T-cell activation by autologous human T-cell leukemia virus type I-infected T-cell clones

(human T-cell leukemia virus type I-associated myelopathy/T-cell activation/adhesion molecules)

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ABSTRACT   A unique feature of both human T-cell leukemia virus type I (HTLV-I) carriers and subjects with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic inflammatory disease of the nervous system, is the presence of large numbers of activated T cells that spontaneously proliferate *in vitro*. We have investigated the mechanisms of T-cell activation by HTLV-I in freshly isolated blood T cells and in naturally infected T-cell clones obtained by direct single-cell cloning from patients with HAM/TSP. Both $CD4^+$ and $CD8^+$ HTLV-I-infected T-cell clones showed the unusual ability to proliferate in the absence of exogenous interleukin 2 (IL-2). Nevertheless, HTLV-I-infected clones were not transformed, as they required periodic restimulation with phytohemagglutinin and feeder cells for long-term growth. Irradiated or fixed HTLV-I-infected clones were found to induce the proliferation of blood T cells when cocultured, which we refer to as T_{HTLV-I}–T cell activation. This T_{HTLV-I}–T cell-mediated activation was blocked by monoclonal antibodies (mAbs) against CD2/lymphocyte function-associated molecule 3 (LFA-3), LFA-1/intercellular cell-adhesion molecule (ICAM), and the IL-2 receptor but not by mAbs against class I or class II major histocompatibility complex molecules, HTLV-I gp46, or a high-titer HAM/TSP serum. Spontaneous proliferation of blood T cells from HAM/TSP patients could also be inhibited by mAbs to CD2/LFA-3, LFA-1/ICAM and to the IL-2 receptor (CD25). These results show at the clonal level that HTLV-I infection induces T-cell activation and that such activated T cells can in turn stimulate noninfected T cells by cognate T_{HTLV-I}–T cell interactions involving the CD2 pathway.

Human T-cell leukemia virus type I (HTLV-I) is a T-cell tropic retrovirus involved in the pathogenesis of adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1–4). HAM/TSP is characterized by focal T-cell infiltrates in the spinal cord and peripheral nerves. The molecular mechanisms by which HTLV-I causes both T-cell leukemia as well as an inflammatory nervous system disease are not understood, but both diseases may be causally related to T-cell activation by the virus.

The presence of large numbers of activated blood T cells as well as a high degree of spontaneous proliferation of *in vitro* cultured T cells is a remarkable feature of HAM/TSP and to a slightly lesser extent of HTLV-I carriers (5–7). T-cell activation as measured by spontaneous proliferation may be of significance in the development of the neurologic disease, as activated but not resting T cells are able to cross the blood brain barrier—a prerequisite to the initiation of an inflammatory response in the central nervous system (CNS) (8).

Recent studies have also demonstrated a high frequency of HTLV-I tax protein-reactive T cells in the blood of patients with HAM/TSP (9).

We have demonstrated that activated T-cell clones can induce the proliferation of resting T cells by cognate interactions involving the CD2/lymphocyte function-associated molecules 3 (LFA-3) and LFA-1/intercellular cell-adhesion molecule (ICAM) pathways (10). As T cells are the major reservoir of HTLV-I (T_{HTLV-I}) (11), we postulated that direct T_{HTLV-I}–T cell interactions may be of importance in inducing spontaneous proliferation of blood T cells. We therefore examined mechanisms of T-cell activation by HTLV-I both in freshly isolated blood T cells and in naturally infected T-cell clones from HAM/TSP patients.

MATERIALS AND METHODS

Cells and Monoclonal Antibodies (mAbs). mAbs used were: anti-IL-2 receptor (anti-Tac, anti-CD25) supplied by T. Waldmann, Bethesda, MD; anti-T11.1 (anti-CD2), anti-CD4, anti-CD8, 9.49 [anti-class II major histocompatibility complex (MHC)], and anti-Ta1 (anti-CD26) supplied by S. Schlossman, Boston; anti-ICAM-1, anti-ICAM-2, anti-LFA-1, and anti-LFA-3 supplied by T. Springer, Boston; anti-B7, anti-CD28, and anti-class I MHC (W6/32) provided by L. Nadler, Boston; anti-gp46 (0.5α) supplied by S. Broder, National Institutes of Health, Bethesda, MD; anti-HTLV-I anti-gp46 (TS101) provided by Thomas Schultz, Institute for Cancer Research, London; and anti-HLA-DQ (S3/4) and anti-HLA-DR (L243) from ATCC. Antibodies to the interleukin 2 (IL-2) receptor, B7, and ICAM-1 were affinity-purified and used at a final concentration of 10 $\mu g/ml$. All other antibodies were used as ascites at a final dilution of 1:100.

Single-Cell Cloning of Peripheral Blood T Cells. Peripheral blood was obtained after informed consent from HAM/TSP patients originating from the West Indies and Central America diagnosed with HAM/TSP. Patients had a chronic progressive myelopathy associated with high titers of anti-HTLV-I antibodies (4). T cells were directly cloned from peripheral blood mononuclear cells at one cell per well in medium containing 1 μg of phytohemagglutinin P (PHA-P; Wellcome) per ml, 5% (vol/vol) IL-2 (Advanced Biotechnology, Silver Spring, MD), and 100,000 irradiated (5000 rad) mononuclear cells per well as feeder cells as described (12). For cloning of T cells from patient Pr, autologous feeder cells were used, while allogeneic feeder cells were used for cloning

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T cells from patient Du. HTLV-I infection of clones was examined by PCR amplification of the *pol* region (patient Du) (13) or of tax cDNA (patient Pr) (14).

Spontaneous proliferation, defined as the incorporation of [^{3}H]thymidine by a clone in the absence of exogenous IL-2, was assessed in proliferation assays set up 7–10 days after last stimulation. Proliferation assays were done in triplicate at 1 $\times$ 10^5 cells per well in 96-well plates. Cells were cultured for 4 days and 1 μCi (37 kBq) of [^{3}H]thymidine was added to each well 18 hr prior to harvesting and assay with an LKB scintillation counter.

Activation of Blood T Cells by Irradiated T$_{HTLV-I}$ Clones. T cells were prepared from the blood of HAM/TSP patients and normal subjects by Ficoll density gradient centrifugation and erythrocyte rosetting. T cells were cocultured with either HTLV-I-infected T-cell clones or control T-cell clones at a 1:1 ratio with 50,000 or 100,000 cells of each population per well. T-cell clones (stimulator cells) were either irradiated with 5000 rad or fixed in 1% formaldehyde in phosphate-buffered saline (PBS) for 15 min at 4°C followed by extensive washing. Cells were incubated for 4 days, and [^{3}H]thymidine incorporation was assessed as above. Inhibition of either T$_{HTLV-I}$–T cell or spontaneous peripheral blood T-cell proliferation by mAbs was performed by adding ascites at a concentration of 1:100 at the initiation of cultures. Alternatively, a polyclonal antiserum from TSP patients was used at a final dilution of 1% or 10%.

Proliferation of T Cells to Purified HTLV-I. T cells from a normal subject were purified by erythrocyte rosetting (10). Twenty-five microliters of T-cell suspension (4 $\times$ 10^6 cells per ml) was incubated with 25 μl of mAb (1:100 final dilution of ascites) or serum (1:10 final dilution). After a 1-hr incubation at 4°C, 50 μl of a purified HTLV-I preparation (gift of M. Duc Dodon) was added. The culture was then incubated 18 hr prior to the addition of 50 μl of medium. Five days later cultures were pulsed for 18 hr with 1 μCi of [^{3}H]thymidine per well, harvested, and assayed for radioactivity in a liquid scintillation counter.

RESULTS

Spontaneous T-Cell Proliferation of CD4$^+$ and CD8$^+$ HTLV-I-Infected T-Cell Clones. A series of T-cell clones were generated from the blood of two patients with HAM/TSP (patient Du, 48 clones; patient Pr, 45 clones). Seven of 40 T-cell clones from patient Du were positive for the HTLV-I *pol* region by PCR (Fig. 1). Southern blot analysis of genomic DNA performed on six clones (Du.4, Du.7, Du.20, Du.26, Du.34, and Du.43) confirmed the presence of HTLV-I provirus and clonality (J.H.R., K.W.W., P.H., D.A.H., and A. Lever, unpublished data). These T-cell clones represent *in vivo* infected T cells, as allogeneic feeder cells were used for cloning and expansion. Five HTLV-I-infected clones were CD4$^+$ while one clone (Du.7) was CD8$^+$, demonstrating that both CD4 and CD8 populations can be infected by HTLV-I *in vivo* (Table 1 and Fig. 2). Eight of 24 clones from subject Pr were positive for HTLV-I tax mRNA by PCR as well as for HTLV-I antigens by Western blotting (data not shown). Since these clones were generated by using autologous feeder cells, *in vitro* infection of T-cell clones cannot be excluded.

The growth characteristics of infected and noninfected T-cell clones were investigated. All clones required restimulation with mitogen and feeder cells at 10- to 14-day intervals for continuous growth. However, only HTLV-I-infected T-cell clones were found to proliferate in the absence of mitogen or IL-2 (spontaneous clonal proliferation) when cultured 7–10 days after the last stimulation (Table 1). HTLV-I-infected clones exhibited a higher growth rate following stimulation by PHA and IL-2 than did uninfected clones and easily could have been grown to large numbers (>1 $\times$ 10^8
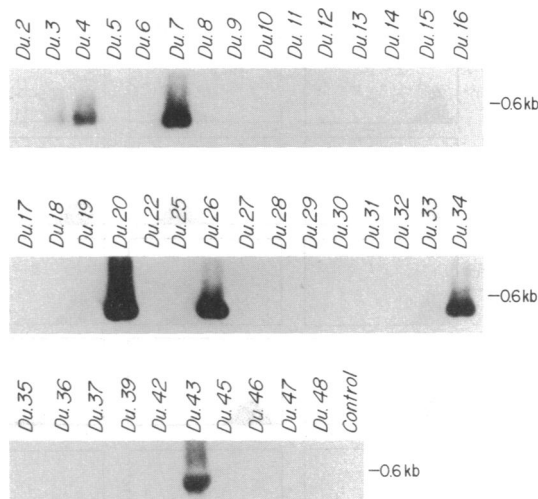

FIG. 1. PCR analysis of T-cell clones from a HAM/TSP patient (Du) for the HTLV-I proviral genome (*pol* region). Amplified products were separated on 1% agarose gels, and Southern blots were hybridized with an internal oligonucleotide probe (11). Seven of 48 clones from this patient were found to carry the HTLV-I proviral genome (the negative control had no DNA but all other reagents). Since T cells were directly cloned with allogeneic feeder cells to prevent *in vitro* contamination, these clones represent *in vivo* infected T cells. kb, Kilobase.

cells). Thus, these T cells exhibited growth characteristics of activated but not transformed cells. Of note is that clone Du.43 was positive for the HTLV-I *pol* region by PCR but did not proliferate spontaneously (Table 1 and Fig. 1). Southern blot analysis of genomic DNA confirmed the presence of HTLV-I proviral genome. However, no viral mRNA could be detected in this clone by Northern blotting (J.H.R., K.W.W., P.H., D.A.H., and A. Lever, unpublished data).

HTLV-I-Infected T Cells Induce Proliferation of Resting T Cells. As large numbers of activated T cells are found in the blood of patients with HAM/TSP (5, 6), we examined if HTLV-I-infected T cells could induce autologous T cells to proliferate. Irradiated or fixed HTLV-I-infected T-cell clones were found to induce proliferation of autologous blood T cells or blood T cells from normal subjects, while noninfected

Table 1. Spontaneous proliferation of CD4$^+$ and CD8$^+$ HTLV-I-infected T-cell clones

Clone	Phenotype	HTLV-I infection	Proliferation, cpm
Du.3	ND	+	16,907
Du.4	CD4	+	31,305
Du.7	CD8	+	46,636
Du.20	CD4	+	2,209
Du.26	CD4	+	18,733
Du.34	CD4	+	5,495
Du.43	CD4	+	353*
Du.2	CD4	−	52
Du.5	CD4	−	187
Du.16	CD4	−	801
Du.19	CD4	−	317
Du.48	CD4	−	70

T-cell clones from HAM/TSP patient Du were established by single-cell cloning with PHA and IL-2. Clones were tested by PCR amplification of the *pol* region and Southern blotting for the presence of the HTLV-I proviral genome (Fig. 1). Spontaneous proliferation was determined by [^{3}H]thymidine incorporation.
*T-cell clone Du.43, which did not show spontaneous proliferation, was found to have integrated the HTLV-I proviral genome (genomic Southern blot) but had no viral RNA (Northern blot). ND, not determined.
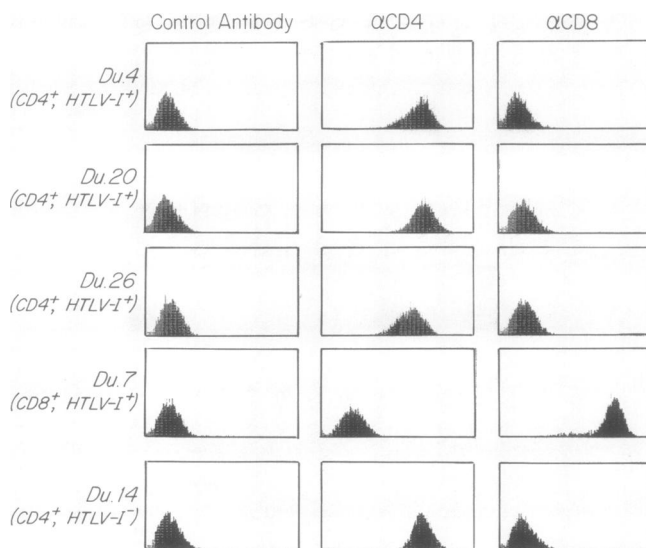
FIG. 2. Fluorescence-activated cell sorter analysis of HTLV-I-infected T-cell clones from a HAM/TSP patient (Du). Clones were stained with fluorescein- or phycoerythrocein-labeled antibodies to CD4 (αCD4) and CD8 (αCD8) antigens or mouse IgG (negative control). Fluorescence intensity was examined by fluorescent activated cell sorting. This analysis demonstrates that HTLV-I-infected T-cell clones Du.4, Du.20 and Du.26 are CD4⁺, while HTLV-I-infected clone Du.7 is CD8⁺.

clones did not (Tables 2 and 3). The ability of fixed HTLV-I-infected T-cell clones to induce proliferation of resting blood T cells indicated that T-cell surface structures and not soluble factors are important in the triggering of proliferation.

A series of experiments was performed to further exclude the possibility that T-cell activation was due to free virus or a soluble factor. Supernatant from two HTLV-I-infected T-cell clones failed to induce T-cell proliferation. Coculturing

of HTLV-I-infected T-cell clones and blood T cells in a transwell system in which stimulator and responder populations are separated by a semipermeable membrane failed to induce T-cell proliferation (data not shown). T_{HTLV-I}-T cell activation was also not blocked by a mAb (0.5α) to the HTLV-I envelope protein (gp46) nor by a high-titer TSP serum with potent neutralizing activity (effective at 1:20,450 in a syncytial inhibition assay) (Table 4). This TSP serum recognized both gag and env antigens on Western blots (Fig. 3). Finally, only a mild degree of T-cell proliferation was induced by the HTLV-I-producing cell line C91/PL, while no proliferation was induced by virus-producing cell line HUT 102 (Table 2).

As purified HTLV-I virions have been reported to directly activate T cells (15, 16), we tested a purified HTLV-I virus preparation (Table 4). The purified virus induced a moderate degree of proliferation that could not be blocked by a mAb (0.5α) to gp46 or by a high-titer HTLV-I antiserum. In contrast, proliferation was blocked (>90%) by mAbs to CD2 (T11.1) and LFA-3, raising the possibility that proliferation is induced by LFA-3 molecules present on contaminating T-cell membranes from activated, HTLV-I-infected cell lines. This interpretation is in agreement with the recent finding that membrane preparations from activated T cells can activate autologous T cells (10). Alternatively, LFA-3 may have been incorporated into the viral lipid envelope during budding. Taken together, these data clearly demonstrate that T-cell activation induced by HTLV-I-infected T-cell clones requires direct cell contact and is not triggered by a soluble factor or free virus.

Antibodies Specific for the CD2/LFA-3 Pathway and the IL-2 Receptor Inhibit the Stimulatory Effect of HTLV-I-Infected T-Cell Clones. The coculture experiments indicated that direct T cell–T cell contact is required for activation to occur. To examine which T-cell surface antigens and activation pathways are involved in triggering T_{HTLV-I}-T cell activation, we performed blocking studies using mAbs to CD2/

Table 2. HTLV-I-infected T-cell clones induce proliferation of autologous T cells

		[³H]Thymidine incorporation, cpm				
			Irradiated stimulator T cells			
Stimulator T cells	HTLV-I infection*	Stimulator T cells alone	Alone	With autologous T cells†	With allogeneic T cells‡	With autologous T cells and autologous serum§
Pr. T cells	+	**584¶**	29	**503¶**	**597**	—
Ctrl T cells	−	42	26	64	34	—
Pr.G clone	+	**10,702**	30	**19,749**	**31,437**	—
Pr.17 clone	+	**4,133**	38	**7,335**	**15,823**	—
Pr.12 clone	−	83	24	60	109	—
Pr.15 clone	−	306	145	183	868	—
Pr.19 clone	−	36	18	63	29	—
C91/PL	+	3,023	805	3,051	4,014	—
Du. T cells	+	**173¶**	151	**173¶**	—	163
Du.4 clone	+	**18,891**	25	**43,507**	—	**60,229**
Du.26 clone	+	**21,474**	46	**63,977**	—	**80,076**
Du.5 clone	−	2,148	36	2,344	—	4,180
Du.6 clone	−	985	36	2,309	—	3,721
Du.12 clone	−	137	41	1,863	—	4,167
Du.14 clone	−	ND†	ND†	753	—	3,079
HUT-102	+	1,952	1,732	1,639	—	1,648

Boldface data indicate spontaneous proliferation (blood T cells, infected clones) or proliferation induced by HTLV-I-infected T-cell clones.
*Stimulator T cells were characterized by PCR and Southern blotting for the presence of tax mRNA (patient Pr) or *pol* retroviral sequences (patient Du).
†Stimulator T cells were irradiated (5000 rad) and cocultured with T cells from a HAM/TSP patient (Pr or Du).
‡Stimulator T cells were cocultured with resting T cells from a normal subject.
§Stimulator T cells were irradiated (5000 rad) and cocultured with Du T cells and 10% Du serum.
¶Frozen T cells from HAM/TSP patients were used, therefore T cells did not proliferate to the same extent as fresh T cells.

Table 3. Inhibition of T-cell activation induced by HTLV-I-infected T-cell clones with mAbs to CD2/LFA-3, LFA-1/ICAM, and the IL-2 receptor

	[³H]Thymidine incorporation, cpm		
	T_{Du} ± clone Du.26	T_{normal} ± clone Du.26	T_{normal} ± clone Du.31
T cells alone	20,428	108	108
Clones alone	10,962	10,962	130
Fixed clones	77	77	62
Fixed clones + T cells	42,201	10,305	90
Irradiated clones	59	59	95
Irradiated clones + T cells	90,965	116,480	452
+ ascites	75,441	49,918	187
+ αT11.1	59,277	**17,871**	125
+ αLFA-3	57,202	**25,444**	156
+ αLFA-1	70,236	36,724	75
+ αICAM-1	80,310	77,089	102
+ αICAM-2	64,080	103,454	260
+ αLFA-3/αICAM-1	47,890	**3,833**	85
+ αLFA-3/αICAM-2	38,221	**14,348**	97
+ αLFA-3/αLFA-1	40,937	**1,971**	76
+ αLFA-1/αLFA-3	27,190	**650**	71
+ αICAM-1/αICAM-2			
+ αB7	74,778	99,438	1052
+ αCD28	77,637	55,802	3077
+ αCD4	68,338	60,297	124
+ αCD8	88,575	114,458	4503
+ αclass I MHC	93,963	41,076	233
+ αclass II MHC	69,608	53,653	504
+ αDR	52,455	39,171	214
+ αDQ	75,043	81,668	289
+ αIL-2 R (CD25)	41,453	**26,195**	101
+ HTLV-I serum (Pr)	106,002	88,592	382
+ control serum	59,567	65,962	303
+ αgp46	58,207	87,939	273

Responder cells were uninfected normal T cells (T_{normal}) and HTLV-I-infected T cells from HAM/TSP patient Du (T_{Du}); stimulator cells were HTLV-I-infected T-cell clone Du.26 and normal T-cell clone Du.31, which was not infected as determined by PCR amplification of the HTLV-I *pol* region. Stimulator T-cell clones were either fixed in 1% formaldehyde/PBS or were irradiated (5000 rad) and then cocultured with autologous T cells from HAM/TSP patient Du or with resting T cells from a normal subject that showed no mixed lymphocyte reaction when cocultured with an activated T-cell clone expressing class II MHC molecules. Cells were cultured for 4 days and pulsed with [³H]thymidine. Proliferation and the inhibition of T_{HTLV-I}-T cell activation by mAbs to T-cell adhesion/activation molecules and the IL-2 receptor were determined by [³H]thymidine incorporation. Antibodies were used at a 1:100 dilution of ascites. Antibodies for ICAM-1, B7, and the IL-2 receptor were affinity-purified and used at a final concentration of 10 μg/ml. The HTLV-I antiserum used was effective at a dilution of 1:20,480 in the syncytia inhibition assay (Table 4). However, this antiserum as well as a mAb to gp46 did not block T_{HTLV-I}-T cell activation. Boldface data show antibodies that gave >75% inhibition of induced T-cell proliferation when using normal T cells (column 2).

LFA-3, LFA-1/ICAM, and CD28/B7 pathways (Table 3). For these experiments, HTLV-I-infected clones were cocultured with autologous T cells or T cells from a normal subject. T cells from the normal subject did not show a mixed lymphocyte reaction when cocultured for 4 days with an uninfected, irradiated T-cell clone (Du.31) that expressed high levels of class II MHC molecules. In contrast, coculture of T cells with an irradiated HTLV-I-infected T-cell clone (Du.26) induced strong T-cell proliferation (Table 3). The proliferation of normal T cells was blocked by mAbs to CD2 (T11.1) and LFA-3 and to a lesser extent by a mAb to LFA-1. In particular, combinations of antibodies specific for CD2/

Table 4. T-cell proliferation induced by a HTLV-I preparation is not inhibited by antibodies to HTLV-I but by mAbs to CD2 and LFA-3

	[³H]Thymidine incorporation, cpm
T cells	148
T cells + HTLV-I virus	**6,666**
+ 10% Pr serum (TSP)	22,660
+ 10% control serum	24,424
+ anti-gp46	8,721
+ anti-CD2 (T11.1)	**157**
+ anti-LFA-3	**344**

Purified T cells from a normal subject were cultured for 6 days with a purified HTLV-I preparation. Proliferation was determined by [³H]thymidine incorporation. The anti-HTLV-I titer of serum from TSP patient Pr used in the proliferation assay was determined in the syncytial inhibition assay by using 10^4 C91/PL (HTLV-I producer) cells and 10^4 C8166 (indicator) cells per well. The assay was scored at 18–24 hr for the presence of syncytia. The end-point titer of the sera that completely blocked syncytia formation was 1/20,480, while a control sera had no blocking activity.

LFA-3 and the LFA-1/ICAM molecules completely blocked the induction of T-cell activation, whereas mAbs to the CD28/B7 pathway had no effect. As expected, a mAb to the IL-2 receptor (CD25) blocked T-cell proliferation, as IL-2 is essential for T-cell proliferation after activation by the T-cell receptor/CD3 complex or the CD2 pathway (17). T_{HTLV-I}-T cell activation was not blocked by a series of mAbs to class I and class II MHC molecules, mAbs to CD4 and CD8 antigens, or a high-titer TSP serum (Table 3). The activation of autologous T cells by the HTLV-I-infected T-cell clone was partially blocked by the same antibodies but to a lesser extent. This may be related to the presence of HTLV-I-infected and -preactivated T cells among the responder population. These results suggest that the CD2 pathway is involved in the activation of T cells by HTLV-I-infected T-cell clones and that the proliferation observed is mediated by IL-2 secretion.

Spontaneous Proliferation of T Cells from HAM/TSP Patients Is Inhibited by Antibodies Specific for CD2/LFA-3 and the IL-2 Receptor. To examine if the spontaneous proliferation of blood T cells was induced by T_{HTLV-I}-T cell interactions involving adhesion molecules, blocking studies were
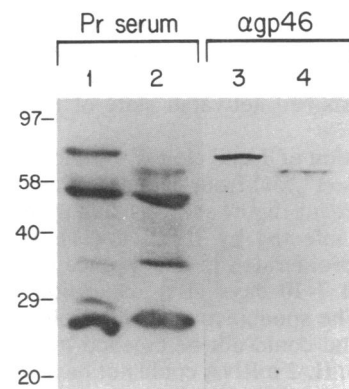
FIG. 3. Western blot (immunoblot) analysis of serum from HAM/TSP patient Pr. HTLV-I antigens were recognized by Pr serum (lanes 1 and 2) and TS101, a rat mAb specific for the HTLV-I envelope protein gp46 (αgp46) and its precursor gp61–68 (lanes 3 and 4). Sizes are shown in kDa. Lysates from HTLV-I-producing cell lines MT2 (env precursor of 68 kDa; lanes 1 and 3) and C91/PL (env precursor of 63 kDa; lanes 2 and 4) were separated by SDS/PAGE and transferred to nitrocellulose. In both cell lines there appears to be very little of the 46-kDa mature env product. The other viral proteins recognized by the Pr serum are mainly gag products: p24, precursor p55, and partially processed intermediates p35 and p28.
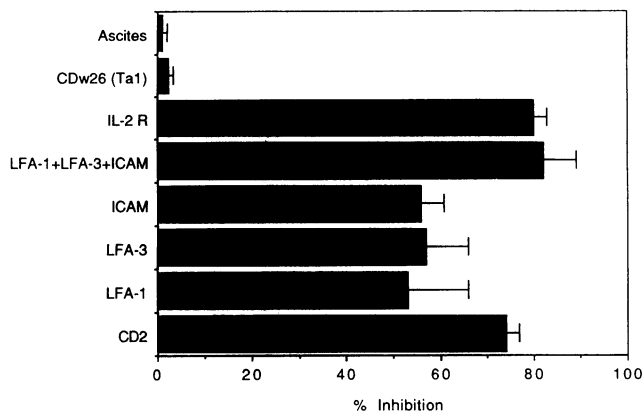
FIG. 4.   Blocking of spontaneous proliferation of blood T cells by mAbs to CD2/LFA-3, LFA-1/ICAM, and the IL-2 receptor. Proliferation was inhibited by mAbs to adhesion molecules and the IL-2 receptor but not by a mAb to CD26 or control ascites. Antibodies were added at a 1:100 dilution of ascites at the initiation of cultures. Proliferation was determined by [^{3}H]thymidine incorporation.

performed using freshly isolated blood T cells from HAM/TSP patients. The seven patients examined were all positive for HTLV-I antibodies by Western blotting as well as for the HTLV-I *pol* region by PCR amplification. As previously demonstrated, T cells from all patients proliferated spontaneously (mean proliferation, 21,112 cpm; control, 1,337 cpm) (5, 6). Significant inhibition of [^{3}H]thymidine incorporation by unstimulated peripheral blood T cells was seen with a mAb to CD2 (T11.1) and to a lesser extent by mAbs recognizing LFA-3, LFA-1, and ICAM. A combination of mAbs to LFA-3, LFA-1, and ICAM had the most pronounced effect. Spontaneous proliferation of T cells could also be blocked by a mAb to the IL-2 receptor (Fig. 4). These results suggest that T_{HTLV-I}–T cell interactions may contribute to the spontaneous proliferation of blood T cells in patients with HAM/TSP.

DISCUSSION

We demonstrate at the clonal level that HTLV-I-infected T cells isolated from the blood of HAM/TSP patients proliferate in the absence of T-cell growth factors, which we term "spontaneous clonal proliferation." HTLV-I-infected T cells induce resting blood T cells to proliferate by a mechanism that involves the CD2/LFA-3 pathway, which may contribute to the pathogenesis of the inflammatory CNS disease by inducing a sustained activated state of HTLV-I-specific T cells.

A large fraction of T-cell clones were found to be infected [7 of 40 clones (17.5%) from patient Du], which is in agreement with a recent report showing that up to 10% of leukocytes can be infected by HTLV-I (11). HTLV-I-infected T-cell clones proliferated in the absence of exogenous IL-2 when cultured 7–10 days after expansion with PHA and feeder cells. The spontaneous clonal proliferation was IL-2 independent and could not be blocked by mAb to the IL-2 receptor. Also, IL-2 mRNA could not be detected by Northern blot analysis (21). We believe that HTLV-I directly activates T cells by an intracellular mechanism.

T-cell activation induced by HTLV-I-infected clones does not appear to be induced by free HTLV-I, as proliferation was not blocked either by a mAb against gp46 or by a high-titer HTLV-I antisera (1:20,450) used at a dilution of 1:10. High concentrations of sera (1:1 to 1:8) were used in previous investigations to partially inhibit virus-induced proliferation (15), which raises the issue of nonspecific inhibitory effects not related to anti-HTLV-I antibodies. As HTLV-I virus preparations induced only a moderate degree of T-cell

proliferation that was blocked by mAbs to both CD2 and LFA-3 but not by a mAb to gp46, it is possible that the induced proliferation is caused by contaminating T-cell membranes containing LFA-3, the ligand for CD2 (10, 18).

As large numbers of activated T cells are present in the blood of HAM/TSP patients, we examined whether HTLV-I-infected clones could activate resting T cells. T cells can be activated by two major pathways, the T-cell receptor/CD3 complex and the CD2 pathway (18, 19). Recent studies have shown that activated T cells can induce the proliferation of resting peripheral blood T cells by CD2/LFA-3 and LFA-1/ICAM interactions (10). HTLV-I-infected T-cell clones were able to activate resting T cells via the CD2/LFA-3 pathway. Moreover, T_{HTLV-I}–T cell activation was found to be involved in the spontaneous proliferation of peripheral blood T cells, as it was blocked by mAbs to CD2/LFA-3 and LFA-1/ICAM. As previously shown, the induced proliferation was mediated by IL-2 secretion and blocked by a mAb to the IL-2 receptor (20).

In summary, these results show at the clonal level that HTLV-I infection induces T-cell activation and that such activated T cells can in turn stimulate noninfected T cells by cognate T_{HTLV-I}–T cell interactions involving the CD2 pathway. We postulate that HTLV-I-activated T cells may contribute to the pathogenesis of TSP by inducing a sustained activated state of T cells that recognize CNS autoantigens or virally infected glial cells.

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