synthesis. Because both the crude enzyme preparation and the combined H and B fractions were free of ribosomes, we are certain that the participation of RNA in formation of ALA was ribosome independent. Involvement of RNA in processes other than the ribosome-dependent protein synthesis has been reported in only a few other instances. An RNA moiety is essential for the functions of ribonuclease P of *Escherichia coli* (*7*) and of signal recognition particles in microsomes (*8*). Aminoacyl-tRNA can add aminoacyl moieties to the α-amino group on the terminal amino acid residue of proteins (*9*).

The proposed pathway for ALA synthesis mentioned above requires that the α-carbon of glutamate be activated. We propose that the activation of glutamate is accomplished by a tRNA-like molecule in the H fraction and an aminoacyl synthetase in the B fraction. We further suggest that the activated glutamate (which could be a glutamyl RNA molecule) is converted to GSA by one or more enzymes in the B fraction. The RNA participating in ALA synthesis has also been isolated from barley plastids and was found to hybridize to the barley plastid genome (*10, 11*).

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Functional Properties of Antigen-Specific T Cells Infected by Human T-Cell Leukemia-Lymphoma Virus (HTLV-I)

Abstract. *Tetanus-toxoid specific helper-inducer T-cell clones, which had been infected and transformed by human T-cell leukemia-lymphoma virus (HTLV-I), were obtained from an antigen-specific human T cell line by using a limiting dilution technique in the presence of the virus. These HTLV-I–infected T-cell clones proliferated specifically in response to soluble tetanus toxoid but, unlike normal T cells, they could do so in the absence of accessory cells. The HTLV-I–infected T-cell clones did not present the antigen to autologous antigen-specific T cells that were not infected with HTLV-I. The capacity of helper-inducer T cells to retain antigen-specific reactivity after infection by HTLV-I, while losing the normal T-cell requirement for accessory cells, has clinical and theoretical implications.*

The term human T-cell leukemia-lymphoma virus (HTLV) refers to a unique family of T-cell tropic retroviruses. Viruses belonging to the HTLV family play a vital role in the pathogenesis of certain adult T-cell neoplasms (*1, 2*) and are believed to be the etiologic agents of acquired immune deficiency syndrome (AIDS) (*3, 4*). A well-recognized property of some subtypes of HTLV is the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro (*5, 6*). However, the effects of HTLV on antigen-driven T-cell responses are not well understood.

We established a tetanus-toxoid reactive T-cell line from a normal blood donor by repeated cycles of in vitro stimulation with soluble antigen and irradiated autologous peripheral blood mononuclear cells (PBM) as a source of accessory cells (*7*). The cells were continuously exposed to T-cell growth factor (TCGF or interleukin-2). We then attempted to propagate clones from this immune T-cell line in the presence and absence of HTLV-I. In the absence of virus, the cloning efficiency was approximately one in 64; however, in the presence of the virus, it was one in five, as detected by Poisson analysis of large numbers of replicate microcultures set up with varying numbers of antigen-responsive cells. The most rapidly growing clones were YTA1, which was derived from a well without virus, and YTH3 and YTH5, which were derived from wells containing virus. These clones, which were obtained by limiting dilution under conditions of one cell per well and have been maintained in continuous culture for more than 160 days after cloning, are the subject of the present report.

Clone YTA1 exhibited a substantial proliferative response against soluble antigen but only in the presence of irradiated autologous PBM that served as accessory cells. In contrast, YTH3 and YTH5 replicated spontaneously without TCGF or accessory cells. Moreover, YTH3 and YTH5 substantially increased their rate of proliferation in response to tetanus toxoid in the presence and also the absence of irradiated autologous PBM (Fig. 1). The overall magnitude of response was generally greater in the presence of accessory cells.

The antigen specificity of the proliferation of these clones was studied by using tetanus toxoid, purified protein derivative, streptokinase and streptodornase, and Formalin-inactivated, zonally purified A2/Aichi/68 influenza virus. None of the clones tested responded to antigens other than tetanus toxoid (data not shown). These data taken together suggest that immune T cells cloned in the presence of HTLV-I retained the capacity to recognize and respond to soluble antigen while acquiring the capacity to respond in the absence of accessory cells.

We then infected YTA1 by recloning in the presence of HTLV-I as described earlier. The resultant T cell population, which was propagated under starting conditions of one cell per well by limiting dilution, is referred to as YTA1H. This HTLV-I–infected clone, unlike the original YTA1 clone, replicated spontaneously without TCGF. YTA1H increased its rate of replication upon exposure to soluble antigen in a dose-dependent fashion, and an antigen-driven response was observed in the absence of accessory cells (Table 1).

Clone YTH3 retained the antigen-specific reactivity described above for approximately 120 days in culture following cloning, but the magnitude of the response gradually decreased. Clone YTH5, in contrast, developed within 60 days a high spontaneous rate of replication in the absence of TCGF. The observable antigen-driven proliferative response was lost and could not be restored by recloning (data not shown).

We wished to know whether clones YTH3 and YTH5 were themselves capable of serving as accessory cells in presenting soluble antigen to normal T cells. We observed that irradiated YTH3 and

YTH5 cells (*8*) did not function as accessory cells in the response of the normal immune T-cell clone YTA1 to soluble antigen (Fig. 2). The addition of purified interleukin-1 did not permit these HTLV-I–infected cells to serve an antigen-presenting function (data not shown). These observations provide evidence that there were no functional accessory cell subsets in the YTH3 and YTH5 populations.

We then confirmed that YTH3 and YTH5 were infected with HTLV-I and determined the characteristics of the proviruses integrated in the genome. DNA from YTA1, YTH3, and YTH5 was analyzed by Southern blot hybridization. No proviral sequences could be detected in the DNA from YTA1 (Fig. 3). (These cells were also negative for the HTLV-I *gag* proteins p19 and p24 as assessed by cytoplasmic indirect immunofluorescence.) In contrast, DNA from YTH3 and YTH5 had, respectively, four and two separate clonally integrated proviruses present, since digestion with Eco RI, which does not cut within the HTLV-I provirus, gave four bands with YTH3 and two with YTH5. Digestion with Bam HI resulted in the presence of a band of 1.1 kb detected with the *pol-env-pX* probe. This is common to all HTLV-I–infected cells, and represents an internal fragment of the provirus, indicating that there is not a large internal deletion of the proviruses. These two clones (YTH3 and YTH5) were also tested for viral protein expression. YTH3 contained 2 to 5 percent cells which were highly positive for the HTLV-I *gag* proteins p19 and p24, while 5 to 10 percent of the cells of YTH5 were highly positive for those proteins (data not shown). As has been observed in other settings (*9*), there appears to be some restriction of viral expression in these cells, since the majority of the cells in these cultures seems to be infected but only a minority is expressing *gag* proteins.

We then analyzed the surface membrane antigens expressed by uninfected and infected antigen-specific T-cell clones by fluorescence-activated cell sorter analysis (*10*). Clones YTA1, YTH3, and YTH5 reacted with OKT3, OKT4, anti-Tac, and anti-HLA-DR monoclonal antibodies. The cells in each population were negative for OKT8 or OKM1. Thus, both the uninfected and infected antigen-specific T-cell clones have the phenotype of mature, activated helper-inducer cells. It is worth noting that this is the phenotype generally expressed in neoplastic cells obtained from patients with HTLV-I–associated leukemias (*2, 11*).

It is a general rule that T cells are not stimulated by soluble antigen alone, and that T-cell activation requires a process of associative recognition that depends on accessory cells bearing major histocompatibility complex (MHC) determinants of the appropriate haplotype (*12–14*). This process is still imperfectly understood despite the dramatic advances

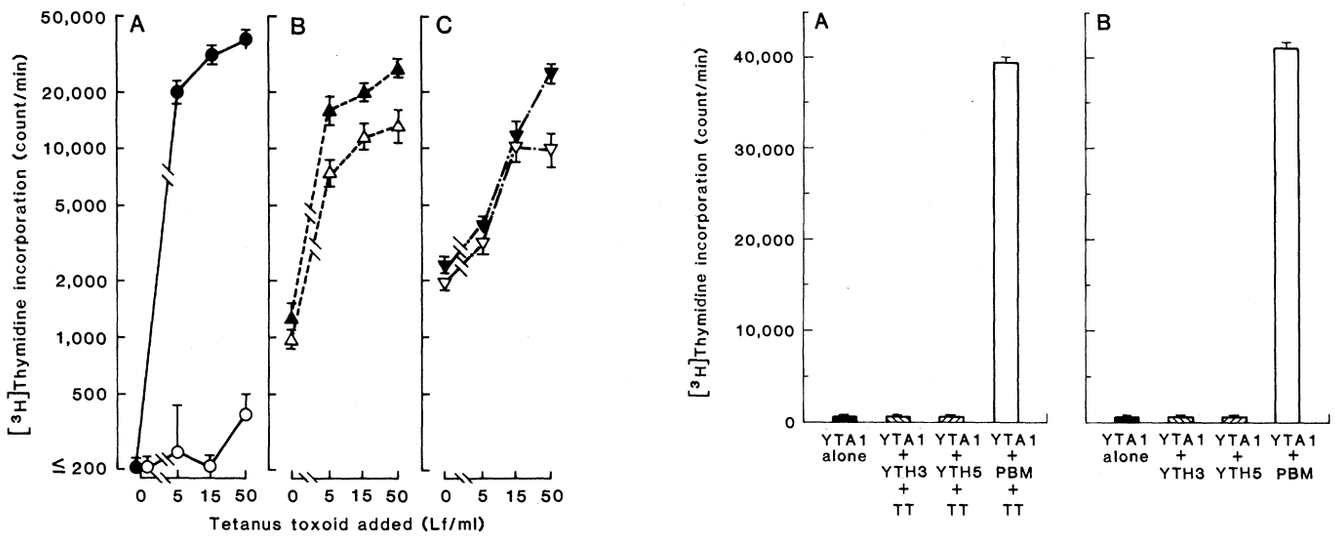

Fig. 1 (left). Stimulation of T-cell clones by soluble tetanus toxoid in the absence of accessory cells. Peripheral blood mononuclear cells (PBM) (10^6) from a normal volunteer (*7*) were cultured with 25 limit flocculation units (Lf) per milliliter of tetanus toxoid (TT) (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain) in 24-well microculture plates (Costar) at 37°C in 5 percent CO_2-containing humidified air in 1 ml of RPMI 1640 medium supplemented with 10 percent autologous plasma, 4 m*M* L-glutamine, and, per milliliter, 50 units of penicillin and 50 μg of streptomycin (complete medium). After 7 days in culture, the cells were continuously exposed to 15 percent TCGF (by volume) (Cellular Products); on day 14 and beyond, 10 percent heat-inactivated fetal calf serum (FCS) was substituted for the autologous plasma. The cells were stimulated every 7 days with the addition of TT (25 Lf/ml) and irradiated (4000 rad) autologous PBM. On day 31 in culture, the cultured cells were cloned by limiting dilution; 0.1-ml portions containing one cell in 15 percent TCGF-containing complete medium were distributed into round-bottomed 96-well microtiter plates (Limbro) with 10^4 irradiated (4000 rad) autologous PBM and TT (25 Lf/ml) in the presence and the absence of 10^4 irradiated (12,000 rad) HTLV-I–producing MJ tumor cells derived from a patient with HTLV-I–associated lymphoma (*5*). The plates were incubated at 37°C in 5 percent CO_2-containing humidified air and fed with 0.1 ml of 15 percent TCGF-containing complete medium every 4 to 5 days. Growing colonies were selected on days 10 to 30 and transferred to 24-well Costar plates. Clones exposed to HTLV-I–producing cells could be expanded in 15 percent TCGF-containing complete medium without further addition of TT and PBM, in contrast to cells not exposed to HTLV-I, which required stimulation with TT and irradiated autologous PBM every 10 to 14 days. Cells (10^5) of the unexposed clone YTA1 (○) (A) and HTLV-I–exposed clones YTH3 (△) (B), and YTH5 (▽) (C) were cultured with various concentrations of tetanus toxoid in the presence (closed symbols) and the absence (open symbols) of 5×10^4 irradiated autologous PBM in 180 μl of complete medium for 4 days, and then exposed to 0.5 μCi of [^{3}H]thymidine for 5 hours and harvested as described (*9*). When cultured alone, irradiated autologous PBM did not proliferate. Each symbol represents the mean ± 1 standard deviation of triplicate determinations. The experiments were performed when cells had been in culture for 36 days after cloning. Fig. 2 (right). YTH3 and YTH5 do not present antigen to tetanus toxoid (TT)-specific T cells. (A) 5×10^4 YTH3, YTH5, or autologous fresh PBM [which were irradiated (*8*) with 4000 rad] were cultured with 10^5 YTA1 cells in the presence of TT (7.5 Lf/ml). (B) Clones YTH3 and YTH5 and fresh autologous PBM were incubated with TT (25 Lf/ml) at 37°C for 3 hours, washed extensively, and irradiated with 4000 rad. These YTH3, YTH5, and PBM (5×10^4) were cultured with 10^5 YTA1 cells without further addition of soluble antigen for 4 days, exposed to [^{3}H]thymidine, and harvested as described in Fig. 1. Irradiated YTH3, YTH5, and PBM, when cultured alone, failed to incorporate [^{3}H]thymidine. Each bar represents the mean ± 1 standard deviation of triplicate determinations.

in characterizing antigen receptor genes and molecules (*15–17*).

Rao *et al.* (*18*) have shown that murine T-cell clones reactive against the *p*-azobenzenearsonate hapten express specific binding sites for radioactively labeled arsanylated ovalbumin. Binding can take place in the absence of accessory cells. Under certain conditions, several structurally related haptens, conjugated to ovalbumin, can competitively inhibit the activation of T cells reactive to the *p*-azobenzenearsonate hapten, although antigen binding per se does not result in T-cell activation. Moreover, Carel *et al.* (*19*) have obtained a T-cell hybridoma that specifically reacts with soluble cytochrome *c* peptide. This hybridoma binds the peptide antigen in the absence of accessory cells, resulting in production of TCGF (*19*). These data imply that some antigenic determinants may bind to specific sites on T-cell clones in the absence of accessory cells. However, it is difficult to categorize the functional consequences of binding alone.

To our knowledge, the HTLV-I–infected T cells generated in the current studies provide the first example of a specific human T-cell proliferative response to a soluble antigen without the addition of accessory cells. It is possible that the integration of HTLV-I into the genome of an antigen-specific T cell qualitatively or quantitatively alters the expression of antigen receptors and class II major histocompatibility (MHC) antigens, thereby conferring on the T cell a self-sufficient capacity for associative recognition. However, these HTLV-I–infected T cells have not gained the ability to present antigen to uninfected, antigen-specific T cells in our experimental conditions. Alternatively, HTLV-I infection might obviate an external signal mediated by MHC proteins, allowing the simple engagement of the T-cell antigen-binding receptors by soluble antigen to serve as a sufficient stimulus for a proliferative response. The availability of HTLV-I–transformed immune lymphocytes might provide an important resource for analyzing antigen recognition by human T cells at a molecular and cellular level. Moreover, it is likely that the capacity of the virus to bring about an altered requirement for accessory cells coupled in some situations with a progressive loss of antigen-specific reactivity in vitro will have relevance in understanding the consequences of HTLV-I infection in vivo.

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Table 1. Dose-dependent proliferative response of HTLV-I–infected, tetanus toxoid–specific clone YTA1H to soluble antigen in the absence of accessory cells. Cells (10^5 YTA1H and 10^5 YTA1) were cultured with various concentrations of soluble tetanus toxoid in the presence or absence of irradiated (4000 rad) autologous PBM for 3 days, exposed to [^{3}H]thymidine, and harvested as described in Fig. 1. YTA1H had never been exposed to autologous PBM (accessory cells) after cloning; while YTA1 (the uninfected counterpart) had been exposed to irradiated autologous PBM for 7 to 10 days prior to these experiments as part of a cycle of restimulation. The data are expressed as means ± 1 standard deviation of triplicate determinations. N.D., not determined.

Clone	Days after cloning	Irradiated PBM	Tetanus toxoid (Lf/ml)			
			0	4	8	16
YTA1H	20	−	1740 ± 156	N.D.	5240 ± 1261	N.D.
	33	−	4181 ± 75	9830 ± 1368	11286 ± 360	8826 ± 385
YTA1	65	−	149 ± 28	218 ± 109	195 ± 88	318 ± 127
	65	+	156 ± 27	19424 ± 743	11914 ± 566	8091 ± 1599
	82	+	230 ± 18	N.D.	26605 ± 1885	N.D.

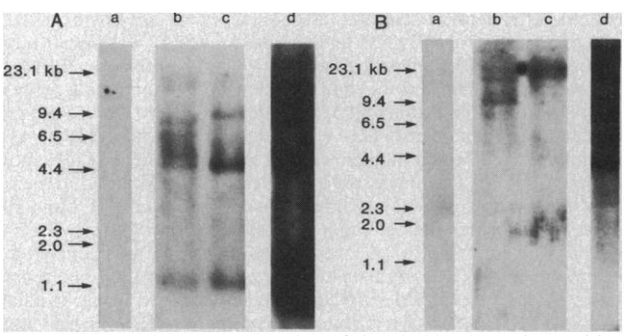

Fig. 3. Proviral sequences of HTLV-I in clones YTH3 and YTH5. The DNA was digested with Bam HI (A) and Eco RI (B), and analyzed by the Southern blotting technique with an HTLV-I probe as described (*9, 20*). The DNA was from YTA1 (lane a), YTH3 (lane b), YTH5 (lane c), or HTLV-I–producing MJ-tumor cells (lane d). Arrows on the left-hand side show the position of the Hind III fragments of λ phage DNA as a marker. DNA digested with Bam HI and Eco RI was subjected to electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P nick-translated DNA insert from a subclone in pBR322 of the portions of the HTLV-I genome defined by the Cla I and Hind III sites of λCR-1 (*21*) and containing *pol*, *env*, and *pX* sequences.

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