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Transactivation of interleukin 2 and its receptor induces immune activation in human T-cell lymphotropic virus type I-associated myelopathy: Pathogenic implications and a rationale for immunotherapy

(retrovirus/spontaneous proliferation/adult T-cell leukemia/tropical spastic paraparesis/polymerase chain reaction)

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ABSTRACT **A state of T-cell activation, reflected by a marked degree of spontaneous proliferation *in vitro*, exists among patients with human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) but not in those with retroviral-induced adult T-cell leukemia (ATL). We wished to define the mechanism by which the immune activation of circulating cells from HAM/TSP is driven, thus gaining insight into the pathogenesis of this HTLV-I-associated disease. By using a modification of the polymerase chain reaction, we compared the levels of interleukin 2 (IL-2) and IL-2 receptor α chain (IL-2Rα) mRNA expression to the transcription of the HTLV-I transactivator gene, pX, in peripheral blood mononuclear cells of HAM/TSP and ATL patients as well as seropositive carriers. Up-regulation of IL-2 and IL-2Rα transcripts was detected in HAM/TSP and seropositive carriers that paralleled the coordinate mRNA expression of the pX transactivator. In addition, IL-2 and soluble IL-2Rα serum levels in HAM/TSP and seropositive carriers were elevated. Despite markedly elevated levels of soluble IL-2Rα in ATL, transcripts for IL-2 and pX were not demonstrable in the circulating cells. Finally, the marked degree of *in vitro* spontaneous proliferation present in HAM/TSP was profoundly inhibited by specific anti-IL-2R or anti-IL-2 blocking antibodies. Collectively, these results suggest that immune activation in HAM/TSP, in contrast to ATL, is virally driven by the transactivation and coordinate expression of IL-2 and IL-2Rα. This deregulated autocrine process may contribute to the evolution of inflammatory nervous system damage in HAM/TSP.**

Infection with the human T-cell lymphotropic virus type I (HTLV-I) is associated with a chronic progressive myelopathy described in the Caribbean basin as tropical spastic paraparesis (TSP) (1, 2). A similar condition termed HTLV-I-associated myelopathy is endemic in Japan (3). It is now recognized that HTLV-I-associated myelopathy and tropical spastic paraparesis (HAM/TSP) are equivalent clinical conditions (4). In addition, the retrovirus associated with HAM/TSP is nearly identical to prototype HTLV-I derived from patients with a malignant clonal proliferation of mature T cells known as adult T-cell leukemia (ATL) (5–8). However, the mechanism by which HTLV-I infection contributes to the pathogenesis of HAM/TSP is unknown. It has been proposed that the panoply of disease states associated with HTLV-I infection may result from the expression of a unique sequence called pX within the proviral genome (9). pX encodes a transcriptional activator known as Tax, which has been shown *in vitro* to induce a variety of host cellular genes including interleukin 2 (IL-2) and the α chain of the IL-2 receptor (IL-2Rα) (10, 11). This altered regulation of cellular genes by the pX transactivating element may begin a process of T-cell activation and proliferation with subsequent events leading to inflammatory nervous system damage in HAM/TSP or malignant transformation in ATL. Recent findings suggest that immune activation may contribute to the histopathological changes in HAM/TSP. Mononuclear inflammatory lesions are observed in the central nervous system with lymphocytic perivascular cuffing (12, 13). In addition, the neurological abnormalities are frequently associated with an altered T-cell phenotype consisting of an increased $CD4^+/CD8^+$ ratio and a high proportion of $IL\text{-}2R\alpha^+$ ($CD25^+$) cells in the peripheral blood (14, 15). The foremost evidence that an ongoing state of lymphocyte activation exists in patients with HAM/TSP is the marked propensity for their circulating mononuclear cells to spontaneously proliferate *in vitro* (16, 17). Although a lesser degree of spontaneous proliferation is noted in HTLV-I-seropositive carriers (16), no such proliferative response has been observed in the circulating cells from ATL patients (18). We undertook this study to determine if the augmented spontaneous proliferation in HAM/TSP is associated with a pX-mediated up-regulation in the expression of the cellular genes that encode IL-2 and its receptor. Utilizing the technique of specific mRNA amplification by the polymerase chain reaction (PCR) (19), we analyzed the *ex vivo* mRNA expression of IL-2, IL-2Rα, and pX in the peripheral mononuclear cells of patients with HAM/TSP, ATL, and seropositive carriers. This was correlated with the serum IL-2 and soluble IL-2R levels as well as the spontaneous proliferative responses, with and without blocking anti-IL-2/IL-2R antibodies, in the three HTLV-I-infected patient populations. Our data suggest that HAM/TSP, in contrast to late-stage acute ATL, represents an autocrine phase of HTLV-I infection in which the pX transactivator induces the production of IL-2 and its receptor leading to a polyclonal T-cell activation.

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Abbreviations: HTLV-I, human T-cell lymphotropic virus type I; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; ATL, adult T-cell leukemia; IL-2, interleukin 2; IL-2Rα, interleukin 2 receptor α chain; PCR, polymerase chain reaction.

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METHODS

Patients and Sample Preparations. Fourteen patients with HAM/TSP, 12 patients with ATL, 12 asymptomatic seropositive carriers, and 10 seronegative healthy individuals were studied. The HAM/TSP group comprised eight females and six males, 12 of Caribbean descent and two Americans, with a mean age of 51 years (range, 34–71 years). A diagnosis of HAM/TSP met the criteria established by the World Health Organization (20). The ATL population consisted of seven females and five males, with mean age of 43 years (range, 30–62 years). Seven were of Caribbean descent and five were American-born. A diagnosis of ATL was firmly established by the presence of a circulating pool of abnormal lymphocytes with a predominant $CD4^+,CD8^-,CD25^+$ T-cell phenotype and the demonstration of a clonal rearrangement of the T-cell receptor β chain gene. In addition, all HAM/TSP and ATL patients were seropositive for anti-HTLV-I antibodies by ELISA. Peripheral blood mononuclear cells were separated from heparinized venous blood by density gradient centrifugation and lysed in a 4 M guanidinium thiocyanate or RNAzol (Cinna/Biotecx, San Antonio, TX) solution, and total cellular RNA was isolated (21, 22).

PCR. Oligonucleotide primers and probes were synthesized by the phosphoramidite method (23). Amplification of pX mRNA was achieved with oligonucleotide primers derived from the long terminal repeat and pX domains, pX/1 and pX/2, respectively (see Fig. 1). The target sequence defined by these primers includes all three regions of the doubly spliced pX transactivating element. pX cDNA synthesis was performed with the pX/2 primer to direct pX-specific first-strand synthesis. Total RNA (5–10 μg) from patient cells or 1 μg from an HTLV-I-infected T-cell line (HuT 102) was resuspended to a final volume of 50 μl containing 50 mM Tris·HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 0.4 mM dATP, 0.4 mM dGTP, 0.4 mM dCTP, 0.4 mM dTTP, 12.5 units of human placental ribonuclease inhibitor (Amersham), 1.0 μM pX/2, and 400 units of Moloney murine leukemia virus reverse transcriptase and then incubated for 60 min at 37°C. Reagent controls consisted of the reaction mixture above but without template RNA. Reverse transcription products were diluted to obtain a final PCR mixture containing 25 mM Tris, 50 mM KCl, 3 mM $MgCl_2$, all four dNTPs (each at 0.2 mM), 1.0 μM pX/1 primer, and 1.0 μM pX/2 primer, and 0.01% gelatin, heated to 94°C for 5 min, quenched on ice, and supplemented with 5 units of *Thermus aquaticus* polymerase (Perkin–Elmer/Cetus). Samples were subjected to 40 cycles of amplification consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 53°C, and polymerization for 2 min at 72°C. An actin-targeted gene sequence served as an internal test control and was defined by 5′-TTCTACAATGAGCTGCGTGT-3′ and 5′-GCCAGACAGCACTGTGTTGG-3′, sense and antisense primers, respectively, and the amplified 636-base-pair (bp) actin signal was detected by an internal 40-base probe, 5′-ACTACCTCATGAAGATCCTCACCGAGCGCGGCTACAGCTT-3′ (24). In a similar manner, IL-2, IL-2Rα, and actin mRNA sequences were reverse-transcribed and amplified together for 30 cycles in a "multiplex" PCR from 1 μg of total RNA samples (see Figs. 2 and 3).

Liquid and Southern Blot Hybridization. For rapid identification, 30-μl samples from each reaction mixture were liquid-hybridized (25) with purified ^{32}P-end-labeled oligonucleotide probes (26), electrophoresed on a 5% polyacrylamide gel, and the gel-retardation preparations were autoradiographed. Further confirmation of the nature of the amplified sequence was obtained from sizing analysis by Southern blot hybridization as follows: 20 μl of each PCR reaction mixture was electrophoresed through a 1% agarose gel and then blot-transferred onto a nitrocellulose filter. Filters were baked at 80°C for 1 hr, prehybridized in a previously described buffer (27), and hybridized overnight at 60°C with 4.5 ml of buffer containing one of the following ^{32}P-labeled nick-translated DNA probes (1×10^7 dpm/ml): a 9-kilobase (kb) *Sac* I clone of the HTLV-I provirus (MT-2, obtained from F. Wong-Staal, National Institutes of Health), a 2.3-kb *Eco*RI full-length cDNA clone of IL-2R (pIL-2R3, obtained from W. Leonard, National Institutes of Health), and a 0.71-kb *Hin*dIII–*Pst* I cDNA clone of human IL-2 (paw55, obtained from the American Type Culture Collection). Filters were washed (27) and autoradiographed at −70°C.

IL-2/IL-2R Assays. Heparinized plasma samples were incubated in 2.8% $CaCl_2$ (1:9 dilution with plasma) for 2 hr at 37°C and then centrifuged at $1000 \times g$ for 15 min to remove fibrin. Sera or treated plasma test samples were diluted 1:3 with isotonic phosphate-buffered saline and assayed for IL-2 in duplicate with a commercial ELISA kit (Intertest 2, Genzyme). A standard curve of recombinant human IL-2 ranging from 62.5 units/ml to 1.0 unit/ml and containing the same percentage of serum/plasma as the test samples was included in each assay. The mean (± SEM) IL-2 concentration was calculated and expressed in units/ml, with 1 unit representing 0.33 ng. Circulating soluble IL-2R levels were measured using a sandwich ELISA (28). The distribution of values for IL-2 and soluble IL-2R among the test populations was compared using the Wilcoxon rank sum statistic.

Proliferative Responses. Cryopreserved peripheral blood mononuclear cells were resuspended at 1×10^6 viable cells per ml in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated human AB serum (Sigma), 4 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) and plated in 100-μl samples in triplicate in sterile flat-bottom 96-well plates. An equal volume of either serum-free RPMI or RPMI containing one of the following antibodies was added: anti-Tac (29) at 20 μg/ml (a monoclonal antibody to IL-2Rα), Mikβ1 (30) at 20 μg/ml (a monoclonal antibody to the β chain of the IL-2 receptor provided by M. Tsudo, Tokyo Metropolitan Institute of Medical Science), a combination of anti-Tac and Mikβ1 each at 20 μg/ml, anti-IL-2 at 20 μg/ml (a neutralizing polyclonal IL-2 antibody provided by Hoffmann–LaRoche), and RPC-5 at 20 μg/ml and 40 μg/ml (a nonspecific murine IgG2a immunoglobulin obtained from Bionetics). After incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air for 6 days, wells were pulse-labeled with 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) for 6 hr and then harvested using a Skatron harvesting system. Statistical comparisons of the proliferation values among the population subgroups were performed using the Student's t and Wilcoxon rank sum tests.

RESULTS

We used the PCR technique to detect the presence of a unique retroviral pX mRNA sequence in the freshly isolated circulating cells of patients with HTLV-I-associated disorders (Fig. 1). By this strategy, a 343-bp sequence, representative of the doubly spliced pX mRNA, was detected in seven out of eight HAM/TSP patients and five out of six seropositive carriers. Despite the demonstration of pX mRNA in the ATL-derived T-cell line HuT 102, there was no evidence of pX expression in the freshly isolated peripheral blood mononuclear cells of 12 ATL patients.

Next we compared the quantitative differences in the production of IL-2 and IL-2Rα mRNA from the peripheral blood mononuclear cells of HAM/TSP patients and seropositive carriers with that of ATL and normal controls (Figs. 2 and 3). IL-2 mRNA was expressed to some degree in the six HAM/TSP patients and seropositive carriers but could not be detected in any of the six ATL patients or normal controls. A representative Southern blot from four patients in each category is displayed in Fig. 2. In comparison to the constitutive low level of IL-2Rα mRNA expression in normal

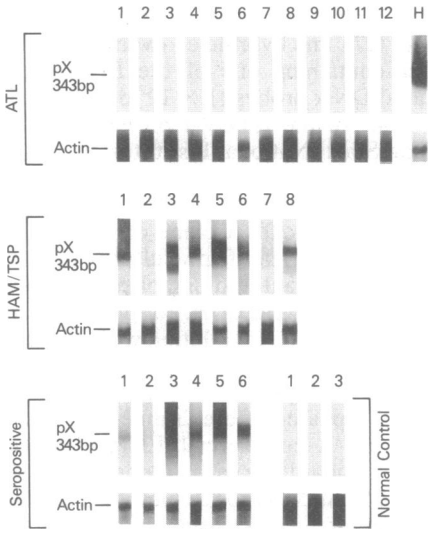

FIG. 1. Southern hybridization for the detection of amplified HTLV-I pX mRNA. Total RNA from patients with ATL or HAM/TSP, seropositive carriers, normal controls, and an HTLV-I-infected T-cell line (lane H) was subjected to pX-specific reverse transcription and then PCR amplification using primer pairs homologous to regions within the pX mRNA. The pX/1 sense primer, 5′-TACCTGAGGGCCCCATCCACGCCGGTTGA-3′, was derived from the R region of the long terminal repeat domain and the pX/2 antisense primer, 5′-ACACAGTCTCGAGACACGTAGACTGGGTAT-3′, was complementary to a 5′ sequence within the pX domain (31). Two internal sense oligonucleotide probes, pX/POL (5′-AGGCTCTCCAAGAAGCTGCCGGCGCTGCTCTCATCCCGGT-3′) and pX/LTR (5′-GCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTC-3′), derived from the pol and long terminal repeat domains, respectively, were used to detect the amplified pX mRNA of 343 bp. A sample of each PCR reaction mixture was electrophoresed, blot-transferred, hybridized to a 9-kb HTLV-I probe, and autoradiographed. pX mRNA was detected as a 343-bp band. An actin sequence was amplified from each RNA sample using primer pairs homologous to regions within the actin mRNA and then detected with an internal oligonucleotide probe.

controls, IL-2Rα transcription was markedly up-regulated in all six HAM/TSP patients and six ATL patients and to a lesser degree in the six seropositive carriers (Fig. 3).

To correlate the observed variations in IL-2/IL-2Rα mRNA expression with their respective translational products, we measured the levels of IL-2 and the soluble form of IL-2R in HAM/TSP, ATL, seropositive carrier, and normal control serum/plasma samples. IL-2 levels in HAM/TSP (12.7 ± 5.1 units/ml) and seropositive carriers (16.6 ± 8.2 units/ml) were significantly elevated ($P < 0.05$) above healthy controls (0.5 ± 0.4 unit/ml). Paralleling the absence of detectable IL-2 transcripts by PCR, there was no measurable IL-2 in eight ATL serum samples. In contrast, very high levels of soluble IL-2R were present in these same ATL samples (117,200 ± 30,600 units/ml). In addition, modest but significantly elevated soluble IL-2R levels were detected in the HAM/TSP patients (1100 ± 220 units/ml, $P < 0.00001$) and seropositive carriers (600 ± 100 units/ml, $P < 0.01$) as compared to healthy controls (290 ± 20 units/ml).

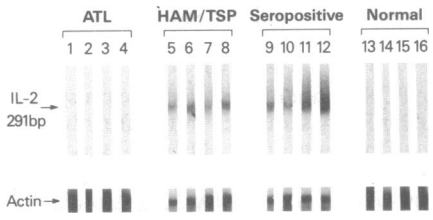

FIG. 2. Southern hybridization for the detection of cellular IL-2 expression. Total RNA from patients with ATL and HAM/TSP, seropositive carriers, and normal controls was subjected to IL-2-specific reverse transcription and then PCR amplification. For amplification of IL-2 mRNA, an IL-2/1 sense primer, 5′-CTAAGTCTTGCACTTGTCAC-3′, and an IL-2/2 antisense primer, 5′-ATTGCTGATTAAGTCCCTGG-3′, were employed (32). A 40-base internal probe, 5′-CCCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAG-3′, was used to detect the amplified IL-2 cDNA fragment of 291 bp. The amplified material was electrophoresed, blot-transferred, hybridized to a 0.71-kb IL-2 cDNA probe, and autoradiographed. IL-2 mRNA was detected by the presence of an amplified 291-bp IL-2 cDNA fragment. In the same PCR reaction mixture, an actin sequence was readily amplified from each RNA sample with actin-specific primers and then detected with an internal oligonucleotide probe.

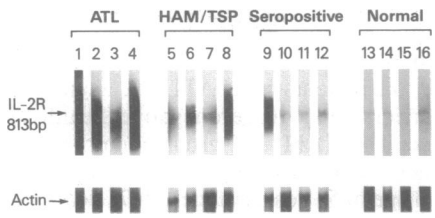

FIG. 3. Southern hybridization for the detection of cellular IL-2Rα expression. Total RNA from patients with ATL and HAM/TSP, seropositive carriers, and normal controls was subjected to IL-2Rα-specific reverse transcription and then PCR amplification as in Fig. 2 with the addition of IL-2Rα-specific primers corresponding to regions within exons 1 and 8, IL-2Rα/1 (5′-GATTCATACCTGCTGATGTG-3′) and IL-2Rα/8 (5′-GATTGTTCTTCTACTCTTCC-3′), respectively (33). These primers directed amplification of an 813-bp signal within the IL-2Rα mRNA, which was detected with an oligonucleotide probe derived from exon 2 (5′-GAGCTCTGTGACGATGACCCGCCAGAGATCCCACACGCCACA-3′). This 813-bp signal representing IL-2Rα mRNA was detected after transfer of the PCR products to a nitrocellulose filter and hybridization of the blot with a 2.3-kb cDNA probe for IL-2Rα. A representative actin sequence, amplified in the same reaction vial as IL-2Rα from each RNA sample, is shown directly below the respective lanes.

To better characterize the lymphoproliferative response in HAM/TSP and seropositive carriers, peripheral mononuclear cells were cultured in the presence of specific antibodies that block the interaction of IL-2 with its binding peptides, the α and β chains of the IL-2 receptor (Fig. 4). The circulating cells from all HAM/TSP patients displayed a marked degree of spontaneous proliferation, assessed by a [^{3}H]thymidine uptake of 40,200 ± 6200 cpm after 6 days in medium alone compared to 690 ± 70 cpm for normal individuals. Addition of anti-Tac, Mikβ1, or an IL-2-neutralizing antibody produced a profound inhibition of the proliferative response [40,200 cpm to 4500 ± 2200 cpm ($P < 0.001$ for anti-Tac), to 10,800 ± 3100 cpm ($P < 0.05$ for Mikβ1), and to 2300 ± 1000 cpm ($P < 0.001$ for anti-IL-2)]. This inhibitory effect from blocking binding of IL-2 to its receptor appeared specific, as the addition of irrelevant monoclonal antibody produced no meaningful suppression of proliferation. Moreover, the combination of anti-Tac and Mikβ1 completely abrogated the augmented HAM/TSP proliferation. Although an increased proliferative response was demonstrated in seropositive carriers (7700 ± 1300 cpm, $P < 0.05$) as compared to healthy controls (690 ± 70 cpm), it was significantly less than in HAM/TSP ($P < 0.01$). However, a similar inhibition of spontaneous proliferation was observed when circulating cells from seropositive carriers were cultured in the presence of anti-Tac (1100 ± 300 cpm, $P < 0.05$), anti-IL-2 antibody (1800 ± 500 cpm, $P < 0.05$), or the combination of anti-Tac and Mikβ1 (1000 ± 300 cpm, $P < 0.05$). Finally, no spontaneous proliferation was observed in the ATL patients (1200 ± 500 cpm).

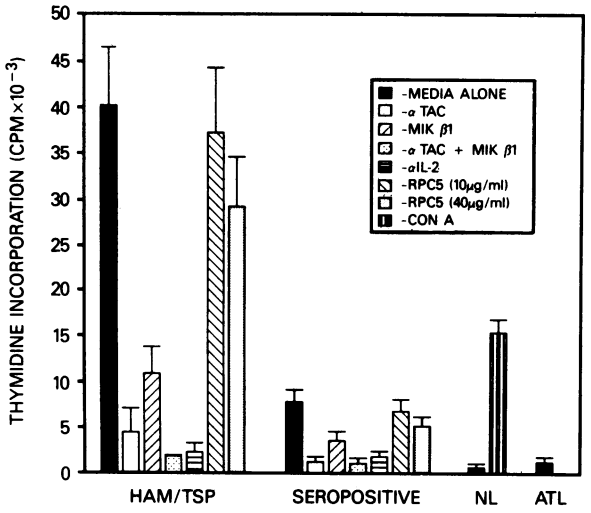

FIG. 4. Spontaneous proliferation in HTLV-I-associated disorders. Peripheral blood mononuclear cells from patients with HAM/TSP and ATL, seropositive carriers, and normal controls (NL) were cultured in medium alone or in the presence of specific IL-2R-blocking antibodies or IL-2-neutralizing antibodies. After 6 days in culture, wells were pulse-labeled with 1 μCi of [^{3}H]thymidine and harvested 6 hr later. Thymidine incorporation is measured as cpm and the results are expressed as mean ± SEM (error bars). αTAC, anti-Tac; αIL-2, anti-IL-2; RPC-5, a nonspecific murine IgG2a; Con A, concanavalin A.

DISCUSSION

The mechanism by which HTLV-I infection causes an inflammatory and demyelinating neurologic process in its host is unknown but is thought to be related to an aberrant activation of T cells in HAM/TSP, reflected by an increased percentage of circulating IL-2R$^+$ cells in HAM/TSP (14) and an enhanced proliferative response from the unstimulated lymphocytes of these patients (16, 17). The initiation of such a T-cell response appears to be critically dependent on the sequential induction of IL-2 and IL-2Rα and the subsequent binding of IL-2 to its high-affinity receptor. An analysis of the HTLV-I-transactivating gene pX suggests a potential mechanism to explain how infection with HTLV-I might drive this chain of events. Cotransfection studies involving the insertion of a pX cDNA into resting Jurkat T cells revealed that the retroviral transactivating gene product is capable of increasing the transcription of host genes governing human IL-2/IL-2Rα expression (10, 11). Thus, the expression of pX *in vivo* could be essential for maintaining a state of lymphocyte activation in HAM/TSP, thereby contributing to the pathogenesis of this disease. However, by using conventional Northern blot analysis, we have failed to detect pX mRNA in the freshly isolated peripheral mononuclear cells of HAM/TSP patients (C.L.T., unpublished data). Therefore, we employed a highly sensitive, yet specific, PCR technique for amplification of minute quantities of retroviral and cellular mRNA *in vivo*. This enabled us to demonstrate that the activated cellular immune state in HAM/TSP is virally driven by the concomitant expression of HTLV-I pX mRNA and the up-regulation of IL-2 and IL-2Rα in these patients. Although we saw a similar pattern of IL-2/IL-2Rα induction in the presence of pX mRNA expression in the seropositive carriers, their levels of IL-2R expression, soluble IL-2R, and spontaneous proliferation were significantly less than those in HAM/TSP. In marked contrast there was no evidence of pX and IL-2 transcription or spontaneous proliferation in fresh ATL mononuclear cells despite very high levels of IL-2Rα expression and soluble IL-2R in these patients. Thus HAM/TSP and seropositive carriers may represent an autocrine phase of HTLV-I infection where pX gene activity induces the production of IL-2 and its receptor leading to polyclonal T-cell proliferation. The polyclonal nature of the T-cell expansion in HAM/TSP has been demonstrated (6) and may be reflected *in vitro* by the spontaneous proliferative responses that others (16, 17) have observed and we have confirmed. Furthermore, the activated T cells in seropositive carriers would then be primed for a second undefined event that would ultimately result in leukemogenesis with a clonal population of IL-2-independent ATL cells (34). Although not in accord with our findings, a pX transcript was detected in fresh mononuclear cells of Japanese patients with ATL (35). Differences in PCR methodologies, pertaining to the reverse-transcription step and to the cDNA sequences targeted in the subsequent gene amplification steps, may account for the discrepancy. Furthermore, the level of pX expression may vary due to differences between Japanese and Caribbean ATL populations studied. Nevertheless, in a subset of ATL patients, we demonstrated pX mRNA in freshly biopsied malignant lymph nodes (36). Therefore, we believe that in ATL pX gene expression may be important in the early stages of leukemogenesis or in certain microenvironments where there is high cell turnover. For the majority of ATL patients in advanced stages of disease an extraordinarily sensitive PCR technique may be necessary to detect pX mRNA in circulating cells. However, it is important to consider the possibility that such a high level of sensitivity permits detection of retroviral transcripts expressed from the smaller polyclonally infected T-cell population, even though retroviral mRNA expression may not be representative of the larger leukemic clone. This view is supported by the experience of establishing cell lines from ATL patients in which, for 70% of the results, a nonleukemic clone predominates (T.A.W., unpublished data). Therefore, the quantitative differences in pX expression in HAM/TSP and ATL patients and seropositive carriers demonstrated with our approach may offer a more realistic assessment of the pathogenic role of pX transcription in HTLV-I-associated disorders.

The data were derived from patient population comparisons, and vertical intrapatient analyses were not performed. Therefore, we were unable to directly study the relationship of pX expression to the production of IL-2 and IL-2Rα or to the degree of spontaneous proliferation within individual patients from a given population group. However, the HAM/TSP patient and seropositive carrier in which we were unable to detect mRNA for the pX gene (lanes 2 in the respective categories from Fig. 1) had significantly lower values of serum IL-2 and soluble IL-2R than the mean levels for their respective population groups. Thus, in HAM/TSP patients and seropositive carriers, the expression of the retroviral-transactivating element seems to be intimately associated with the induction of IL-2 and its receptor. Moreover, our data would suggest that the presence of pX mRNA in the circulating cells plays an important role in the elevated level of spontaneous proliferation noted in HAM/TSP and the HTLV-I carrier state, but not in ATL where neither pX expression nor spontaneous proliferation were detected.

We also were able to demonstrate that the proliferative response in HAM/TSP, and to a lesser degree in the HTLV-I carrier state, is mediated by the binding of IL-2 to its receptor. This was accomplished by culturing peripheral mononuclear cells in the presence of a IL-2-neutralizing antibody or with antibodies that block the interaction of IL-2 with its high-affinity receptor (anti-Tac and Mikβ1). In HAM/TSP, under both conditions, there was a >90% inhibition in thymidine incorporation compared to the addition of a nonspecific antibody to the culture. In addition to the induction of IL-2 and its receptor in HAM/TSP, there are genetic factors that may confer a heightened immune response against HTLV-I in these patients. For example,

HAM/TSP-associated HLA haplotypes were found in 70% of Japanese patients with HAM/TSP but not in any individuals with ATL (37). Peripheral blood lymphocytes bearing the HAM/TSP-associated haplotypes exhibited a high immune response to HTLV-I antigen whereas the ATL-associated haplotypes had a low response. Since the combination of immune activation and genetic predisposition may contribute to the mononuclear inflammation and demyelination present in the central nervous system of HAM/TSP patients, down-modulating this process provides a rationale for therapy with immunosuppressive agents.

A role for immune intervention in HAM/TSP is supported by the observation that treatment with oral prednisolone has been associated with clinical improvement in many cases (38). However, others have reported less consistent effects with corticosteroids (39). Unfortunately, its long-term use in a chronic illness, such as HAM/TSP, can produce serious complications. We have, therefore, turned to the IL-2R as a target for immunotherapy in patients with HAM/TSP. The foundation for such an approach is the observation that an increased proportion of circulating cells from HAM/TSP patients express surface-bound IL-2Rα, identified by the anti-Tac monoclonal antibody, whereas normal resting cells do not (14). Furthermore, we have shown that *in vitro* anti-Tac treatment of peripheral mononuclear cells from HAM/TSP patients markedly inhibits spontaneous proliferative responses. In addition, anti-Tac therapy has recently been safely and effectively utilized in humans for the treatment of ATL (40) and the prevention of allograft rejection (41). In a similar fashion, anti-Tac may suppress the immune activation in HAM/TSP and potentially ameliorate the inflammatory process leading to nervous tissue damage in this disease.

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1. Roman, G. C., Schoenberg, B. S., Madden, D. L., Sever, J. L., Hugon, J., Ludolph, A. & Spencer, P. S. (1987) *Arch. Neurol.* **44**, 605–607.
2. Vernant, J. C., Maurs, L., Gessain, A., Barin, F., Gout, O., Delaporte, J. M., Sanhadji, K., Buisson, G. & de-The, G. (1987) *Ann. Neurol.* **21**, 123–130.
3. Osame, M., Matsumoto, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Tara, M. & Igata, A. (1987) *Ann. Neurol.* **21**, 117–122.
4. Roman, G. C. & Osame, M. (1988) *Lancet* **i**, 651.
5. Yoshida, M., Osame, M., Usuku, K., Matsumoto, M. & Igata, A. (1987) *Lancet* **i**, 1085–1086.
6. Greenberg, S. J., Jacobson, S., Waldmann, T. A. & McFarlin, D. E. (1989) *J. Inf. Dis.* **159**, 741–744.
7. Kwok, S., Ehrlich, G. D., Poiesz, B. J., Bhagavati, S. & Sninsky, J. J. (1988) *J. Inf. Dis.* **158**, 1193–1197.
8. Bangham, C. R. M., Daenke, S., Phillips, R. E., Cruickshank, J. K. & Bell, J. I. (1988) *EMBO J.* **7**, 4179–4184.
9. Murphy, E. L. & Blattner, W. A. (1988) *Ann. Neurol.* **23**, Suppl., 174–180s.
10. Siekevitz, M., Feinberg, M. B., Holbrook, N., Wong-Staal, F. & Greene, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5389–5393.
11. Cross, S. L., Feinberg, M. B., Wolf, J. B., Holbrook, N. J., Wong-Staal, F. & Leonard, W. J. (1987) *Cell* **49**, 47–56.
12. Piccardo, P., Ceroni, M., Rodgers-Johnson, P., Mora, C., Asher, D. M., Char, G., Gibbs, C. J. & Gajdusek, D. C. (1988) *Ann. Neurol.* **23**, Suppl., 156–160s.
13. Azizuki, S., Nakazato, O., Higuchi, Y., Tanabe, K., Setoguchi, M., Yoshida, S., Miyazaki, Y., Yamamoto, S., Sudou, S., Sannomiya, K. & Okajima, T. (1987) *Lancet* **i**, 156–157.
14. Itoyama, Y., Minato, S., Kira, J. I., Goto, I., Sato, H., Okochi, K. & Yamamoto, N. (1988) *Neurology* **38**, 816–818.
15. Kitajima, I., Osame, M., Izumo, S. & Igata, A. (1988) *Autoimmunity* **1**, 125–131.
16. Jacobson, S., Zaninovic, V., Mora, O., Rodgers-Johnson, P., Sheremata, W. A., Gibbs, C. J., Gajdusek, D. C. & McFarlin, D. E. (1988) *Annu. Neurol.* **23**, Suppl., 196–200s.
17. Itoyama, Y., Minato, S., Kira, J., Goto, I., Sato, H., Okochi, K. & Yamamoto, N. (1988) *Neurology* **38**, 1302–1307.
18. Tsuda, H. & Takatsuki, K. (1985) *J. Clin. Immunol.* **5**, 109–114.
19. Newman, P. J., Gorski, J., White, G. C., Gidwitz, S., Cretney, C. J. & Aster, R. H. (1988) *J. Clin. Invest.* **82**, 739–743.
20. Regional Office for the Western Pacific of the World Health Organization (1989) *Scientific Group on HTLV-I Infections and Its Associated Diseases* (World Health Organization, Manila, Philippines).
21. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
22. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
23. Beaucage, S. L. & Caruthers, M. H. (1981) *Tetrahedron Lett.* **22**, 1859–1862.
24. Ponte, P., Ng, S. Y., Engel, J., Gunning, P. & Kedes, L. (1984) *Nucleic Acids Res.* **12**, 1687–1696.
25. Greenberg, S. J., Ehrlich, G. D., Abbott, M. A., Hurwitz, B. J., Waldmann, T. A. & Poiesz, B. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2878–2882.
26. Richardson, C. C. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 158–165.
27. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Preisinger, A. C., Willard, H. F., Michelson, A. M., Riggs, A. D. & Orkin, S. H. (1987) *Cancer Res.* **47**, 4806–4813.
28. Rubin, L. A., Kurman, C. C., Fritz, M. E., Biddison, W. E., Boutin, B., Yarchoan, R. & Nelson, D. L. (1985) *J. Immunol.* **135**, 3172–3177.
29. Uchiyama, T., Broder, S. & Waldmann, T. A. (1981) *J. Immunol.* **126**, 1393–1397.
30. Tsudo, M., Kitamura, F. & Migasaka, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1982–1986.
31. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3618–3622.
32. Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. & Hamuro, J. (1983) *Nature (London)* **302**, 305–310.
33. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312**, 768–771.
34. Broder, S., Bunn, P. A., Jaffe, E. S., Blattner, W., Gallo, R. C., Wong-Staal, F., Waldmann, T. A. & DeVita, V. T. (1984) *Ann. Intern. Med.* **100**, 543–557.
35. Kinoshita, T., Shimoyama, M., Tobinai, K., Ito, M., Ito, S. I., Ikeda, S., Tajima, K., Shimotohno, K. & Sugimura, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5620–5624.
36. Greenberg, S. J., Tendler, C. L., Manns, A., Bartholomew, C., Hanchard, B., Blattner, W. & Waldmann, T. A. (1989) in *Current Issues in Human Retrovirology: HTLV-I*, ed. Blattner, W. (Raven, New York), pp. 87–104.
37. Usuku, K., Sonoda, S., Osame, M., Yashiki, S., Takahashi, K., Matsumoto, M., Sawada, T., Tsuji, K., Tara, M. & Igata, A. (1988) *Ann. Neurol.* **23**, Suppl., 143–150s.
38. Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M. & Tara, M. (1986) *Lancet* **i**, 1031–1032.
39. Dalakas, M. C., Stone, G., Elder, G., Ceroni, M., Madden, D., Roman, G. & Sever, J. L. (1988) *Ann. Neurol.* **23**, Suppl., 136s–142s.
40. Waldmann, T. A., Goldman, C. K., Bongiovanni, K. F., Sharrow, S. O., Davey, M. P., Cease, K. B., Greenberg, S. J. & Longo, D. L. (1988) *Blood* **72**, 1805–1816.
41. Kirkman, R. L., Shapiro, M. E., Carpenter, C. B., Milford, E. L., Ramos, E. L., Tilney, N. L., Waldmann, T. A., Zimmerman, C. E. & Strom, T. B. (1989) *Transplant. Proc.* **21**, 1766–1768.