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Peripheral T-Lymphocyte Activation by Human T-Cell Leukemia Virus Type I Interferes with the CD2 but Not with the CD3/TCR Pathway

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Human T-cell leukemia virus type I (HTLV-I) is etiologically associated with adult T-cell leukemia, an aggressive lymphoproliferative disorder, and with chronic neurological diseases. In vitro it can infect several types of cells but transforms only human T lymphocytes. We have previously shown that HTLV-I viral particles, even when noninfectious, were able to activate human resting T lymphocytes, suggesting that this activation step may be important in the initiation of the lymphoproliferative process. In the present study, we first demonstrate that in contrast to other mitogenic stimuli, HTLV-I has the unique property to activate human resting T cells in the absence of accessory cells. We then investigate the relationship between HTLV-I-induced T-cell activation and the classical well-known pathways of activation, namely, the CD3/TCR and CD2 pathways. Competitive blocking experiments were performed in which the effects of monoclonal antibodies (MAb) to the CD3/TCR complex or to the CD2 molecule were evaluated on the HTLV-I activation of T cells and compared with that obtained on phytohemagglutinin (PHA)-stimulated cells. It was found that anti-CD3 or -TCR MAb strongly suppress the proliferative response of T cells to PHA, but are significantly less efficient in inhibiting the activation initiated by HTLV-I. By contrast, MAb recognizing specific epitopes of the CD2 molecule inhibit the proliferative response of T cells to PHA or to HTLV-I to the same extent. The results provide evidence that HTLV-I virions interfere mainly with activation via CD2 but not via the CD3/TCR complex. Considering the earlier expression of the CD2 molecule on human T-cell precursors, these observations might be relevant to the characterization of the differentiation stage at which viral infection could interfere with the development and the maturation of T lymphocytes.

Human T-cell leukemia virus types I and II (HTLV-I and HTLV-II) are closely associated with specific T-cell disorders in humans. The latter has been linked to at least three cases of hairy-cell leukemia. The former is the etiological agent of adult T-cell leukemia/lymphoma and tropical spastic paraparesis. In vitro, these viruses can infect a number of cells of both human and animal origin, including sarcoma cell lines and epithelial and endothelial cells (16, 29, 44; for a review, see reference 31). Both viruses use the same cell surface receptor attributed to human chromosome 17 (35). However, HTLVs have been shown to immortalize only human normal T lymphocytes in vitro.

These observations suggest that T-cell-specific events triggered by viral infection may be at the onset of the lymproliferative process. Activation of resting T cells could be one of the first events (11, 15). Indeed, HTLV-I particles were shown to be mitogenic for resting T cells obtained from human peripheral blood (either CD4 or CD8) or for thymocytes (12). This initial activation should, in turn, be essential for subsequent events of viral replication, such as reverse transcription and proviral integration. At that stage, cell activation should favor proviral transcription and the synthesis of the Tax ($p40^x$) protein, which activates the proviral transcription in *trans* (21, 33). Furthermore, since the Tax protein is able to induce genes involved in T-cell activation and proliferation, it should also be involved in the maintenance of the activation state (1, 20, 24, 27, 34). This initial virus-induced proliferation was shown to be mediated by the external envelope glycoprotein gp46, since human sera containing antibodies to HTLV-I as well as a monoclonal antibody to HTLV-I gp46 neutralize the mitogenic effect (15). Furthermore, the fact that virus inactivated with UV light was still mitogenic for T cells suggests that virus binding to cell surface receptors is sufficient for triggering T-cell activation (15). Finally, mitogenic stimulation by noninfectious HTLV-I virions was recently shown to induce the production of large quantities of human immunodeficiency virus type 1 by human peripheral blood leukocytes infected with human immunodeficiency virus type 1 (45). Therefore, cell surface receptors for HTLV-I could deliver a mitogenic signal after contact with the viral particles. Alternatively, viral receptors could be associated with membrane antigens involved in T-cell activation.

On human T lymphocytes, two main cell surface receptors have been identified that define two distinct activation pathways (26, 43). The first pathway is mediated by the T-cell receptor (TCR) (CD3/Ti) complex, which acts as receptor for the antigen. This pathway can be triggered by a single anti-CD3 monoclonal antibody (MAb) (40). The second pathway, also referred to as the alternate pathway, can be triggered by specific combinations of antibodies against the CD2 molecule (3, 6). These CD2 or CD3 MAb could also inhibit T-cell activation under specific experimental conditions (9, 10, 41, 42).

In the present study, we have investigated T-cell activation by HTLV-I and its relationship to the major pathways of activation. Competitive-blocking experiments were performed in which the effects of MAb directed against the TCR

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complex or the CD2 molecule were evaluated on the activation of T cells induced by HTLV-I. The results provide evidence that HTLV-I interferes with the activation pathway mediated via the CD2 molecule, but not with the pathway initiated by the TCR complex.

MATERIALS AND METHODS

Reagents. The CD3 MAb used were OKT3 (immunoglobulin G2a [IgG2a]; Ortho Diagnostics), X357 (IgG2a; a generous gift from D. Bourel, Centre de Transfusion Sanguine, Rennes, France) and anti-CD3 (IgG2b; generous gift from H. Spits, Unicet, Dardilly, France). The MAb reacting with the T-cell receptor-dependent epitopes (clone WT31) was a generous gift of H. Spits (36). The CD2 MAb used in this study were from our own laboratory (A. Bernard) and displayed all the criteria that characterize MAb belonging to the 9.6/T11$_1$ group; MAb CD2 "D66" (IgG2b) and "GT2" (IgG2) were also from our laboratory.

Cell preparation. Mononuclear cells were obtained after Ficoll-Hypaque centrifugation of peripheral blood from normal healthy donors (Centre de Transfusion Sanguine, Lyon, France). They were then incubated for 1 h at 37°C in RPMI 1640 medium with 10% fetal calf serum in plastic culture flasks. The nonadherent cells were depleted of phagocytic cells by carbonyl iron ingestion at 37°C for 1 h and passaged over a magnet. The fraction of cells that had not ingested carbonyl iron were washed and incubated with 2.5 mM L-leucine methyl ester (Sigma Chemical Co.), a procedure shown to selectively lyse accessory cells (39). Under these conditions, the T-cell population obtained was able to proliferate in response to phytohemagglutinin (PHA), but not in response to the CD3 MAb.

When needed, monocytes were used as accessory cells. They were recovered at the adherence step of T-cell purification. They were then fixed by treating them with 1% paraformaldehyde for 20 min at room temperature. Cells were then washed four times and were kept in RPMI medium at 4°C until 1 week before use.

To obtain the purified CD2$^+$ CD3$^-$ T cells, residual human leukocyte antigen DR and CD3-positive T cells were eliminated by two cycles of treatment with nontoxic rabbit complement and a mixture of the murine MAb (lot 2b) anti-human leukocyte antigen DR (Immunotech, Marseille, France) and X-357 CD3 MAb. After this procedure, these cell preparations were carefully checked for purity, and the only preparations retained (99% CD2 positive cells) were those which showed no significant [^{3}H]thymidine incorporation when cultivated with optimal concentrations of PHA or CD3 MAb.

Immunofluorescence assays. Cells were stained with saturating amounts of MAb, and staining was revealed by affinity-purified fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Meloy laboratories, Springfield, Va.) as described previously (18). Cells were analyzed by using cytofluorometry (System 50; Ortho-Becton Dickinson). The negative control used was taken from the same cell preparation but stained with ascites containing an irrelevant antibody.

Virus preparation. HTLV-I was prepared from the culture medium of HTLV-I-producing cells (C91/PL) (30). After 48 h of culture, media were harvested and centrifuged at low speed to remove cells. The virus-containing supernatant was concentrated 20-fold by centrifugation for 3 h at 32,000 × g in a type 35 rotor (Beckman Instruments, Inc., Fullerton, Calif.) and by resuspending the viral pellet in an appropriate volume of RPMI medium; 50 μl of these viral preparations was added to each well.

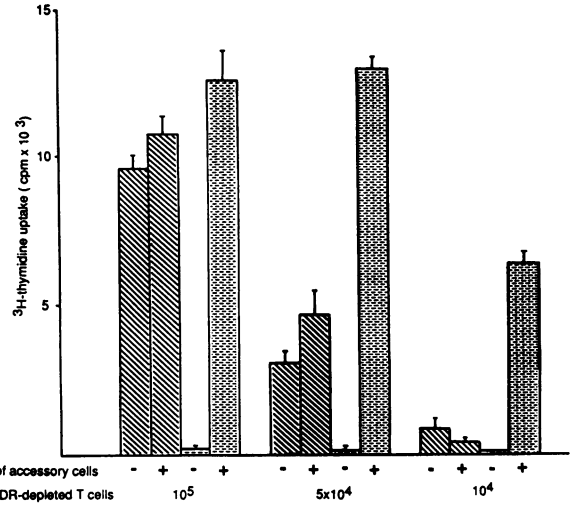

FIG. 1. Comparison of the proliferative response of resting T cells to HTLV-I virions or to PHA in the absence or in the presence of accessory cells. DR-depleted T cells were prepared as indicated in Materials and Methods and were seeded at the indicated cell density into a 96-well microdilution plate in a final volume of 100 μl, in the absence (−) or presence (+) of 5 × 10^3 accessory cells obtained as indicated in Materials and Methods. HTLV-I particles (▨) were prepared from the culture medium of an HTLV-I-producing cell line (C91/PL). PHA (▩) was added at a final concentration of 1 μg/ml. Incorporation of [^{3}H]thymidine was measured on day 5 of the culture during the last 6 h. Incorporation by triplicate cultures is reported as mean ± SD.

Proliferation assays. T lymphocytes were seeded in triplicate 96-well flat-bottomed microdilution plates at a ratio of 10^5 cells per well in 100 μl of medium in the absence or presence of either HTLV-I virions or PHA (Wellcome, England) added at a final concentration of 1 μg/ml. Proliferation was assessed 3 to 5 days after the beginning of the culture. Thymidine incorporation (1 μCi per well; specific activity, 5 Ci/mmol; CEA, Saclay, France) during the last 6 h of culture was measured by standard liquid scintillation counting techniques after harvesting with a Skatron harvester. Data are reported as mean of triplicate cultures ± standard deviation (SD).

RESULTS

Numerous studies have shown that T-cell proliferation responses to various mitogenic stimuli require the presence of accessory cells. Indeed, the number of accessory cells needed to achieve a plenary level of a mitogenic response is a specific feature of each stimulus. For example, T cells require far fewer accessory cells to be stimulated by PHA than by anti-CD3 MAb (2, 6, 9, 18). When the requirement of accessory cells for T cells stimulated by HTLV-I was determined, it was found (Fig. 1) that the activation of resting T cells by HTLV-I is observed even in the absence of accessory cells. Moreover, the addition of accessory cells, which is mandatory for T cells incubated with PHA, did not increase the proliferative response of these T cells to HTLV-I. Thus, this accessory-cell independence represents an unique feature of the HTLV-I mitogenic system.

MAbs against the CD3-TCR complex cannot efficiently block HTLV-I-induced T-cell proliferation. In a first series of

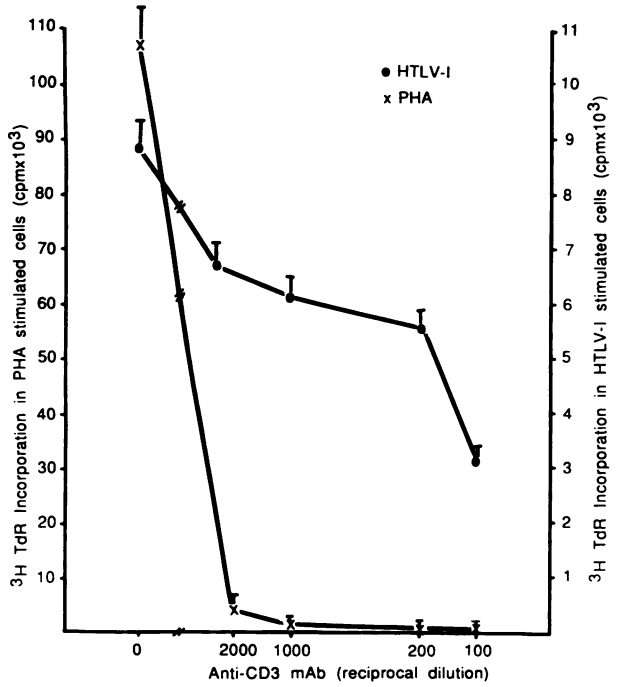

FIG. 2. Concentration-dependent effect of a CD3 MAb on DNA synthesis by T cells stimulated by either PHA or HTLV-I virions. T cells (1×10^5 per well) partially depleted of monocytes were cultured alone or with various dilutions of the MAb. PHA (at a final concentration of 1 μg/ml) or HTLV-I virions were added to the culture 18 h later. [^{3}H]thymidine (^{3}H TdR) incorporation was assessed on day 4 of the culture during the last 6 h. Incorporation by triplicate cultures is reported as mean ± SD.

experiments, the inhibitory effect exerted by CD3 MAbs on T-cell proliferation was evaluated in terms of the mitogenic ability induced by HTLV-I virions. For this purpose, human peripheral blood T cells were partially depleted of monocytes. Under these conditions, T cells were no longer response to CD3 MAb, but could still proliferate in response to PHA (9, 10, 42). These cultures were first treated with various dilutions of a CD3 MAb. After overnight incubation, either PHA or HTLV-I was added to these cultures. As expected, the CD3 MAb fully inhibited thymidine incorporation in PHA-stimulated cultures over the whole range of dilutions tested (Fig. 2). In contrast, preincubation of T cells with the CD3 MAb did not impair the proliferative response of these cultures to HTLV-I to the same extent. Indeed, in the presence of the highest MAb dilution (5×10^{-4}), 70% of DNA synthesis was still observed in HTLV-I-incubated cultures, compared with the untreated cultures. At the lowest dilution (1×10^{-2}), this was reduced by 35%. The same results were obtained with other CD3 MAb (Table 1). The higher inhibition of PHA-induced proliferation in the presence of the CD3 MAb compared with that of HTLV-I may be due to a much higher excess of the virus than the lectin. To assess this possibility, the effect of the CD3 MAb on the proliferative response of T cells incubated with various concentrations of either HTLV-I or PHA was investigated. The results indicate that when 50% of the maximal response was observed with both mitogens, the inhibition of the proliferative response to PHA in the presence of the CD3 MAb was complete, whereas the inhibition of the HTLV-I response was only partial (Fig. 3). Thus, thymidine incorporation in cells incubated with the fourfold-diluted viral preparation in the presence of CD3 MAb was reduced by only 42%, when compared with that in virus-incubated cultures in the absence of the MAb.

TABLE 1. Effect of CD3 MAb on PHA- or HTLV-1-induced T-cell proliferation[a]

MAb[b]	Stimulus[c]	Mean [^{3}H]thymidine incorporation (10^3 cpm) ± SD[d]
None	None	1.3 ± 0.0
	PHA	97.0 ± 7.1
	HTLV-I	35.9 ± 9.2
OKT3 (IgG2b)	None	1.1 ± 0.1
	PHA	1.7 ± 0.4 (1.8)
	HTLV-I	16.2 ± 5.2 (45.1)
X357 (IgG2a)	None	0.7 ± 0.3
	PHA	0.5 ± 0.2 (0.5)
	HTLV-I	12.5 ± 0.8 (34.8)
Control	None	1.1 ± 0.0
	PHA	92.0 ± 2.0 (94.8)
	HTLV-I	30.1 ± 7.0 (83.8)

[a] T cells partially depleted of monocytes and prepared as indicated in Materials and Methods were seeded at 1×10^5 cells per well in 100 μl into a 96-well microdilution plate.
[b] MAb were added at the onset of the cultures.
[c] PHA at a final concentration of 1 μg/ml or HTLV-I was added after an overnight incubation with the MAb.
[d] T-cell responsiveness was monitored 4 days later by measuring the [^{3}H]thymidine uptake during the last 6 h of the culture. Numbers in parentheses are the percentages of the thymidine incorporation measured in MAb-treated and stimulated cultures with respect to that measured in MAb-untreated stimulated cultures.

In a second series of experiments, T lymphocytes were incubated with CD3 MAb added either together with PHA or after 18 h of incubation with the lectin. The antibody reduced the PHA-induced T-cell activation in both cases (Table 2). No such decrease could be observed in HTLV-I cultures incubated with the MAb under the same conditions. Finally, a MAb to the T-cell receptor-dependent epitopes, clone WT31 (36), did not decrease the proliferative response to HTLV-I, whereas it fully inhibited the PHA response (Table 3).

These data support the conclusion that the CD3/Ti complex does not play an essential role in T-cell activation triggered by HTLV-I.

CD2 MAb can efficiently block HTLV-I-induced T-cell activation. CD2 MAb have been shown to react with either exposed epitopes or hidden epitopes (CD2R). The former usually block rosettes quite efficiently, whereas the latter block rosettes poorly or not at all (3, 4). It has been established that defined pairs of CD2 MAb are mitogenic for T lymphocytes (6, 23, 26). However, a single CD2 MAb can inhibit T-cell proliferation induced by various stimuli (41).

We studied the effect of a CD2 MAb (directed to an exposed epitope) on the T-cell response to HTLV-I and found that HTLV-I-stimulated uptake of thymidine was inhibited by the anti-CD2 MAb in a concentration-dependent manner (Fig. 4). The same was true for PHA-induced T-cell activation.

Furthermore, five CD2 MAb (four recognizing exposed epitopes and one directed against a CD2R epitope) were compared for their specific effects on HTLV-I-induced proliferation. T lymphocytes were incubated with the MAb overnight and then the HTLV-I virions were added. The

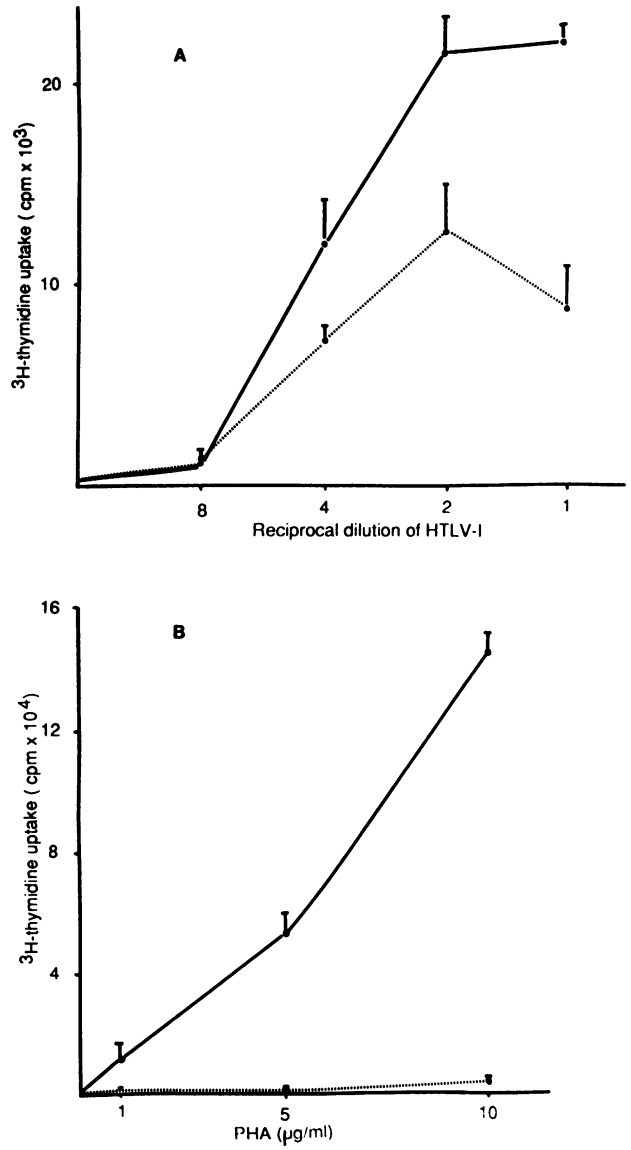

FIG. 3. Concentration-dependent effect of HTLV-I (A) or PHA (B) on the inhibitory effect on DNA synthesis by T cells incubated in the presence of a CD3 MAb. T cells (1×10^5 per well) partially depleted of monocytes were cultured alone (——) or with the X357 MAb (····), at a final dilution of 10^{-3}. PHA or HTLV-I virions were added to the culture 18 h later. [^{3}H]thymidine incorporation was assessed on day 4 of the culture during the last 6 h. Incorporation by triplicate cultures is reported as mean ± SD.

effect of these MAb on HTLV-I-induced proliferation always correlates with that obtained for the PHA-stimulated response (Table 4). Three types of response were observed: (i) MAb 0275 and G144 strongly inhibited both proliferative responses; (ii) CD2X11 and GT2 did not significantly decrease the thymidine uptake by cells stimulated by either PHA or HTLV-I, but the combination of CD2X11 and HTLV-I appeared to be more mitogenic than HTLV-I alone; and (iii) the effect of MAb D66 was intermediate compared with those observed with the other MAb. These results indicate that the ability to block rosette formation could not account for the inhibition of stimulation. However, the contrary is not true, since the possibility cannot be ruled out that

TABLE 2. Response of T lymphocytes to HTLV-I or to PHA in the presence of a CD3 MAb and effect of the MAb relative to the time of addition

MAb (dilution)	Time of addition[a]	[^{3}H]thymidine incorporation (cpm) in cells stimulated with[b]:		
		Nothing	PHA	HTLV-I
Control IgG	Before	2,153	54,264	31,585
X357 (10^{-3})	Before	2,749	12,758	28,354
	Together	ND[c]	17,308	38,046
	After	ND	16,820	40,524
X357 (10^{-4})	Before	2,991	26,057	41,758
	Together	ND	33,245	45,288
	After	ND	21,715	44,454

[a] T cells partially depleted of monocytes and prepared as indicated in Materials and Methods were seeded at 1×10^5 cells per well in 100 µl into a 96-well microdilution plate. The MAb was added either 18 h before the stimulus, together with it, or 18 h after it.
[b] T-cell responsiveness was monitored 4 days later by measuring [^{3}H]thymidine uptake during the last 6 h of the culture.
[c] ND, Not done.

CD2 MAb, displaying an inhibitory action on T-cell proliferation, would always be able to block rosette formation.

Finally, when the 0275 MAb was added to the cultures together with PHA or HTLV-I or 18 h after initial activation by these stimuli, it still inhibited the proliferative response (Table 5), whereas results obtained with the D66 MAb or the CD2X11 MAb confirm that these MAb did not significantly interfere with the proliferative response.

To further investigate the role of the CD2 molecule on the mitogenic ability of HTLV-I, human peripheral blood T lymphocytes were subjected to two consecutive cycles of CD3 MAb and rabbit complement to isolate T cells expressing only CD2 epitopes. These $CD2^+$ $CD3^-$ T cells were unable to proliferate in response to PHA or to the CD2 MAb, but still incorporated thymidine after incubation with the

TABLE 3. Effect of a MAb reacting with a T-cell receptor-dependent epitope on PHA- or HTLV-I-induced T-cell proliferation[a]

MAb[b]	Dilution	Stimulus[c]	Mean [^{3}H]thymidine incorporation (10^3 cpm) ± SD[d]
Control IgG	10^{-2}	None	1.4 ± 0.9
		PHA	140.1 ± 6.2
		HTLV-I	30.5 ± 3.8
WT31	10^{-2}	None	2.4 ± 1.3
		PHA	23.3 ± 0.3 (16.6)
		HTLV-I	26.8 ± 1.2 (87.8)
WT31	10^{-3}	None	4.2 ± 1.1
		PHA	32.4 ± 6.3 (23.1)
		HTLV-I	26.6 ± 1.0 (87.4)

[a] T cells partially depleted of monocytes and prepared as indicated in Materials and Methods were seeded at 1×10^5 cells per well in 100 µl into a 96-well microdilution plate.
[b] MAb were added at the onset of the cultures.
[c] PHA at a final concentration of 1 µg/ml or HTLV-I was added after an overnight incubation with the MAb.
[d] T-cell responsiveness was monitored 4 days later by measuring [^{3}H]thymidine uptake during the last 6 h of the culture. Numbers in parentheses are the percentages of the thymidine incorporation measured in WT31 MAb-treated stimulated cultures with respect to that measured in control MAb-treated stimulated cultures.

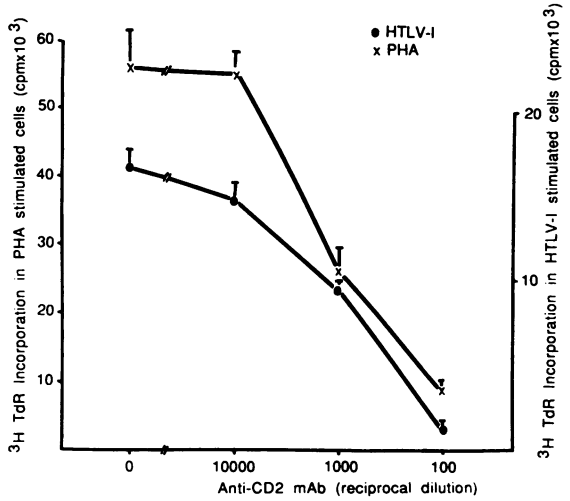

FIG. 4. Concentration-dependent effect of a CD2 MAb on DNA synthesis by T cells stimulated by either PHA or HTLV-I virions. T cells (1×10^5/well) partially depleted of monocytes were cultured alone or with various dilutions of the MAb. PHA (at a final concentration of 1 μg/ml) or HTLV-I virions were added to the culture 18 h later. [^{3}H]thymidine (^{3}H TdR) incorporation was assessed on day 4 of the culture during the last 6 h. Incorporation by triplicate cultures is reported as mean ± SD.

virus (Table 6). As expected, the addition of accessory cells did not increase thymidine incorporation by cells incubated with HTLV-I and did not restore the proliferative response of these cells to either CD3 MAb or PHA.

Collectively, these data establish that the activation pathway triggered by HTLV-I interferes with the CD2 pathway and suggest, therefore, that the expression of the CD2 molecule on the T-cell surface seems to be essential for the activation of T lymphocytes by HTLV-I.

DISCUSSION

The results presented in this study confirm that HTLV-I particles are directly mitogenic for human resting T lymphocytes. HTLV-I represents a potent mitogenic agent with unique features, since it is able to trigger T-cell proliferation in the absence of accessory cells. HTLV-I may be classified into a new category of T-cell mitogens, since all other known mitogens (lectins, CD3 MAb, or appropriate pairs of CD2 MAb) need accessory cells to trigger T-cell proliferation (H. Valentin, S. Huet, J. Morizet, and A. Bernard, *in* W. Knapp, ed., *Leukocyte Typing IV*, in press).

We also showed that HTLV-I interferes with the CD2 pathway but not with the CD3/TCR pathway. This was demonstrated by performing competitive blocking experiments in which the ability of CD2 MAb or CD3 MAb to block HTLV-I-induced T-cell proliferation was assessed. Thus, some MAb recognizing exposed epitopes of the CD2 molecule were able to inhibit significantly the proliferation of T cells incubated with HTLV-I. Conversely, CD3 MAb were shown to be inefficient in inhibiting T-cell proliferation triggered by HTLV-I, in contrast to their inhibitory effect on PHA-stimulated T cells. Furthermore, HTLV-I was shown to induce a significant [^{3}H]thymidine incorporation on $CD2^+$ $CD3^-$ T cells purified from peripheral blood. However, this $CD2^+$ $CD3^-$ T-cell population was unable to proliferate in response to CD3 MAb or to PHA, even in the presence of significant numbers of accessory cells. Taken together, these

TABLE 4. Effect of CD2 MAb on PHA- or HTLV-I-induced T-cell proliferation[a]

MAb[b]	Rosette blockade	Stimulus[c]	Mean [^{3}H]thymidine incorporation (10^3 cpm) ± SD[d]
Control IgG	−	None	1.3 ± 0.4
		PHA	97.0 ± 7.1
		HTLV-I	35.9 ± 3.9
0275	+	None	0.3 ± 0.0
		PHA	0.3 ± 0.0 (0.3)
		HTLV-I	2.4 ± 0.0 (6.7)
G144	+	None	0.3 ± 0.0
		PHA	5.2 ± 2.0 (5.4)
		HTLV-I	5.4 ± 1.8 (15.0)
D66	+	None	0.2 ± 0.0
		PHA	25.1 ± 2.0 (25.8)
		HTLV-I	13.7 ± 0.2 (38.1)
CD2X11	+	None	0.5 ± 0.0
		PHA	61.8 ± 4.2 (63.7)
		HTLV-I	45.5 ± 3.0 (126.7)
GT2	−	None	0.3 ± 0.1
		PHA	133.1 ± 12.1 (137.2)
		HTLV-I	23.8 ± 1.9 (66.2)

[a] T cells partially depleted of monocytes and prepared as indicated in Materials and Methods were seeded at 1×10^5 cells per well in 100 μl into a 96-well microdilution plate.

[b] MAb were added at the onset of the cultures.

[c] PHA at a final concentration of 1 μ/ml or HTLV-I was added after an overnight incubation with the MAb.

[d] T cell responsiveness was monitored 4 days later by measuring [^{3}H]thymidine uptake during the last 6 h of the culture. Numbers in parentheses are the percentages of the thymidine incorporation measured in MAb-treated and stimulated cultures with respect to that measured in MAb-untreated stimulated cultures.

results suggest that the expression of the CD3/Ti complex is not a prerequisite for activation of T lymphocytes by HTLV-I but that the expression of the CD2 molecule may be relevant to the T-cell activation induced by this virus.

As mentioned above, HTLV-I is able to infect a wide variety of cells including lymphocytes and fibroblasts. The CD2 glycoprotein whose expression is restricted to the T-cell membrane could not therefore be considered the viral receptor. Likewise, the CD2 molecule has been mapped on chromosome 1 (7), whereas the viral receptor for HTLV-I has been localized on chromosome 17 (35). Furthermore, the delayed addition of CD2 MAb (0275) to T cells previously exposed to HTLV-I is still followed by an inhibition of the T-cell response, indicating that the action of CD2 MAb did not interfere with the binding of viral particles. However, it is possible that HTLV-I viral receptors are associated with the CD2 molecule on resting T lymphocytes. Alternatively, they could become associated with the CD2 molecule as a consequence of the binding of viral particles. Such possibilities might explain why HTLV-I preferentially interferes with the signaling pathway via the CD2 molecule. In that respect, the observation that a combination of a CD2 MAb, namely CD2X11, with HTLV-I is more mitogenic for T cells than HTLV-I alone (Table 4) is reminiscent of the comitogenic action of the CD2 ligand, leukocyte function antigen type 3, and certain single CD2 antibodies (5, 13, 19, 38).

A link between the mitogenic ability of HTLV-I and the events leading to the development of leukemia is still open to debate. The present study might shed light on the nature of

TABLE 5. Response of T lymphocytes to HTLV-I or to PHA in the presence of CD2 MAb and effect of the MAb relative to the time of addition

MAb	Dilution	Time of addition[a]	[^{3}H]thymidine incorporation (cpm) in cells stimulated with[b]:		
			Nothing	PHA	HTLV-I
Control IgG		Together	3,189	54,264	31,585
0275	10^{-3}	Together	1,099	15,018	1,278
		After	ND[c]	11,260	2,380
0275	10^{-4}	Together	604	12,165	1,438
		After	ND	10,754	3,000
D66	10^{-2}	Together	272	2,582	24,730
		After	ND	4,134	14,635
D66	10^{-3}	Together	710	27,961	25,520
		After	ND	32,716	36,168
CD2X11	10^{-2}	Together	1,885	53,726	59,443
		After	ND	26,042	59,540
CD2X11	10^{-3}	Together	1,741	34,363	32,619
		After	ND	26,334	43,312

[a] T cells partially depleted of monocytes and prepared as indicated in Materials and Methods were seeded at 1×10^5 cells per well in 100 μl into a 96-well microdilution plate. The MAb was added either together with the stimulus or 18 h after it.

[b] T-cell responsiveness was monitored 4 days later by measuring [^{3}H]thymidine uptake during the last 6 h of the culture.

[c] ND, Not done.

the HTLV-I target cell for leukemogenesis, which might be exclusively recruited from among precursors of the T-cell lineage, providing that they express the CD2 molecule. This molecule is acquired at 8 weeks of gestation after T-cell precursors enter the human thymic epithelial rudiment. Virtually all thymocytes express CD2, including the less mature $CD3^-$ population, which lacks the CD3-TCR complex (17). The role of the CD2 molecule is therefore important throughout the T-cell life, not only during the maturation process in the thymus, but also in the activation of T lymphocytes. Thus, the stimulation of $CD2^+$ $CD3^-$ thymocytes by a pair of anti-CD2 MAb has been shown to trigger the interleukin-2 receptor expression without interleukin-2 synthesis (14). In this regard, we have previously demonstrated that thymocytes could be activated by HTLV-I in the presence of submitogenic doses of PHA (12).

TABLE 6. Response of $CD2^+$ $CD3^-$ T cells to PHA, HTLV-I, or CD3 MAb[a]

Stimulus	[^{3}H]thymidine incorporation (cpm) in expt[b]:			
	A	B[c]	C	D
None	504	86	566	384
PHA	601	289	769	501
CD3 MAb	429	154	402	396
HTLV-I	13,000	14,239	23,559	5,309

[a] $CD2^+$ $CD3^-$ T cells were obtained after two consecutive cytotoxicity cycles by using a mixture of MAb CD3 and HLA-DR MAb and newborn-rabbit complement. They were seeded at 1×10^5 cells per well in 100 μl into a 96-well microdilution plate.

[b] T-cell responsiveness was monitored 3 days later by measuring [^{3}H]thymidine uptake during the last 6 h of the culture.

[c] In this experiment, 2×10^4 accessory cells prepared as previously described (15) were added per well.

Retroviral glycoproteins have several crucial functions in viral infection. They bind the cell surface receptors and intervene into the penetration process (8, 22, 28, 32). They interact with the immune system to elicit neutralizing antibodies. The *env* gene of HTLV-I codes for two glycoproteins, gp46 and gp21, which are products of the cleavage of a precursor protein, gp61. On mature viral particles, the gp46 represents the outer component of the viral envelope. It is attached via a disulfide linkage to gp21, the transmembrane envelope component which anchors the glycoprotein complex to the viral nucleocapsid. From comparative studies with other retroviruses, it is assumed that gp46 contains the binding domain to the cell surface receptor. Thus, when HTLV-I virions were incubated with an anti-gp46 MAb (25), the mitogenic ability of these particles was inhibited (15). Likewise, the gp21 contains the fusion domain involved not only in syncitium formation, but also in virus penetration (37). It would be of interest to delineate the specific contribution of each envelope glycoprotein to the mitogenic stimulation of T lymphocytes. Such studies may reveal the mechanisms by which the interactions of these glycoproteins with the T-cell membrane trigger T-cell activation.

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