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T-Cell Growth Factor Gene: Lack of Expression in Human T-Cell Leukemia-Lymphoma Virus-Infected Cells

Abstract. *Activated mature T cells require T-cell growth factor (TCGF) for continuous proliferation. However, many mature T cells infected with human T-cell leukemia-lymphoma virus grow independently of exogenously added TCGF. It is now reported that cells infected with this virus also lack detectable TCGF messenger RNA (less than one copy per cell) and thus do not produce their own growth factor. The results apparently rule out an autostimulation mechanism of growth control.*

T-cell growth factor (TCGF, interleukin 2) is required for the continuous proliferation of specifically activated mature T lymphocytes (*1*). Similarly, some neoplastic mature T cells require TCGF for their growth in vitro, although in some cases without the requirement of prior antigen and lectin activation (*2*). This is apparently because at least some of these cells already possess TCGF receptors (*3*). Some mature T cells infected with human T-cell leukemia-lymphoma virus (HTLV) require TCGF for growth early in culture. Subsequently, many of the HTLV-infected cell lines become independent of exogenously added TCGF (*4*). Some of these cell lines constitutively produce and respond to their own TCGF (*3*), while other cell lines do not elaborate detectable extracellular TCGF (*5*). The question thus arises whether the latter cell lines are truly independent of TCGF or produce small quantities of TCGF, sufficient for growth but undetectable by conventional assay procedures. Moreover, it is possible that these HTLV-infected cells produce TCGF that is not externalized and that would not be detected in extracellular medium. A more direct answer to this question could be obtained from a study of TCGF messenger RNA (mRNA) in these cells. This is now possible because a human TCGF complementary DNA (cDNA) sequence has been molecularly cloned (*6*, *7*), providing a sensitive probe for evaluating the expression of the TCGF gene. We have previously demonstrated that the production of TCGF is regulated at the transcriptional level (*7*). Therefore, if these cell lines synthesize TCGF, whether externalized or not, they would be expected to contain TCGF mRNA. Here we show that several of the TCGF-independent cell lines do not contain detectable TCGF mRNA, suggesting that the immortalization of mature T cells by HTLV may sometimes be by mechanisms that bypass the TCGF-TCGF receptor system.

The TCGF gene recently cloned from a normal human lymphocyte cDNA library (*7*) was identical to the clone from the human lymphoma Jurkat cell line (*6*) and contained the complete coding sequence for TCGF, in addition to 5′ and 3′ untranslated sequences. We have shown that this gene is transcribed into an 11*S* to 12*S* mRNA in all human TCGF-producing cells (*7*). To determine if such an mRNA species was also present in HTLV-infected cells, we analyzed polyadenylate [poly(A)]-containing RNA from these cells by the Northern blot procedure, using cloned TCGF DNA as a probe (Fig. 1). The cell lines examined included those derived from HTLV-positive T-cell malignancies (HUT 102, MO, MI, and MJ), those obtained by HTLV infection of normal human cord blood and bone marrow cells in vitro (C5/MJ, C10/MJ, B2/UK, and MT-2), and an uninfected mature neoplastic T-cell line (HUT 78) (see legend to Fig. 1). The Jurkat cell line, which produces abundant TCGF upon stimulation with phytohemagglutinin (PHA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (*7*, *8*), served as a positive control.

As expected, 11*S* to 12*S* TCGF mRNA was readily detected in stimulated Jurkat cells. A similar mRNA species was also detected in HTLV-infected HUT 102 and MO cells, and also in HUT 78 cells. We estimate (*9*) that Jurkat, MO, and HUT 102 cells, respectively, contain about 40 to 50, 5 to 10, and less than 5 copies of TCGF mRNA per cell. None of the other HTLV-infected cells contained detectable TCGF mRNA. Since TPA in combination with PHA increases the level of TCGF mRNA in some cells (*7*), a number of HTLV-infected cells were also examined after treatment with TPA and PHA. This treatment did not cause

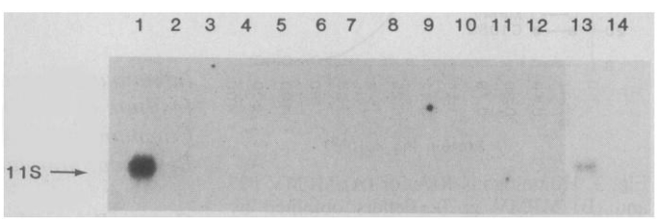

Fig. 1. Expression of TCGF gene in HTLV-infected cells. Poly(A)-selected RNA, size-separated by agarose gel electrophoresis, was hybridized with labeled cloned TCGF DNA. Lanes 1 to 14 are for RNA from: 1, PHA plus TPA-stimulated Jurkat cells; 2, TCGF-independent HUT 78 cells; 3, TCGF-independent HUT 102 cells; 4, another preparation of TCGF-independent HUT 102 cells; 5, TCGF-independent C5/MJ cells; 6, TCGF-dependent C5/MJ cells; 7, TCGF-independent C10/MJ cells; 8, TCGF-independent C10/MJ cells treated with TPA-PHA; 9, TCGF-independent B2/UK cells; 10, TCGF-independent B2/UK cells treated with TPA-PHA; 11, TCGF-independent MT-2 cells; 12, TCGF-dependent MI cells; 13, TCGF-independent MO cells; and 14, TCGF-independent Molt 4 cells (immature T). Poly(A)-selected RNA was obtained as described (*15*). After denaturation at 65°C in 50 percent formamide, RNA (10 μg per lane) was subjected to electrophoresis in 1 percent agarose slab gel containing 6 percent formaldehyde and transferred to a Zeta Probe membrane (Bio-Rad Laboratories) by electroelution (*7*). Hybridization with ^{32}P-labeled nick-translated cloned TCGF DNA was performed at 37°C for 16 hours in a mixture containing 50 percent formamide, five times standard sodium chloride and sodium citrate (SSC, 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7), 0.05*M* sodium phosphate buffer (*p*H 7), five times PM (0.02 percent each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll 400), yeast RNA (200 μg/ml), denatured DNA (20 μg/ml), 0.1 percent sodium dodecyl sulfate (SDS), and 10 percent dextran sulfate. The membrane was subsequently washed repeatedly with SSC and 0.1 percent SDS at 65°C, air dried, and exposed to a Kodak XAR film with the use of intensifying screens. Where indicated, cells were treated with TPA (10 ng/ml) and PHA (1 μg/ml) for 20 hours.

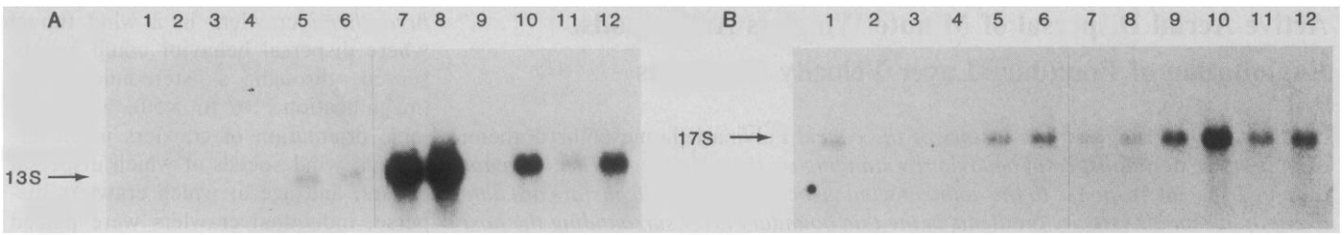

Fig. 2. Expression of (A) γ-interferon gene and (B) JD15 gene in HTLV-infected cells. Analysis was performed as described in the legend to Fig. 1. Lanes 1 to 12 contain the same RNA's as in lanes 1 to 12 of Fig. 1.

the induction of TCGF mRNA in cells that were previously negative (Fig. 1). It was possible that some of these cells synthesized TCGF mRNA that was only distantly related to our cloned TCGF sequence. Such an mRNA could possibly arise from the use of an alternative splicing mechanism or possible polymorphism of the TCGF gene. This was tested by lowering the stringency of hybridization and hybrid detection. Instead of the 50 percent formamide used under standard conditions (see legend to Fig. 1), 40 percent formamide was used. This is equivalent to reducing the temperature at which hybridization was performed by about 7°C. In addition, the filters were washed at 45°C instead of 65°C as under standard conditions. While TCGF mRNA was readily detected in cell lines that gave positive results under standard conditions, such mRNA was not detected in negative cell lines even under reduced stringency conditions (data not shown).

To ensure that the integrity of RNA was maintained during the hybridization procedure, we used two additional probes spanning the 12*S* to 18*S* mRNA range: namely, cloned γ-interferon (γ-IFN) DNA and a cloned sequence designated JD15. The latter cloned DNA was obtained from a Jurkat cDNA library and is specifically expressed in stimulated lymphocytes (*10*). As shown in Fig. 2, the γ-IFN probe detected a specific 12*S* to 13*S* mRNA species in C5/MJ, C10/MJ, B2/UK, and MI cells, and the level of this mRNA was increased by treatment with PHA and TPA. Further, all the HTLV-infected cell lines contained varying amounts of 17*S* to 18*S* mRNA corresponding to cloned JD15 DNA. Thus, the lack of detectable TCGF mRNA in some HTLV-infected cells was not due to the degradation of RNA. It is interesting that there was a reverse correlation between the synthesis of TCGF and γ-IFN mRNA in these cells. Though TCGF may stimulate γ-IFN production by normal peripheral blood lymphocytes (*11*), γ-IFN mRNA was synthesized in these HTLV-infected cells without the concomitant synthesis of TCGF mRNA. Conversely, Jurkat cells stimulated with PHA and TPA synthesized TCGF mRNA in the absence of γ-IFN mRNA (Figs. 1 and 2). As expected, PHA-stimulated normal lymphocytes synthesized both TCGF and γ-IFN mRNA's (data not shown).

Two primary HTLV-infected neoplastic cell lines (HUT 102 and MO) contained TCGF mRNA; the level in HUT 102 was barely detectable. These cells are known to produce low levels of TCGF (*3*) and it has been postulated that the continued abnormal proliferation of these transformed cells may be due to the concomitant presence of cells bearing TCGF receptors and cells producing TCGF (*12*). Two other primary HTLV-infected cell lines (MI and MJ), whether dependent or independent of added TCGF for growth, did not contain detectable TCGF mRNA. The cell lines infected with HTLV in vitro, such as C5/MJ, C10/MJ, B2/UK, and MT-2, which grow independently of added TCGF (*4, 13*), also did not contain detectable levels of TCGF mRNA. The lack of detectable TCGF mRNA in these cells was not due to the poor quality of RNA preparations or other artifacts. It was also not due to a selective loss of this mRNA during poly(A) selection, since total unselected RNA gave the same results as poly(A)-selected RNA (data not shown). Thus, these cells appeared to be truly independent of TCGF for growth. Since the procedure used here can detect one or a few copies of TCGF mRNA per cell (*9*), the results rule out an autostimulation mechanism in which each cell produces and responds to its own TCGF (*12*). Some of these cell lines have been examined by in situ hybridization. Of hundreds of cells examined, not a single cell displaying a specific hybridization signal with the TCGF probe was detected (*14*). Thus, no cell in the population appeared to express TCGF mRNA, suggesting that a parastimulation mechanism, in which a few cells in a population produce sufficient TCGF to sustain the growth of the population, was also not evident.

These results suggest that TCGF production is not always needed for the growth of HTLV-infected T cells. Since all cells possess TCGF receptors (*3*), it is possible that changes in or influences on TCGF receptors are sufficient for continued T-cell growth without the need for any growth factor. Alternatively, it is possible that a protein not related to TCGF produced by normal lymphocytes stimulates proliferation of some neoplastic T cells. If this is the case, this protein must also be significantly different from the TCGF released by such neoplastic T cells as Jurkat, since the normal and Jurkat TCGF gene and mRNA are identical (*7*). In either case, cells transformed by HTLV may use other mechanisms to regulate control.

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