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Human T-cell growth factor (interleukin 2) and γ-interferon genes: Expression in human T-lymphotropic virus type III- and type I-infected cells

(mRNA abundance/gene induction/transcriptional regulation/acquired immune deficiency syndrome/leukemia)

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ABSTRACT **Acquired immune deficiency syndrome (AIDS) is characterized by severe depletion of OKT4$^+$ T lymphocytes and leukemia is associated with abnormal proliferation of maturation-arrested lymphocytes. Human T-lymphotropic virus type III (HTLV-III) or lymphoadenopathy virus (LAV) and type I (HTLV-I) are etiologically linked to AIDS and adult T-cell leukemia/lymphoma, respectively. T-cell growth factor (TCGF, also known as interleukin 2) is required for the growth of activated T-cells, which play an important role in immune regulation. γ-Interferon (IFN-γ) is also implicated in immune modulation. It was possible that T-cell depletion in acquired immune deficiency syndrome could be due to an impairment of TCGF synthesis and that adult T-cell leukemia could be due to unregulated production of TCGF. The results reported here show that the transcription of the *TCGF* gene was not impaired in cultured HTLV-III-infected cells. Paradoxically, the *TCGF* gene in HTLV-I-infected cells was transcriptionally inactive. The reverse was the case for the γ-interferon gene—it was actively transcribed in HTLV-I-infected cells but not in the HTLV-III-infected and virus-producing H9 and H4 cell line. No evidence was obtained suggesting abnormal regulation of the *TCGF* or of the *IFN-γ* gene consequent to HTLV-III infection. It thus appears that in both HTLV-III and HTLV-I infection, growth control and immune regulatory mechanisms may bypass a modulatory role of TCGF or of IFN-γ.**

Human T-lymphotropic viruses (HTLV) constitute a group of exogenous retroviruses associated with T-cell disorders of man. Its members include HTLV-I, HTLV-II, and HTLV-III (LAV). These viruses share a number of properties which include tropism for OKT4$^+$ lymphocytes (1–3), ability to induce the formation of giant multinucleated cells (3–6), a similar mode of transmission, weak immunologic crossreactivity of some virally encoded proteins (7–9), distant nucleic acid homologies (10–12), a similar pattern of viral gene expression (12–14), and the phenomenon of transacting transcriptional activation (15, 16). Despite these similarities, HTLV-III differs from HTLV-I and -II in some important respects, most notably in their biological properties. HTLV-I is etiologically linked to human adult T-cell leukemia (ATLL) (17–20), and HTLV-III is causatively associated with acquired immune deficiency syndrome (AIDS)—a disease characterized by the depletion of the OKT4$^+$ lymphocyte population (20–24). Whereas infection of human T lymphocytes with HTLV-I often results in transformation and immortalization of cells (17–20), HTLV-III is generally cytotoxic (3, 20, 22). HTLV-II has not yet been associated with a human disease, though it is transforming *in vitro* (7). Moreover, the genetic structure of HTLV-III differs significantly from HTLV-I and -II (25–28).

Human T-cell growth factor (TCGF), also termed interleukin 2, is produced by a subset of T-lymphocytes upon appropriate antigen or mitogen stimulation (29–31). It is required for the proliferation of activated T cells which play a central role in immune regulation (30, 31). γ-interferon (IFN-γ) has also been implicated in immune regulation (32). It was thus possible that immune deficiency and T-cell depletion in HTLV-III-infected AIDS patients was due to the suppression of TCGF synthesis. This possibility was further encouraged by the observation that many of the HTLV-I-infected cells do not transcribe the *TCGF* gene (33), raising the prospect that HTLV infection in general may influence TCGF synthesis. In addition, it has been suggested that TCGF modulates the synthesis of IFN-γ (34), and that there is a cascading linkage among TCGF, IFN-γ, and other lymphokines (35). HTLV infection thus could impair the immune regulatory functions of IFN-γ by affecting its synthesis. We have previously shown that the production of TCGF is primarily regulated at the level of *TCGF* gene transcription (36, 37). We therefore investigated the expression of *TCGF* and *IFN-γ* genes in HTLV-III- as well as HTLV-I-infected T-cells and show here that the transcription of the *TCGF* gene is not impaired in HTLV-III-infected cells and that there is no necessary link between the expression of *TCGF* and *IFN-γ* genes in HTLV-infected cells.

MATERIALS AND METHODS

Cell Culture. HTLV-III-infected and uninfected human H4 and H9 cells (3) and Jurkat and CEM cells were induced with phytahemagglutinin-M (PHA) (1%) and phorbol myristate acetate (PMA) (10 ng/ml) for 18–20 hr. HTLV-I-infected C2/MJ and MI cells (38) were similarly induced. All cells were cultured at 37°C under humidified 5% CO_2/95% air at a density of 1–2 × 10^6 cells per ml in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum and antibiotics.

RNA Isolation and Blot Hybridization. Poly(A)-containing RNA was obtained from freshly harvested cells by a procedure involving guanidine·HCl extraction (39) and cesium chloride centrifugation (40) followed by oligo(dT)-cellulose chromatography (41) as described (42, 43). For RNA blot hybridization, poly(A)-selected RNA (10 μg per lane) was size-separated by denaturing formaldehyde-agarose gel electrophoresis and transferred to a membrane by electroelution. Dot blots were prepared by applying serial dilutions (1:5) of

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Abbreviations: HTLV, human T-cell leukemia/lymphotropic virus; AIDS, acquired immune deficiency syndrome; TCGF, T-cell growth factor; IFN-γ, γ-interferon; PHA, phytohemagglutinin; PMA, phorbol myristate acetate.

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poly(A)-selected RNA to a nitrocellulose membrane such that the first sample of a series contained 10 μg of RNA. Hybridization with ^{32}P-labeled probes was performed as described (36, 44). TCGF and IFN-γ probes consisted of full-length cloned cDNA (36, 45) labeled *in vitro* by nick-translation.

Nuclear Transcription and Blot Hybridization. Nuclei were prepared essentially according to Mulvihill and Palmiter (46). They were allowed to elongate RNA chains *in vitro* in the presence of [^{32}P]UTP. The labeled RNA thus obtained was hybridized to serial dilutions of TCGF and IFN-γ cDNA (both cloned in pBR322) along with vector pBR322 DNA immobilized on a nitrocellulose sheet. The details of these procedures have been described (37, 44).

Actinomycin D Treatment. Cells were induced with PHA (1%) and PMA (10 ng/ml) for 16 hr. They were washed and recultured in fresh medium containing actinomycin D (5 μg/ml) and (*i*) no inducer, (*ii*) PHA, (*iii*) PMA, and (*iv*) PHA plus PMA. They were incubated for an additional 8 hr and processed for RNA isolation and blot hybridization as described above.

RESULTS

***TCGF* Gene Expression.** Transcription of the *TCGF* gene and its inducibility by PHA and PMA in HTLV-III- and HTLV-I-infected cells was analyzed by RNA blot hybridization procedure. Fig. 1 shows the hybridization of TCGF DNA probe with size-separated poly(A)-selected cellular RNAs. As in normal human lymphocytes (36, 47), the size of TCGF mRNA in H4 (and H9) cells was about 0.9 kilobase, and this was the only species present in either uninfected or HTLV-III-infected cells. Low steady-state level of this message was detectable in uninduced H4 (and H9) cells, and this level was increased markedly upon induction of both uninfected and HTLV-III-infected cells. HTLV-I-infected cells on the other hand lacked detectable TCGF mRNA, confirming our results (33), and they did not contain TCGF mRNA even after induction with PHA and PMA. To obtain a better quantitative appreciation of the magnitude of induction, poly(A)-selected RNAs from H4 and H9 cells were analyzed by dot blot hybridization (Fig. 2). Uninduced and uninfected cells contained some TCGF mRNA, and the abundance of this mRNA appeared to decrease somewhat upon infection with HTLV-III. When uninfected cells were induced with PHA and PMA, the abundance of TCGF mRNA increased 2- to 3-fold. The magnitude of this induction for HTLV-III-infected cells was 10- to 12-fold. In other words, though the constitutive levels of TCGF mRNA in HTLV-III-infected cells were lower than in uninfected cells, the induced levels were much higher in infected than in uninfected cells.

We also evaluated the inducibility of *TCGF* gene in two other HTLV-III-infected cell lines, CEM and a selected clone of Jurkat cells (clone D6). These cells were specifically chosen because we knew from our other studies that they were not inducible for *TCGF* expression prior to HTLV-III infection. When analyzed by RNA blot hybridization, these two cell lines did not reveal detectable TCGF mRNA upon induction whether they were infected with HTLV-III or not (data not shown).

***IFN-γ* Gene Expression.** We have shown that some of the HTLV-I-infected T-cell lines in culture synthesize IFN-γ mRNA constitutively. We have now extended these studies to other HTLV-I- as well as HTLV-III-infected cell lines and specifically investigated the effect of PHA and PMA on the induction of IFN-γ mRNA synthesis. As shown in Fig. 3, IFN-γ mRNA was readily detected in HTLV-I-infected C2/MJ and MI cells. The size of this mRNA was about 1.2 kilobases, identical to the size found in normal human T lymphocytes (44, 47) and no additional or aberrant forms of IFN-γ mRNA were detected. The abundance of this mRNA was significantly increased when HTLV-I-infected cells were induced with PHA and PMA. In contrast, HTLV-III-infected or uninfected H4 cells neither synthesized IFN-γ mRNA constitutively nor could be induced to do so (Fig. 3). The case

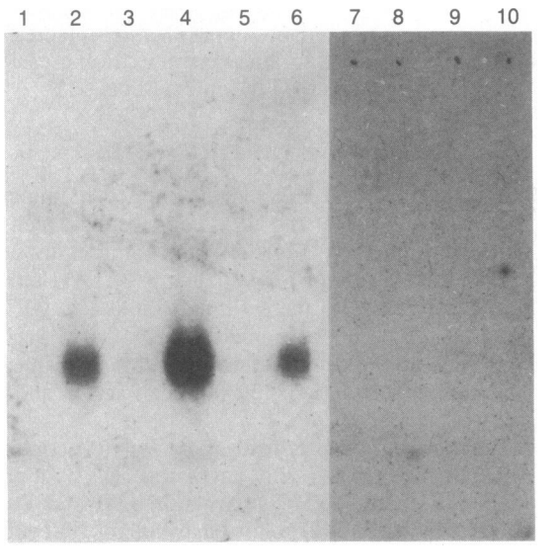

FIG. 1. RNA blot analysis of TCGF mRNA in HTLV-III- and HTLV-I-infected cells. Poly(A)-selected RNA (10 μg per lane) was size-separated by denaturing agarose-gel electrophoresis, transferred to a membrane, and hybridized with ^{32}P-labeled cloned TCGF DNA probe. Lanes: 1, uninduced H9 cells; 2, induced H4 cells; 3, uninduced, HTLV-III-infected H4 cells; 4, induced, HTLV-III-infected H4 cells; 5, uninduced Jurkat cells; 6, induced Jurkat cells; 7, uninduced, HTLV-I-infected C2/MJ cells; 8, induced HTLV-I-infected C2/MJ cells; 9, uninduced, HTLV-I-infected MI cells; 10, induced, HTLV-I-infected MI cells. Induction was carried out by treating the cells with PHA (1%) and PMA (10 ng/ml) for 18–20 hr.

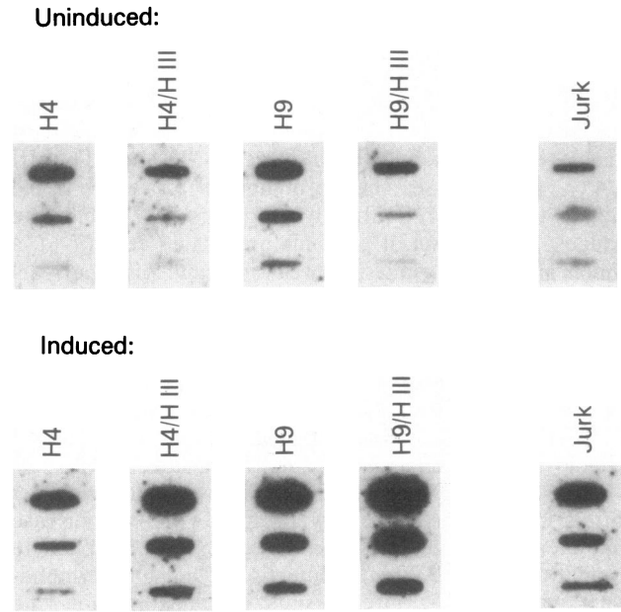

FIG. 2. Relative abundance of TCGF mRNA in uninfected and HTLV-III-infected cells. Serial dilutions (1:5) of poly(A)-selected RNA, starting with 10 μg of RNA, were applied to a nitrocellulose membrane and hybridized with ^{32}P-labeled cloned TCGF DNA probe. The lanes for the respective RNA preparations are as indicated; H4/HIII and H9/HIII refers to HTLV-III-infected H4 and H9 cells, respectively, and Jurk refers to Jurkat cells.

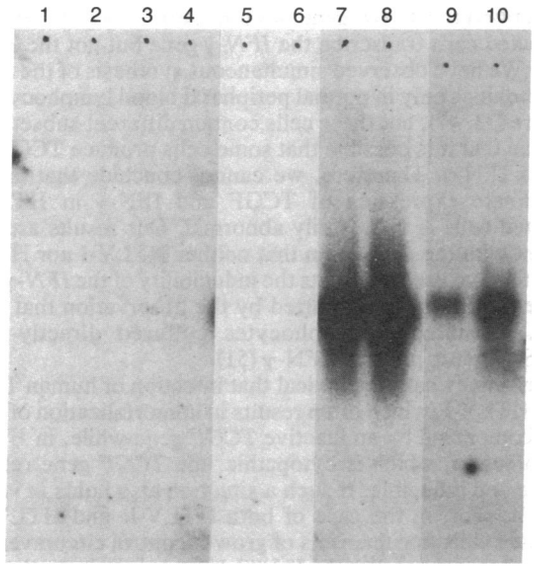

FIG. 3. RNA blot analysis of IFN-γ mRNA in HTLV-III- and HTLV-I-infected cells. The analysis was performed as described in Fig. 1, substituting ^{32}P-labeled cloned IFN-γ DNA probe for TCGF probe. Lanes 1–10 contain the same RNAs as in Fig. 1.

for HTLV-III-infected H9 cells, CEM cells, and Jurkat cells was similar (data not shown).

Transcription in Isolated Nuclei. To determine if the inducing agents acted primarily by influencing *TCGF* gene transcription, nuclear transcription or "run-off" experiments were performed. *In vitro* nuclear transcription assays have been used to study transcriptional activation. Such nuclear transcription systems support elongation of RNA polymerase II transcripts initiated *in vivo* but do not allow reinitiation of transcription. Thus, incorporation of radiolabeled nucleotides by nuclei *in vitro* into specific RNA provides an estimate of the number of RNA polymerase II molecules in the process of transcribing a specific gene (44, 48, 49). For these experiments, HTLV-III-infected H4 cells were treated with inducing agents alone or in combination and the nuclei from each culture were allowed to elongate the preinitiated RNA chains *in vitro*. Fig. 4 shows the results of hybridization of labeled RNA thus obtained with cloned TCGF and IFN-γ DNAs along with vector pBR322 DNA. It was apparent that the *TCGF* gene was transcriptionally active in uninduced cells while there was little or no transcription of the *IFN-γ* gene. Upon induction with PHA and PMA, there was a marked increase in the transcription of the *TCGF* gene with no noticeable concomitant increase in the transcription of the *IFN-γ* gene. The induction was thus specific for the *TCGF* gene consistent with our results on the steady-state levels of these mRNAs. Interestingly, the magnitude of the response induced by PMA was approximately equivalent to that induced by PHA, and the effect of these two inducers was not additive or synergistic. This is in contrast to our results with Jurkat cells where PMA and PHA acted synergistically in enhancing *TCGF* gene transcription (37).

Persistence of TCGF mRNA. To ascertain if PHA or PMA affected the half-life of TCGF mRNA in HTLV-III-infected cells, the cells were induced with PHA plus PMA for 16 hr and then incubated for an additional 8 hr with or without inducers in the presence of actinomycin D. The intracellular concentration of TCGF mRNA was analyzed by dot blot hybridization (Fig. 5). There was no apparent difference in the levels of TCGF mRNA in cells incubated in the absence or presence of PHA, PMA, and PHA plus PMA. Apparently, neither PHA nor PMA significantly affected the turnover of TCGF mRNA for 8 hr after induction.

DISCUSSION

HTLV-III-induced immune suppression in AIDS is at least in part a consequence of the depletion of T-cell populations, particularly OKT4$^+$ cells (3–6). This is mirrored by cytopathic effects of HTLV-III infection of T lymphocytes in culture (3, 22). TCGF, produced by a subset of T cells, is required for the proliferation of activated T lymphocytes (29–31). It was therefore reasonable to think that T-cell depletion in AIDS patients may be related to the impaired synthesis of TCGF induced by HTLV-III. This possibility was supported by the observation that many of the HTLV-I-infected cells do not synthesize TCGF (ref. 33 and this report).

We have shown that the synthesis of TCGF in human lymphocytes is primarily regulated at the level of *TCGF* gene transcription (36, 37). We show here that HTLV-III-infected H4 and H9 cells not only transcribed the *TCGF* gene constitutively, but also could be induced with PHA and PMA to synthesize abundant quantities of TCGF mRNA. This was accompanied by the synthesis and release of TCGF into the culture medium (data not shown). This was in contrast to

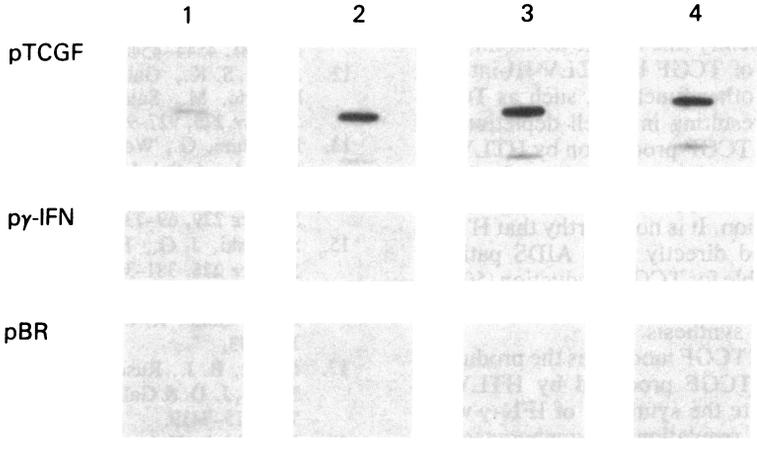

FIG. 4. Analysis of TCGF mRNA synthesized by nuclei from uninduced and induced HTLV-III-infected H4 cells. Nuclei from uninduced and induced cells were allowed to elongate RNA chains *in vitro* in the presence of [^{32}P]UTP and labeled RNA thus obtained was hybridized to serial dilutions (1:5) of cloned TCGF DNA (pTCGF), cloned IFN-γ DNA (pγ-IFN), and vector pBR322 DNA (pBR). Lanes: 1, uninduced cells; 2, cells induced with PHA; 3, cells induced with PMA; 4, cells induced with PHA plus PMA.

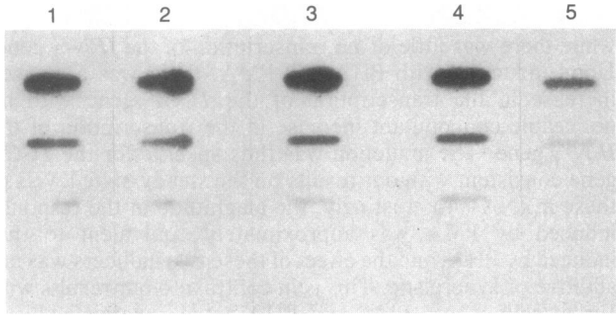

FIG. 5. Persistence of TCGF mRNA in induced HTLV-III-infected H4 cells. Cells were induced with PHA plus PMA for 16 hr, washed, and recultured for an additional 8 hr in the presence of actinomycin D (5 μg/ml) with or without inducers. Poly(A)-selected RNA was isolated from respective cultures, serial dilutions (1:5) starting with 10 μg of RNA were applied to nitrocellulose membrane and hybridized with ^{32}P-labeled cloned TCGF cDNA probe. Lanes 1–4 are for RNA from actinomycin D-treated cultures incubated with the following: 1, no inducer; 2, PHA; 3, PMA; and 4, PHA plus PMA. Lane 5 shows constitutive levels of TCGF mRNA.

some HTLV-I-infected cells which could neither synthesize nor be induced to synthesize TCGF mRNA. To test if HTLV-III-infection had a direct positive effect on the inducibility of the *TCGF* gene, we examined those human T cells that were known not to be inducible for TCGF synthesis, namely, CEM and Jurkat clone D6 cells. These latter cells were negative for detectable TCGF mRNA either prior or subsequent to HTLV-III infection and could not be induced to synthesize TCGF. These results taken together suggest that HTLV-III infection by itself does not necessarily affect either the constitutive synthesis or inducibility of the *TCGF* gene.

It was possible that the synthesis of TCGF in HTLV-III-infected cells was unregulated and involved mechanisms other than those operating in uninfected or normal cells. Nuclear transcription experiments with HTLV-III-infected cells showed that elevated steady-state levels of TCGF mRNA in induced cells were primarily due to the increased transcriptional activity of the *TCGF* gene—a situation similar to what we have described for other inducible T cells (Jurkat) (37). However, in the case of HTLV-III-infected H4 cells, transcription of the *TCGF* gene was enhanced by both PMA and PHA, and their effects were not synergistic. For Jurkat cells, PHA induced transcriptional activation of the *TCGF* gene but we were unable to determine if PMA alone also had this effect (36, 37). Further, the effect of PHA and PMA in Jurkat cells was synergistic (36, 37). Whether these differences are real and biologically meaningful remains to be determined. We cannot presently rule out the possibility that an unregulated production of TCGF by HTLV-III-infected cells down-regulates some other functions, such as TCGF-receptor expression (50), resulting in T-cell depletion and cytotoxicity. Alternatively, TCGF production by HTLV-III-infected cells could stimulate the proliferation of other T cells, such as cytotoxic and/or suppressor T cells, eventually leading to immune suppression. It is noteworthy that HTLV-III-infected T cells cultured directly from AIDS patients' lymphocytes are also inducible for TCGF production (50, 51). Clearly, lymphocytes can be infected with HTLV-III and remain inducible for TCGF synthesis.

It has been suggested that TCGF modulates the production of IFN-γ (34, 35). Thus, TCGF produced by HTLV-III-infected cells could stimulate the synthesis of IFN-γ which may in turn affect immune regulation of lymphocytes. We find that the synthesis of TCGF and IFN-γ is not positively linked in HTLV-infected cells. On the contrary, there appeared to be a reciprocal relation between the synthesis of these two lymphokines. Whereas HTLV-III-infected cells transcribed the *TCGF* gene but not the *IFN-γ* gene, HTLV-I-infected cells transcribe the *IFN-γ* gene but not the *TCGF* gene. We have observed simultaneous synthesis of these two lymphokines only in normal peripheral blood lymphocytes in culture (33, 47), but these cells contain different subsets of T cells so that it is possible that some cells produce TCGF and others IFN-γ. Therefore, we cannot conclude that lack of coordinate expression of TCGF and IFN-γ in HTLV-I-infected cells is necessarily abnormal. Our results are consistent with the suggestion that neither HTLV-I nor HTLV-III infection directly affects the inducibility of the *IFN-γ* gene expression. This is supported by the observation that some HTLV-III-infected lymphocytes cultured directly from AIDS patients produce IFN-γ (51).

It is somewhat paradoxical that infection of human T cells with HTLV-I, which often results in immortalization of cells, is accompanied by an inactive *TCGF* gene while, in HTLV-III infection, which is cytopathic, the *TCGF* gene remains active and inducible. If such a situation also holds *in vivo*, it may be that, in the case of both HTLV-I- and HTLV-III-infected cells, mechanisms of growth control circumvent the regulatory role of TCGF. If HTLVs *trans*-activate the transcription of cellular genes relevant to their pathobiology, as has been suggested (14, 16), *TCGF* and *IFN-γ* genes are not likely to be activated.

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