Constitutive Production of Interleukin-6 and Tumor Necrosis Factor-α From Spontaneously Proliferating T Cells in Patients With Human T-Cell Lymphotropic Virus Type-I/II

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The human T-cell lymphotropic viruses (HTLV) type I and type II are capable of inducing a variety of cellular genes, including many of the cytokines that regulate cell proliferation. To determine if the spontaneous proliferation of peripheral blood mononuclear cells from patients infected with HTLV-I and HTLV-II was related to coordinate expression of cytokines, we analyzed the levels of interleukin-1β (IL-1β), IL-2, IL-3, IL-4, IL-6, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in culture supernatants derived from spontaneously proliferating cells. Significantly elevated levels of IL-6 and TNF-α were present in culture supernatants from HTLV-I/II-infected individuals when compared with normal controls (P < .01). Kinetic experiments showed that both IL-6 and TNF-α were elevated by day 5. None of the other cytokines (IL-1β, IL-2, IL-3, IL-4, and IFN-γ) were detectable in any of the culture. These data suggest that release of IL-6 and TNF-α may regulate lymphocyte proliferation in HTLV-I/II-infected individuals.

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RESULTS

Soluble HTLV antigen production. The production of cytokines from unstimulated cultures was determined by commercial ELISA kits for IL-1β, IL-2, IL-4, IL-6 (Genzyme, Boston, MA), TNF-α (T cell Sciences, Cambridge, MA), and interferon-γ (IFN-γ) (Amgen, Thousand Oaks, CA).

DISCUSSION

Various receptor ligand interactions between surface molecules on lymphocytes and other cells trigger the release of a variety of soluble mediators. Among such mediators are ILs, IFNs, and TNFs, all of which are important in the initiation and regulation of the immune response. In the present investigation, we analyzed the constitutive production of different cytokines from sponta-
CONSTITUTIVE EXPRESSION OF IL-6 AND TNF-α

Fig 2. Kinetics of IL-6 and TNF-α release in the cultures derived from normal controls (■) or from patients infected with HTLV-I/II (□). Culture supernatants were collected at time points indicated and assayed for IL-6 and TNF-α.

nenously proliferating lymphocytes of patients infected with HTLV-I and HTLV-II.

In concordance with previous reports,5,12,17 we observed spontaneous proliferation in both HTLV-I- and HTLV-II-infected persons. That the HTLV antigen released in the culture supernatant might stimulate the proliferation is consistent with earlier reports of HTLV mitogenicity.18 However, absence of detectable levels of p24Ag antigen in culture supernatants from spontaneous proliferating cells argues against this possibility. Presence of other gene products, in particular tax, either alone or in synergy with cytokines, may be responsible for cellular proliferation.

Among the battery of cytokines evaluated, no differences in the levels of IL-1β, IL-2, IL-4, and IFN-γ were observed in the spontaneously proliferating cells. Conflicting reports of IL-2 in culture supernatants from spontaneously proliferating cells have been reported. Although Tendler et al12 reported significantly elevated levels of IL-2 in both asymptomatic and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients, Matsumoto et al17 found increased levels only in patients with HAM/TSP. Our inability to detect increased levels of IL-2 in spontaneously proliferating lymphocytes from HTLV-I/II-infected persons may be due to sequestration of IL-2 by its receptor. Indeed, elevated levels of soluble IL-2 receptor (IL-2Rα) were found in the culture supernatants from these patients (Lal RB, Rudolph DL, Rowe T, Folks TM: submitted for publication, April 1991).

Of greater significance is the finding that levels of IL-6 and TNF-α were significantly increased in the culture supernatants derived from HTLV-infected persons when compared with those of normal controls. Both IL-6 and TNF-α have pleotropic activities in vitro and in vivo, in particular stimulation of various immune effector functions.19,20 TNF-α can also induce its own secretion and serve as a cofactor in the production of several cytokines, including IL-6.8 Therefore, overall cytokine levels in culture supernatants may result from virus infection as well as the autocrine and paracrine induction of these cytokines in infected cells. With regard to the relationship of IL-6 with HTLV, long-term T-cell lines infected with HTLV have been shown to induce gene expression and secretion of IL-6.13 In addition, increased levels of IL-6 have been demonstrated in both the plasma and the cerebrospinal fluid of HTLV-infected persons.9 Both IL-6 and TNF-α have also been shown to regulate HIV replication at transcriptional and posttranscriptional levels.20

The mechanism by which spontaneously proliferating cells induce the secretion of TNF-α and IL-6 might be mediated by transactivation through the HTLV regulatory gene product, tax.17 Tax has been shown to regulate in trans a variety of cellular genes, such as IL-2R, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and TGF-β12,15 and this transactivation is mediated via one or more transcription factors and cellular proteins.11,21 For example, studies on transcriptional regulation of IL-6 have identified sequence elements in its promoter22 that have the potential to bind cellular transcription factors (NF-IL-6) in response to tax. Similarly, TNF-α induces IL-2Rα gene expression via nuclear proteins that specifically interact with a nuclear factor enhancer element (NF-kB), which is capable of binding to the HTLV-LTR.11 TNF has also been shown to modulate growth and differentiation by inducing nuclear protooncogenes such as c-fos and c-jun.23 Both of these oncogenes encode for DNA binding proteins with a leucine zipper structure, that binds to the tax-responsive element within the HTLV-LTR.24 These transcription factors may function as intermediary transcriptional regulators in signal transduction, thereby sustaining the proliferation of lymphocytes from HTLV-I and HTLV-II infected individuals. Constitutive expression of both fos and jun transcripts have recently been observed in HTLV transformed long-term T-cell lines (Hooper CW, Rudolph DL, Laimore MD, Folks TM, Lal RB, manuscript in preparation, 1991). Further understanding of the role of IL-6 and TNF-α in the regulation of HTLV expression in infected cells may provide important insights into the basis of immune dysfunction in these patients.

REFERENCES


11. Lowenthal JW, Ballard DW, Bohlein E, Greene WC: Tumor necrosis factor-α induces proteins that bind specifically to kB-like enhancer elements and regulate interleukin 2 receptor α-chain gene expression in primary human T lymphocytes. Proc Natl Acad Sci USA 86:2331, 1989
22. Akira S, Issyhihi H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T, Kishimoto T: A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J 9:1897, 1990
24. Yoshimura T, Fujisawa J, Yoshida M: Multiple cDNA clones encoding nuclear proteins that bind to the tar-dependent enhancer of HTLV-I: All contain a leucine zipper structure and basic amino acid domain. EMBO J 9:2237, 1990
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