Alteration of T-Cell Functions by Infection with HTLV-I or HTLV-II

Abstract. *Two functionally different types of human T-cell clones, one with helper function and two with specific cytotoxic activity, were infected with different isolates of HTLV-I and HTLV-II. Both types of human T cells showed alterations in specific function after infection with either of the HTLV subgroups. Before HTLV infection, the T-cell clone with helper function proliferates and provides "help" to B cells only in the presence of both a specific soluble antigen (keyhole limpet hemocyanin) and histocompatible antigen-presenting cells. After HTLV infection, these cells respond with increased proliferation and indiscriminant stimulation of polyclonal immunoglobulin production by B cells, regardless of the histocompatibility of the antigen-presenting cells or the presence of the soluble antigen. Infection of the normal cytotoxic T-cell clones led to a diminution or loss of the cytotoxic function. The results of these studies suggest some possible mechanisms for induction of immune deficiency and of polyclonal B-cell activation by viruses of the HTLV family.*

Since the first isolation of a human retrovirus (*1*), designated human T-cell leukemia-lymphoma virus subgroup I (HTLV-I), a large number of human T-lymphotropic retroviruses (HTLV) have been isolated (*2–6*). This was made possible by the development of sensitive methods for virus detection (*7*) and growth of the T cells with T-cell growth factor (TCGF) (*8*). HTLV-I is closely associated with malignancies of mature T cells occurring in adults of various countries (*2*), particularly with clusters of adult T-cell leukemia (ATL) in Japan (*4*) and a related syndrome in the Caribbean (*5*). The disease has an aggressive clinical course frequently accompanied by opportunistic infections (*9*), including *Pneumocystis carinii* pneumonia (*9*), which is also a common opportunistic infection in acquired immune deficiency syndrome (AIDS) (*10*). Some earlier results were consistent with the notion that a member of the HTLV family causes AIDS (*11*), and there is now eviden that a new subgroup of HTLV (HTL III) is the primary cause (*6*). It is possit that infection of a certain subset of ly phocytes with the leukemoger HTLV's (HTLV-I and HTLV-II) c occur in vivo, ultimately leading to immunological deficiency by abrogati of the specific functions of the lymph cytes or by selective cell killing. Th combined with the ability of HTLV-I immortalize some infected T cells, m explain the apparent efficient leukem genic activity of these viruses.

The ability of HTLV-I or HTLV-II alter specific T-cell functions was stu ied by in vitro infection of T-cell clon of predefined function. One T-cell clon SR-2, both proliferates and provid "help" to B lymphocytes when expos to the soluble antigen keyhole limp hemocyanin (KLH) in association wi antigen-presenting cells (APC) expres ing a defined histocompatible (HL class II antigen (DR4 and LD40). Tv other clones, DM322A and AE15.3, e hibit specific cytotoxic responses towa cells expressing the HLA DR2 and DI target antigens, respectively. Their d velopment and characterization in vit

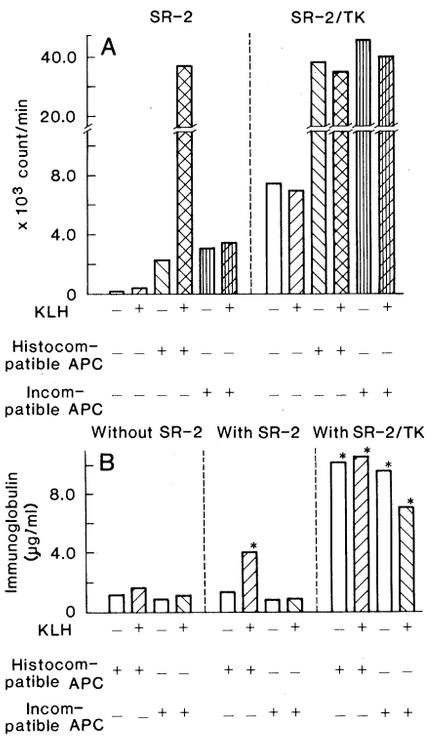
Table 1. Properties of KLH-specific helper T-cell clone and the cytotoxic T-cell clones before and after infection with HTLV. The developmer growth properties in vitro, and functional characterization of KLH-specific and cytotoxic T-cell clones have been described (*12, 13*). The T-c clones were infected by cocultivation with three different HTLV isolates: HTLV-I_{TK} isolated from the mother of a Japanese patient with ATL (; HTLV-I_{EP} from a patient with AIDS (*2*) and HTLV-II_{MO} from a patient with a T-cell variant of hairy cell leukemia (*14*). The cocultivation ass and identification of the HTLV-infected T-cell population by histocompatibility (data not shown) and karyotyping have been described (*3, 1*. Cell-surface markers were identified in three independent experiments and representative data are shown. The percentage and mean fluoresce units (mfu) of positive cells reacting with monoclonal antibodies OKT4 (helper-inducer), OKT8 (suppressor-cytotoxic), antibody to TAC (TC receptor), and 4D12 (detecting an epitope common to the HLA-B cross-reactive group antigens) were described (*3, 18, 20*). Positive cells a those that show greater fluorescence than the P-3 myeloma protein. The values are lower than previously described (*18, 20*) because of P binding. However, there are no essential changes in reaction with OKT4 and OKT8 monoclonal antibodies before and after HTLV infection. T percentage of HTLV-infected T cells was determined by immunofluorescence assay (IFA) with a mouse monoclonal antibody against HTLV p (*16*). The core protein p24 was detected by homologous radioimmunoprecipitation assay (RIPA) with goat serum directed against HTLV-I p (*17*) and by heterologous RIPA with rabbit serum directed against HTLV-II p24 (*14*). RIPA was carried out with ^{125}I-labeled HTLV-I p24 and limiting dilution of the goat (homologous) or the rabbit (heterologous) antibodies to p24. Reverse transcriptase (RT) activity in culture fluids w assayed (*15*) and the activity expressed as picomoles of ^{3}H-labeled deoxythymidine monophosphate incorporated into trichloracetic aci precipitable DNA per milliliter of 20-fold-concentrated culture fluids. Virus particles were detected by electron microscopy. N.D., no detected.

T-cell clone	Sex chromosomes	Cell surface markers								HTLV expression				Virus particles
		OKT4		OKT8		TAC		4D12		IFA for p19 (% positive)	RIPA for HTLV p24 (% competition)		RT (pmol/ml)	
		%	mfu	%	mfu	%	mfu	%	mfu		Homologous	Heterologous		
KLH-specific														
SR-2	XX	69	773	0.6	21	30	245	0	1.0	0	0	N.D.	0	N.D
SR-2/HTLV-I_{TK}	XX	74	881	1.2	26	48	773	45	588	85	100	100	50	+
Cytotoxic to HLA-DR2														
DM322A-13	XX	51	599	6	56	27	689	7.3	59	0	0	N.D.	0	N.D
DM32A-13/HTLV-I_{TK}	XX	61	781	6	49	72	2158	25	386	18–75	100	100	25	+
DM322A-13/HTLV-I_{EP}	XX	44	408	4.3	57	48	883	23	315	75	100	100	12	+
DM322A-13/HTLV-II_{MO}	XX	46	621	3.7	50	42	1229	7.4	39	18–72	<50	100	32	+
Cytotoxic to HLA-DR7														
AE15.3	XY	62	489	4	56	35	306	22	189	0	0	N.D.	0	N.D
AE15.3/HTLV-I_{TK}	XY	37	564	12	174	39	2456	58	2081	45–90	100	100	410	+
AE15.3/HTLV-II_{MO}	XY	65	502	6	33	46	448	43	210	15–45	<50	100	22	+

have been described (*12, 13*). The properties of these clones, virus expression, karyotype, and cell-surface characteristics before and after infection with HTLV are summarized in Table 1. All three T-cell clones were infected by cocultivation with different HTLV isolates (*3*). The SR-2 clone was infected with an isolate of HTLV-I known as HTLV-I_{TK} (*3*). The cytotoxic DM322A T-cell clone was infected with the HTLV-I_{TK} and HTLV-I_{EP}, which is an isolate from a patient (E.P.) with AIDS (*2*); and with HTLV-II_{MO}, a virus belonging to a separate subgroup of HTLV and, therefore, called subgroup II (*14*). The cytotoxic clone AE15.3 was infected with HTLV-I_{TK} and HTLV-II_{MO}.

Table 1 shows that HTLV was fully expressed in all three T-cell clones, as determined by reverse transcriptase activity in culture fluids (*15*) and by electron microscopic examinations. The identity of HTLV-infected clones and their normal (noninfected) counterparts was determined by HLA typing (data not shown) and chromosomal analysis. The percentage of HTLV-infected cells was determined by an indirect immunofluorescence assay in which monoclonal antibodies to the HTLV structural protein p19 were used (*16*). The major core protein, p24, was detected by homologous and heterologous radioimmunoprecipitation assays performed with goat and rabbit sera directed against p24 of HTLV-I and HTLV-II, respectively (*17*). In addition, the expression of new antigens related to HLA class I antigens was induced by the HTLV-I_{EP} and HTLV-I_{TK} isolates and not by HTLV-II (*18*). These findings taken together demonstrated that the cells were infected by particular isolates of each subgroup.

The blastogenic responses of the uninfected and HTLV-infected KLH-specific T-cell clone SR-2 were studied next. Uninfected SR-2 showed little or no thymidine incorporation (Fig. 1A). Low levels of thymidine incorporation occurred with APC alone, but increased by a factor of more than 14 in the presence of both KLH and compatible APC. Moreover, the T cells did not respond to KLH in association with incompatible APC. In contrast, HTLV-I–infected cells (SR-2/TK) proliferated in the absence of both



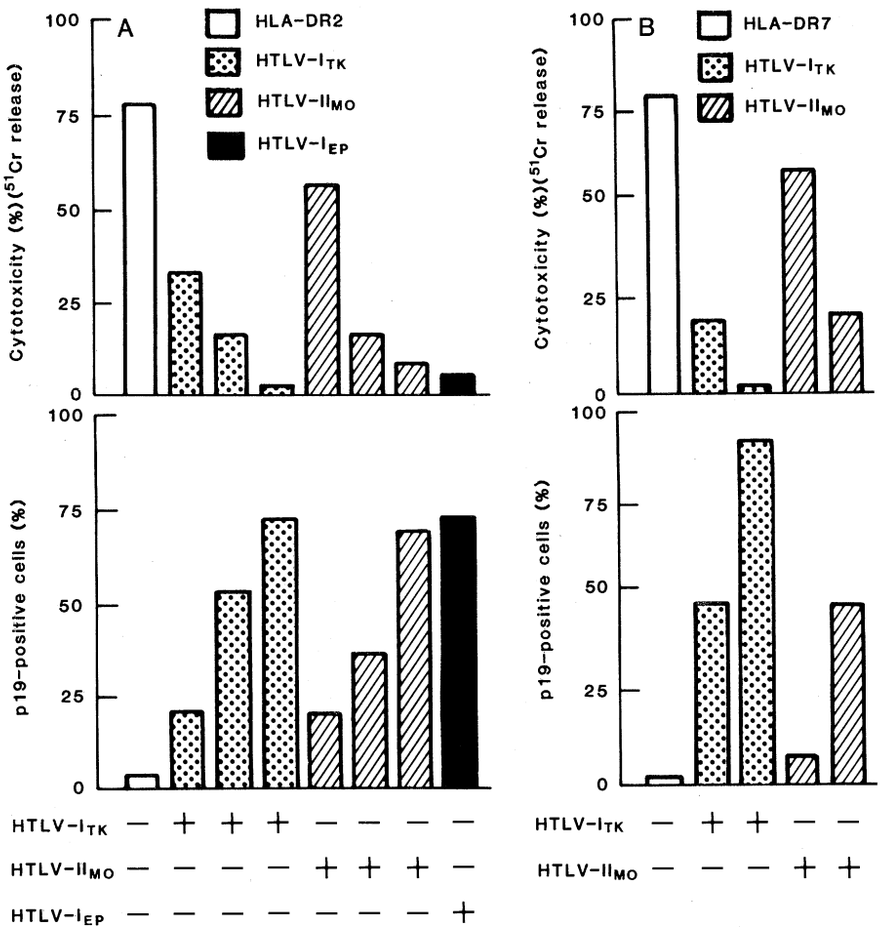


Fig. 1 (left). Function of a KLH-specific human T-cell clone (SR-2) before and after infection with HTLV. (A) Proliferative responses of (i) normal (uninfected) KLH-specific SR-2 and (ii) HTLV-I_{TK}–infected SR-2 cells (SR-2/TK) were determined in the presence (+) or absence (−) of KLH and histocompatible or incompatible APC. Briefly, 2.5×10^5 irradiated (3500 rad) mononuclear cells were incubated with 10^4 T cells, with or without KLH (100 μg/ml). After 3 days, cultures were treated with 1 μCi of [^{3}H]thymidine for 18 hours. Data represent mean counts per minute of triplicate cultures. Mononuclear cells from compatible or incompatible persons above, with or without KLH, gave ≤700 count/min. (B) Immunoglobulin production of B cells in presence (+) or absence (−) of KLH and histocompatible or incompatible APC was followed (i) without KLH-specific SR-2 cells, (ii) with SR-2 cells, and (iii) with HTLV-I_{TK}–infected SR-2/TK cells. In the assay, 2.5×10^5 T cells (SR-2 or SR-2/TK) for 10 days and the supernatant was measured for immunoglobulins G and M. Data represent mean immunoglobulin levels of triplicate cultures. Cultures with added SR-2 or SR-2/TK cells were compared to those without added T cells, and the significance of the values was determined by Student's *t*-test (*$P < 0.05$). The standard error of the mean was less than 20 percent of the total immunoglobulin level in each case. Fig. 2 (right). Cytotoxic activity of DM322A-13 and AE15.3 T-cell clones before and after HTLV infection. Cytotoxicity assays and immunofluorescence assays for HTLV p19 were performed as described (*17, 21*). Cytotoxic T-cell clones infected with particular HTLV isolates were analyzed for cytotoxicity and for HTLV p19 from the same cultures and compared at various intervals after infection to normal (uninfected) counterparts. (A) Clone DM322A-13 with cytotoxicity directed toward HLA-DR2 was infected with HTLV-I_{TK}, HTLV-II_{MO}, and HTLV-I_{EP}, respectively. (B) Clone AE15.3 with cytotoxicity directed toward HLA-DR7 was infected with HTLV-I_{TK} and HTLV-II_{MO} isolates, respectively. (−) Negative for particular HTLV isolate; (+) positive for particular HTLV isolate. Data represent the mean of triplicate determinations of ^{51}Cr release, and variability for each value does not exceed 20 percent.

KLH and APC. The addition of KLH alone had a negligible effect, but the presence of APC increase proliferation more than fivefold whether histocompatible (DR4 and LD40) or incompatible APC were used, with or without KLH antigen. Thus, HTLV infection of SR-2 alters these cells so that they proliferate spontaneously and are further stimulated by allogeneic APC in an unregulated fashion regardless of the presence of histocompatibility or antigen. HTLV-I–transformed T cells can respond to a common structure of HLA class II antigens by proliferation (*19*); this would explain the ability of irradiated APC to augment proliferation of SR-2/TK cells. Further studies are needed to determine whether the nonspecific stimulation of these cells could be mediated through soluble lymphokines.

The ability of SR-2 and SR-2/TK to stimulate polyclonal B-cell activation is illustrated in Fig. 1B. Both compatible and incompatible allogeneic mononuclear cells containing B- and T-lymphocytes and monocytes were obtained from individuals who were not immune to KLH. These cells were cultured alone with uninfected SR-2 helper cells or with infected SR-2/TK cells. Supernatant immunoglobulin production was measured (see legend to Fig. 1B). Immunoglobulin production increased by a factor of almost 3 when SR-2 cells were cultured with compatible APC in the presence of KLH but not when incompatible APC were used or when compatible or incompatible APC were used in the absence of KLH antigen. In contrast, the SR-2 cells infected with HTLV-I_{TK} (SR-2/TK) stimulated B-cell immunoglobulin production to a greater degree, and this did not depend on activation of the infected T cells by KLH or on the presence of histocompatible APC. The nonspecific nature of the B-cell help shown by the HTLV-infected helper T cells indicates that these cells stimulate in an unregulated manner, independent of either the HLA or antigen recognition that were characteristic of the uninfected clone.

Two T-cell clones, DM322A and AE15.3, with specific cytotoxic activity directed toward HLA class II antigens DR2 and DR7, respectively, were infected with HTLV, and their cytotoxic function was compared to that of the uninfected clones. The percentage of HTLV-positive T cells (measured as the percentage of cells positive for the HTLV-specific antigen p19) and cytotoxic activity were determined in parallel experiments at various intervals after infection. As the number of HTLV-positive cells increased, there was a parallel decrease in cytotoxic activity in both HTLV-infected clones (Fig. 2). When the percentage of virus-infected cells was over 70 percent, cytotoxic T-cell activity decreased to 1/10 or less. In some instances there was complete loss of function. The cytotoxic activity in both T-cell clones could not be restored by restimulation with antigen-specific cells. The data indicate that cytotoxic function in both the T-cell clones infected with different virus isolates is irreversibly lost.

Previous studies of human T cells infected with HTLV-I and HTLV-II showed that some of the infected T cells are transformed and differ from normal uninfected T cells in several well-defined characteristics in vitro (*20*). In addition, as we have shown with two types of functionally defined T-cell populations, the morphologic, growth, and cell-surface alterations of HTLV-infected T cells are accompanied by alterations in function in the case of KLH-specific helper T cells and by significant decrease or complete loss of cytotoxic function in the case of cytotoxic T cells. Although proliferation and B-cell help in uninfected, KLH-specific SR-2 cells are regulated by the presence of both the soluble antigen and the histocompatibility of APC, cells infected with HTLV manifest a loss of this regulation of induction. Thus, functions of SR-2 cells such as proliferation and B-cell help become indiscriminant after HTLV-I infection, resulting in polyclonal induction of immunoglobulin production. If HTLV-III, the apparent cause of AIDS, has effects similar to those of HTLV-I in these systems, the results might be relevant to the observation that AIDS patients exhibit polyclonal activation of B cells (*21*). The capability of HTLV-I and HTLV-II to induce loss of specific responses of the infected T cells is consistent with observations suggesting that HTLV-I–associated T-cell malignancies are linked with immune deficiency manifested in various opportunistic infections, including those produced by agents like *Pneumocystis carinii* (*9*), and, as recently shown, with an increased risk of B-cell lymphoid valignancies (*22*).

Retroviruses associated with neoplastic diseases may also cause other diseases. In cats, for example, an acquired immune deficiency may result from infection with feline leukemia virus (FeLV) (*23*), and abrogation of lymphocyte functions in vitro by FeLV has also been demonstrated (*23*). Clinical observations as well as in vitro data suggest that the now widening family of human T4 lymphotropic retroviruses can induce alterations in T cells that could lead to an immune deficiency. In addition, our present results show that HTLV-I and HTLV-II can infect and immortalize T cells having a specific immune function. HTLV-infected T cells cultured in vitro are frequently mono- or oligoclonal, representing a selected cell population (*20*). Therefore, it is possible that, at the time of infection of the normal clones, some T cells were in "a nonfunctional phase," and these HTLV-transformed T cells became dominant. However, this is unlikely because the alteration of specific function after HTLV infection followed the same pattern in six independent experiments with three different HTLV isolates (Figs. 1 and 2). It is of interest to attempt immortalization of various types of functional human T cells for molecular analysis of various specific T-cell structures. For instance, Mitsuya *et al.* (*24*) recently provided evidence for only partial abrogation of a specific cytotoxic T-cell function in a T-cell clone positive for HTLV-I. Thus, the establishment of immortalized T-cell clones with various degrees of altered T-cell function originated from functionally defined T-cell populations could provide a system suitable for the molecular analysis of T cells with specific function.

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Separation of Morphine Analgesia from Physical Dependence

Abstract. *Intravenous infusion of morphine sulfate in rats for 24 hours produced marked opioid dependence, manifested by a series of well-documented signs appearing after injection of the opiate antagonist naloxone. Treatment of rats with naloxonazine significantly reduced the analgesia associated with the morphine infusions for more than 24 hours. Furthermore, 14 of 16 withdrawal signs observed in naloxonazine-treated rats were virtually identical to those in rats that received morphine alone. These results raise the possibility that different receptor mechanisms mediate morphine analgesia and many of the withdrawal signs associated with morphine dependence.*

Repeated administration of morphine produces tolerance and physical dependence. A number of typical signs and symptoms of this dependence are seen after the morphine is withdrawn or an opiate antagonist such as naloxone is administered, Although analgesia and physical dependence are discrete pharmacological effects, they have a close association. A major question in opiate pharmacology is whether analgesia can be dissociated from the production of physical dependence. Binding studies have identified two subtypes of μ receptors: the μ_1 or common high-affinity site, which binds opiates and the enkephalins equally well, and the μ_2 site, which binds morphine far more potently than the enkephalins (*1*). The development of irreversible antagonists of μ_1 binding sites (*2*), such as naloxonazine, has permitted investigations of the pharmacological actions mediated by these sites. Naloxonazine selectively antagonizes in a long-acting manner a variety of opioid actions, including analgesia, prolactin release, catalepsy, acetylcholine turnover, and hypothermia without affecting a number of other actions, such as respiratory depression, growth hormone release, bradycardia, sedation, inhibition of electrically stimulated guinea pig ileum, and dopamine turnover (*3*). We examined the effects of naloxonazine on morphine analgesia and physical dependence.

Male Sprague-Dawley rats (250 to 300 g; Charles River) were surgically prepared with cranial cannula connector pedestals (*4*). Four days later, indwelling jugular cannulas were emplaced (*5*). The animals were then treated intravenously with naloxonazine (20 mg/kg) or saline, and 24 hours later intravenous infusions of morphine sulfate (15 or 50 μg/kg-min) or saline were started at the rate of 6ml/day. Analgesia was assessed with the radiant heat tail-flick method with a maximum cutoff of 10 seconds (*3, 6*). The infusions were discontinued after 24 hours and the animals then received a single dose of naloxone HCl (4 mg/kg, subcutaneously). Rats were monitored continuously for any signs associated with the morphine withdrawal syndrome for 1 hour after the naloxone injection (*7*). Body weights were also measured. Nonparametric statistical techniques were used to assess the significance of differences in withdrawal signs observed among groups (*8*).

Morphine infusion at 15 μg/kg-min (group 4) and 50 μg/kg-min (group 2) significantly increased tail-flick latencies over baseline values (Table 1). Peak latencies were observed 2 hours into the infusion, after which latencies decreased, presumably because of the development of tolerance. After a 24-hour infusion, the mean latency of group 4 returned to baseline (2.8 ± 0.3 seconds) and that of group 2 decreased to 6.3 ± 0.6 seconds. Naloxonazine pretreatment (group 3) markedly attenuated the peak analgesic effect of morphine at 50 μg/kg-min, reducing it to that produced by morphine at the lower dose. The mean latency of group 3 remained much lower than that of group 2 throughout the 24-hour infusion. However, little tolerance was noted, with an average latency at 24 hours of 4.0 ± 0.2 seconds. Tail-flick latencies of rats infused with saline only (group 1) did not change significantly from baseline. Similarly, naloxonazine treatment did not produce a significant change in latency.

Group 2 became dependent on mor-