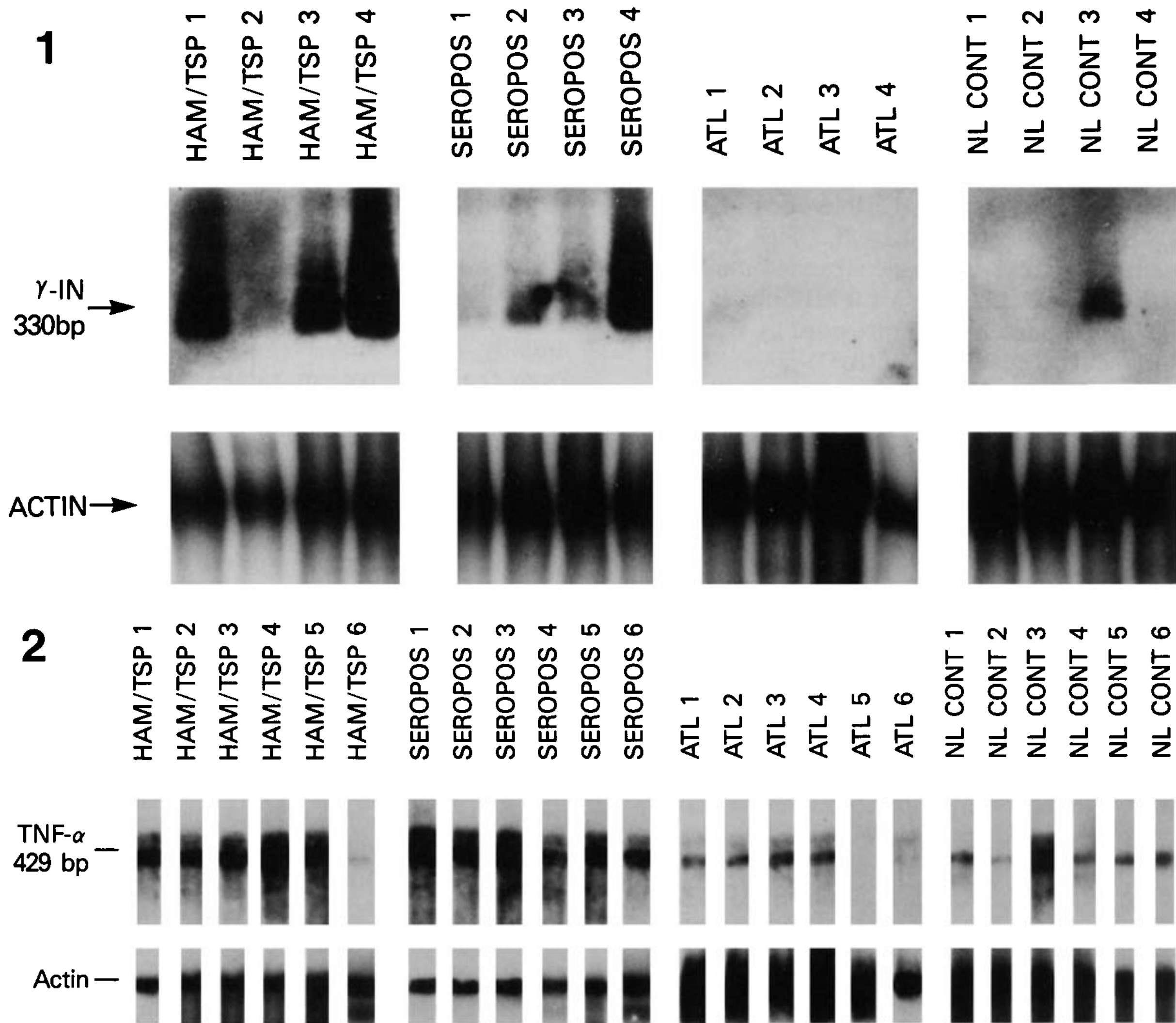

Fig. 1. Expression of IFN-γ mRNA in HTLV-I associated disorders. Total RNA from patients with HAM/TSP or ATL, seropositive carriers (SEROPOS), and normal controls (NL CONT) was subjected to reverse transcription followed by PCR amplification using primer pairs homologous to regions within the IFN-γ mRNA (see Table I). An aliquot of each patient's PCR reaction mixture was electrophoresed, blot-transferred, and hybridized with a ^{32}P-end labeled internal oligonucleotide probe, IFN-γ/P. IFN-γ mRNA was detected by the presence of an amplified 330-bp fragment. An actin sequence was amplified from each RNA sample, in the same reaction vial as IFN-γ, using the actin/1 sense and the actin/2 antisense primers. The amplified actin signal was detected with a 40-base internal probe.

Fig. 2. Detection of cellular TNF-α expression in HTLV-I associated disorders. Total RNA from patients with ATL or HAM/TSP, as well as from seropositive carriers (SEROPOS) and normal controls (NL CONT), was subjected to TNF-α-specific reverse transcription and then PCR amplification using the TNF-α/1 and TNF-α/2, sense and antisense primers, respectively. These primers directed amplifications of a 429-bp signal within the TNF-α mRNA. After transfer of the PCR reaction mixtures to nitrocellulose, the amplified TNF-α mRNA was detected by hybridization of the filter with a ^{32}P-nick-translated cDNA probe for human TNF-α. An actin sequence was also targeted for amplification in the same reaction vial as TNF-α using primer pairs homologous to regions within the actin mRNA. ATL patients 1, 2, and 4 were hypercalcemic with serum calcium values > 5.3 meq/L.

(ATL 2, 4, and 1 in Fig. 3). In contrast, a significant induction of TGF-β_1 mRNA was seen in the PBMC of ATL patients as compared to the PBMC isolated from HAM/TSP patients, seropositive carriers, normal controls, and a population of resting T-cells (Fig. 4). Moreover, TGF-β_1 expression in freshly isolated ATL PBMC was comparable to that seen in HUT 102 or PHA-activated T-cells (data not shown).

Serum Levels of IFN-γ, TNF-α, and IL-1β in HTLV-I Infected Patients

Dysregulation of IFN-γ, TNF-α, and IL-1β expression was correlated with their respective

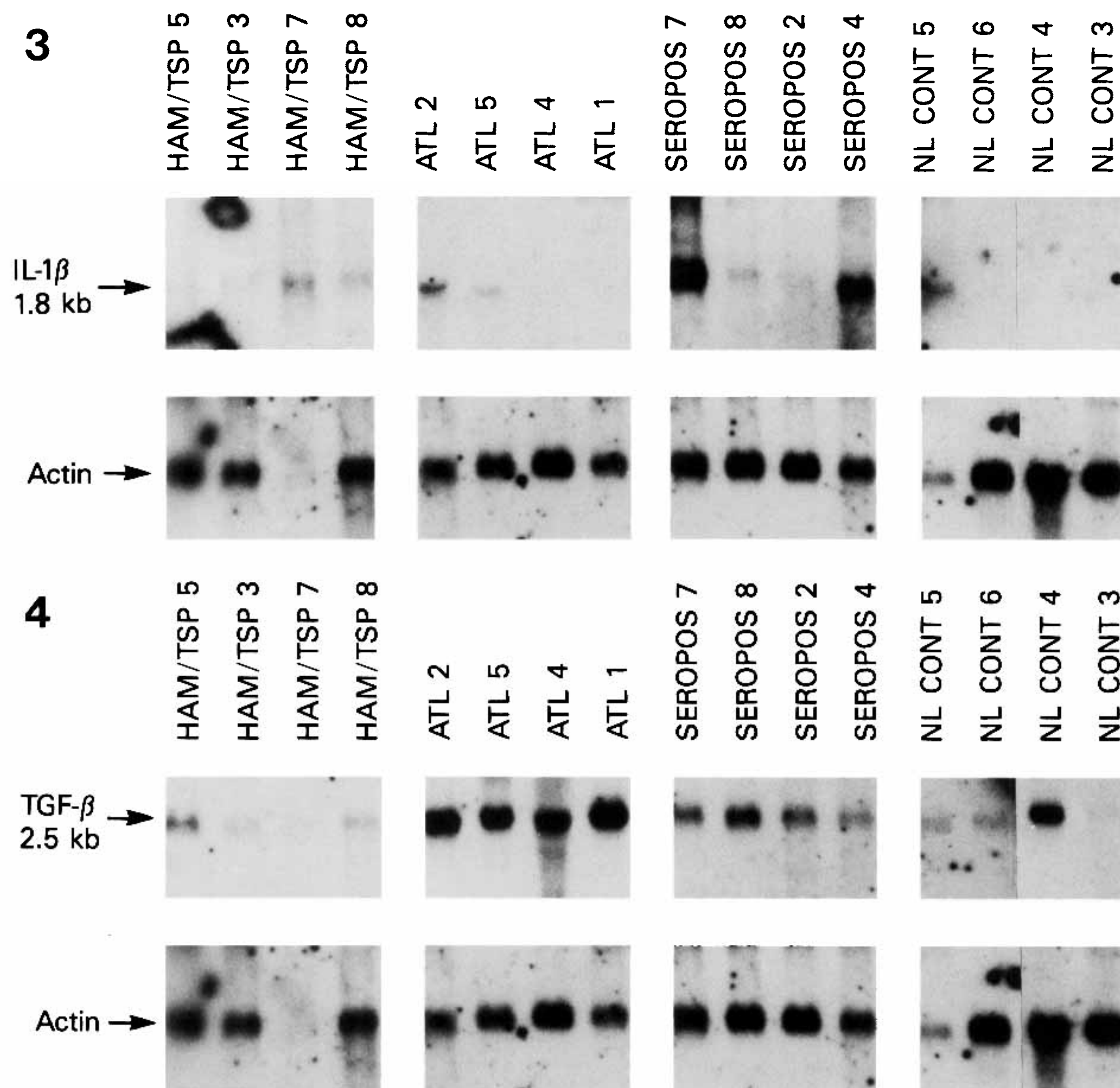

Fig. 3. Northern blot analysis of IL-1β mRNA expression in patients with HTLV-I related disorders. Ten μg of total RNA from the PBMC of HAM/TSP, ATL, seropositive carriers (SEROPOS), or normal patients (NL CONT), as well as from control resting T-cells (NL CONT 3), was subjected to electrophoresis and blotted onto a nitrocellulose filter. The filter was hybridized with a random-primed human IL-1β cDNA probe to detect the 1.8 kb IL-1β mRNA and autoradiographed for 72 hours. **Bottom panel:** The same filter rehybridized with a cDNA probe for β-actin. ATL patients 2, 4, and 1 were hypercalcemic.

Fig. 4. Northern blot analysis of TGF-β_1 mRNA expression in patients with HTLV-I related disorders. The filter in Figure 3, containing total RNA from the PBMC of HTLV-I infected or normal patients, as well as from control resting T-cells (NL CONT 3), was hybridized with a radiolabelled antisense TGF-β_1 probe to detect the 2.5 kB TGF-β_1 mRNA. Following hybridization, the Northern blot was washed and autoradiographed for 24 hours. **Bottom panel:** Corresponding levels of actin transcription detected by rehybridizing filter with a β-actin cDNA probe.

translational products by measuring the levels of these cytokines in HAM/TSP, ATL, seropositive carrier, and normal control serum samples. There was no measurable IFN-γ in any of the serum samples assayed. In contrast, TNF-α levels in HAM/TSP (129 ± 52 pg/mL) were significantly elevated ($P < .03$) above ATL (15 ± 5 pg/mL), seropositive carriers (15 ± 4 pg/mL), and normal controls (14 ± 4 pg/mL). Furthermore, modest but significantly elevated IL-1β levels were detected in the HAM/TSP patients (305 ± 258 pg/mL) as compared to ATL (33 ± 12

pg/mL, $P < .03$), seropositive carriers (23 ± 17 pg/mL, $P < .03$), and normal controls (3 ± 5 pg/mL, $P < .03$).

TGF-β_1 Secreted by CD4$^+$ T-Cells From Patients With HTLV-I Associated Disorders

We measured the level of TGF-β_1 protein secreted by the circulating cells from the HTLV-I infected patient populations to correlate the observed variations in TGF-β_1 mRNA expression with its respective gene product. Since the source of TGF-β_1 transcription detected by Northern analysis might have been due to a subset of monocytes, platelets, or CD8$^+$ cells "contaminating" the HTLV-I infected T-cells in the PBMC pool, we measured TGF-β_1 production in a purified population of CD4$^+$ T-cells isolated from patients with HTLV-I related disorders and normal controls as well as in ATL PBMC (Table II). The enriched CD4$^+$ malignant T-cells from 2 ATL patients, and PBMC from two additional ATL patients, secreted approximately ten times the level of TGF-β_1 protein than those CD4$^+$ populations isolated from 2 HAM/TSP patients, a seropositive carrier, and 2 normal controls.

TABLE II. TGF-β_1 Levels in HTLV-I Infected Patients and Normal Controls*

Patient		TGF-β_1 (pM)
ATL	1	39.12 ± 2.3[18]
ATL	2	8.91 ± 0.7[18]
ATL	7	7.90 ± 0.2
ATL	8	5.80 ± 0.5
HAM/TSP	7	<0.30
HAM/TSP	8	0.71 ± 0.1
SEROPOS	8	<0.30
NL CONT	4	<0.30
NL CONT	5	<0.30

*CD4$^+$ T-cells or PBMC [18] from each of the patients above were cultured for 72 hours in serum-free media as described in Materials and Methods. TGF-β_1 levels were assayed by a specific sandwich ELISA for TGF-β_1. All values represent the mean of three measurements ± SD. NL CONT, normal control; SEROPOS, seropositive carrier.

DISCUSSION

In this report we have described alterations in host gene expression and cytokine production which accompany HTLV-I infection in vivo. Specifically, we have identified an array of cytokines, including IFN-γ, TNF-α, and IL-1β, whose transcription was up-regulated in HAM/TSP and seropositive carriers as compared to ATL. This up-regulation was accompanied by an elevation of TNF-α and IL-1β exclusively in the sera of the HAM/TSP patient population. Conversely, the ATL patients expressed higher levels of both TGF-β_1 message and protein than their HTLV-I infected counterparts with neurological disease. These observed differences in retroviral induction of host cytokine genes may underly the diverse pathophysiological conditions associated with the respective HTLV-I disease states. We have previously demonstrated an increased expression and production of interleukin-2 leading to a state of T-cell activation in HAM/TSP, but not in late-stage acute ATL [20]. Analogous to interleukin-2 expression in patients with HTLV-I associated diseases, IFN-γ transcription was also up-regulated in HAM/TSP and seropositive carriers, but could not be consistently detected in ATL. This variation in IFN-γ mRNA expression between HTLV-I infected patients may have been anticipated because IFN-γ is induced by interleukin-2 and TNF-α [33], both of which were shown to be up-regulated in HAM/TSP and seropositive carriers compared to ATL. Similar to interleukin-2, one would have predicted an accumulation of the IFN-γ gene product in HAM/TSP sera as well, since IFN-γ is also regulated at the transcriptional level [34]. However, we were unable to detect any IFN-γ in the sera of these patients. Given the fact that IFN-γ induces class II major histocompatibility antigens [35], and HAM/TSP patients have an increased expression of class II antigens in their central nervous system [36], the IFN-γ protein may circulate in their cerebrospinal fluid. Thus, induction of IFN-γ in HAM/TSP may still play a role in eliciting a detrimental T-cell response leading to inflammatory central nervous system damage in these patients. The inappropriate cell-mediated inflammation associated with HAM/TSP may be augmented by TNF-α which has been shown to directly potentiate the T-cell response to antigenic challenge [37]. Neurological damage in HAM/TSP may also depend upon the ability of TNF-α to induce morphological changes in the vascular endothelium [38,39], creating a breach in the blood-brain barrier by which cytotoxic T-cells can enter. Clearly IFN-γ, TNF-α, and IL-1β are not the sole factors contributing to the pathogenesis of HAM/TSP since we have demonstrated an equivalent up-regulation of these cy-

tokines in asymptomatic seropositive carriers. However, the dysregulation of these inflammatory cytokines, was accompanied by a post-transcriptional increase of serum TNF-α and IL-1β in HAM/TSP providing a fertile environment for precipitating severe neurological injury in the genetically susceptible seropositive carriers.

In addition to IFN-γ we were interested in studying the gene expression of TNF-α and IL-1β in patients with HTLV-I related disorders because both cytokines are capable of stimulating bone resorption by activating osteoclasts. Thus, either cytokine may be a potential mediator of hypercalcemia in ATL [40]. Furthermore, TNF-α can induce the production of IL-1β in vitro [41]. Using Northern analysis, Tschachler et al. could only detect TNF-α mRNA in cell lines established from ATL patients, but not from their fresh leukemic cells [12]. With a more sensitive PCR technique, we were unable to demonstrate quantitative differences in TNF-α gene expression between the freshly isolated circulating cells from hypercalcemic ATL patients and normal controls. In agreement with Wano et al. [17] and Kodaka et al. [42] we also observed IL-1β mRNA expression in the PBMC of some ATL patients. However, consistent with the previous studies there was no association between IL-1β expression and clinical hypercalcemia. Moreover, the up-regulation of both TNF-α and IL-1β in HAM/TSP compared to ATL suggests that these cytokines are more likely contributing to the immune activation of HAM/TSP rather than the hypercalcemia of ATL.

Although we were unable to correlate ATL-associated hypercalcemia with a dysregulated cytokine, the expression of TGF-β_1 in HTLV-I related disorders appears to have important clinical relevance. We previously demonstrated that the TGF-β_1 promoter was transactivated by HTLV-I Tax protein and that ATL PBMC secreted high levels of TGF-β_1 comparable to mitogen-activated B or T-cells [18]. In this study we further determined that increased levels of TGF-β_1 mRNA and protein were produced by the ATL cells, but not by the PBMC or HTLV-I infected T-cells of HAM/TSP patients and seropositive carriers. This observed variation in TGF-β_1 production between ATL and HAM/TSP correlated with the immune status of our patients. All of our ATL patients were immunosuppressed and many had opportunistic infections such as parasitic gastroenteritis [43]. In contrast, our HAM/TSP population showed no evidence of immunosuppression. In fact, patients with ATL often display impaired helper and suppressor T-cell function, as well as diminished mitogen responsiveness and natural killer cell induction [44,45]. Moreover, ATL cells or cultured supernatants from ATL cell lines will suppress the lectin-induced immunoglobulin synthesis by control PBMC [46,47]. The immunosuppressive features observed in ATL bears a striking resemblance to the effect of TGF-β_1 on the cells of the immune system. TGF-β_1 is a multifunctional molecule with significant immunosuppressive potential. It has been shown to inhibit IL-2 dependent T-cell proliferation, B-cell immunoglobulin biosynthesis, activation of natural killer cells, and cytotoxic T-cell induction [48–50]. Furthermore, the levels of TGF-β_1 which we have measured in short term cultures of ATL PBMC [18] or leukemic T-cells, in contradistinction to HAM/TSP, seropositive carrier, or normal T-cells, are of a magnitude where functional immunosuppression has been observed [48–50]. Thus excess TGF-β_1 secreted by the leukemic cells in vivo may be important in the pathogenesis of immunosuppression in ATL.

Finally, many have suggested that the balance between IL-2 and TGF-β_1 may be important in the regulation of the T-cell response. This is based on the fact that approximately 24 hours after activated T-cells make IL-2, TGF-β_1 protein begins to accumulate sending an inhibitory signal to the proliferating T-cell [48]. Thus in HAM/TSP the continued production of IL-2 by activated T-cells, without the feedback inhibition from TGF-β_1, may culminate in an autoimmune inflammation of the central nervous system. Conversely in ATL, the leukemic cells' ability to secrete large amounts of TGF-β_1 in the absence of IL-2 may confer a selective advantage for the tumor cells enabling them to escape host immune-mediated destruction.

In our analysis of altered cytokine expression in HTLV-I associated diseases we have identified potential mediators of the physiological derangements accompanying ATL and HAM/TSP. Our data suggest that the up-regulation of IFN-γ, TNF-α, and IL-1β in HAM/TSP, in contrast to late-stage acute ATL, may contribute to the ongoing state of central nervous system inflammation in these patients. Furthermore, the increased levels of TGF-β_1 secreted by the

malignant T-cells may be responsible for the global immunosuppression seen in our ATL patients. Since the altered regulation of these cytokines may contribute to the pathogenesis of both ATL and HAM/TSP, reversing their effect may profoundly alter the clinical course of these diseases and may thus provide a foundation for therapeutic protocols in the future.

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