T-Cell Malignancies With Mature Phenotypes: Altered Cell Cycle Regulation by HLA Class I Molecules

By Maria Caterina Turco, Fiorella Alfinito, Mario De Felice, Annalisa Lamberti, Soldano Ferrone, and Salvatore Venuta

Soluble anti-HLA class I monoclonal antibodies (MoAbs) modulate normal T-lymphocyte proliferation induced via the CD3/Ti and the CD2 pathway, but do not induce proliferation of normal T lymphocytes in the absence of additional mitogenic stimuli. In this report, we show that anti-HLA class I MoAbs induce DNA synthesis in peripheral blood mononuclear cells from a patient with a CD4+/CD8+ T-prolymphocytic leukemia (T-PLL) and from a patient with a CD4+/CD8+ T-chronic lymphocytic leukemia (T-CLL), in the absence of detectable additional mitogenic stimuli. Proliferation of leukemic T cells is induced by both whole lgs and Fab fragments of anti-HLA class I MoAbs, arguing in favor of their direct interactions with the proliferating cells as the mechanism underlying the mitogenic effect. This interpretation is also supported by the ability of anti-HLA class I MoAbs to induce proliferation of leukemic T-cell preparations, depleted of accessory cells. DNA synthesis in T-CLL and T-PLL cells is preceded by expression of G1-specific messenger RNAs, ie, c-myc, 2F1, Tac, and interferon-y, in activated cells. Cell proliferation is inhibited by the protein kinase C inhibitor H7, indicating that activation of this enzyme is required for the mitogenic effect of anti-HLA class I MoAbs. The latter inhibit the proliferation of T-CLL cells as well as that of normal T cells stimulated with anti-CD3 MoAbs and enhance that of both types of cells stimulated with anti-CD2 MoAbs. In addition, anti-HLA class I MoAb Q6/64 in combination with anti-CD2 MoAb 9.6 or MoAb 9-1 induces proliferation of leukemic T cells to a greater extent than the individual MoAbs, but is not mitogenic for normal T cells. Anti-HLA class I MoAbs restore the cytolytic activity of T-CLL cells that is lost after 5 days of incubation in control medium, suggesting that HLA class I antigens may mediate a signal contributing to the activation state. The present results indicate that leukemic T-cell proliferation can be triggered via HLA class I molecules and suggest a potential role for these antigens in the in vivo growth of malignant clones.

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In normal T lymphocytes, triggering of the T-cell receptor (CD3/Ti) or the CD2 molecule induces G1→G0 switch and initiates cell progression towards G1 and S phase. G1 phase is regulated by interleukin-2 (IL-2) and IL-4 and their receptors, and by other signal-mediating molecules present on the T cell surfaces. Among the latter are HLA class I antigens. Anti-HLA class I monoclonal antibodies (MoAbs) have been shown to inhibit T-cell proliferation induced via the CD3/Ti pathway and to enhance that induced via the CD2 pathway. Anti-HLA class I MoAbs exert their effect on gene expression in G1 phase, after G0→G1 switch. Although the natural ligand of HLA class I antigens is presently unknown, it has been suggested to correspond to structure(s) expressed on regulatory cells, ie, accessory or helper/suppressor cells.

Malignant T cells, and in particular T-chronic lymphocytic leukemia (T-CLL) lymphocytes, often respond to normal mitogenic stimuli, such as those mediated via the CD3/Ti or the CD2 pathway. They can also directly respond to costimulatory signals, such as IL-2 and IL-4, that are not mitogenic for normal resting T lymphocytes. The latter phenomenon may reflect the escape of malignant cells from regulatory mechanisms that control cell proliferation.

In the course of the study of the effect of anti-HLA class I MoAbs on the proliferation of leukemic T cells stimulated via the CD3/Ti or the CD2 pathway, we observed that anti-HLA class I MoAbs could induce the proliferation of two leukemic T-cell samples in the absence of detectable additional mitogenic stimuli. The aim of the present study is to characterize this deregulated pathway of leukemic T-cell proliferation.

**MATERIALS AND METHODS**

*Cells.* Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood of patients and normal donors by centrifugation through a Ficoll-Hypaque (Sigma Chemical Co, St Louis, MO) density gradient at 400g for 30 minutes, washed twice with phosphate-buffered saline (PBS), and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). PBMC were depleted of monocytes by sequential incubation with saturating amounts either of anti-HLA class II MoAb Q5/13 or of anti-CD14 MoAb anti-Leu-M3 for 30 minutes at 4°C and with an excess of rabbit complement for 60 minutes at 37°C. Dead cells were eliminated by centrifugation through a Ficoll-Hypaque density gradient at 400g for 30 minutes. Then cells were washed twice with PBS and resuspended in RPMI 1640 medium supplemented with 10% FCS. The depletion of monocytes was monitored by immunofluorescence staining with anti–HLA-DR MoAb OKDR and with anti-CD14 MoAb anti-Leu-M3.

Cultured erythromyeloid cells K562 were maintained in RPMI 1640 medium supplemented with 10% FCS. Phychotohemagglutinin (PHA), chemicals, and rabbit complement. PHA-P (Leucoagglutinin) was purchased from Pharmacia (Uppsala, Sweden). Protein kinase C inhibitor 1-(s-isooquinolinesulfonfyl)-2-methyl-piperazine, dihydrochloride (H7), and the calcium antagonist (N-2-guanidinoethyl)-s-isooquinolinesulfonamide, dihydroycholine, were used as received.

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drochloride) (HA1004) were purchased from Calbiochem Corp (La Jolla, CA). The fluorescein isothiocyanate (FITC)-conjugated MoAbs OKT3, OKT8, OKT11, OKDR (all IgG2a), and OKT4 (an IgG2b) were purchased from Ortho Diagnotic Systems (Milan, Italy), FITC-conjugated MoAb antitransferrin receptor (TTR) (an IgG2a), anti-IL-2 receptor (IL-2R) (an IgG1), and anti-CD14 MoAb anti-Leu-M3 (an IgG2b) were purchased from Becton Dickinson (Milan, Italy). MoAb Q6/64 (an IgG2a), recognizing a determinant restricted to HLA-B antigens,30 MoAb TP25.99 (an IgG1), determining a determinant expressed on beta-2m-free HLA class I heavy chain (unpublished results), MoAb W6/32, recognizing a nonmonomorphic determinant expressed on beta-2m-associated HLA-A, B, and C heavy chains,22 and anti-HLA-DR,DQ,DP MoAb Q5/13 (an IgG2a2) were developed and characterized as described.

MoAbs were purified from ascitic fluid by affinity chromatography on protein A-Sepharose.22 Fab' fragments were prepared from MoAbs as described.22 The purity of MoAB and Fab' fragment preparations was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).24

Rabbit complement was purchased from Istituto Behring S.p.A. (Scoppito, Italy).

cDNA probes. The PstI fragment from c-myc cDNA clone pRyc 7.4 was kindly provided by Dr. K.B. Marcu (Department of Biochemistry, SUNY, Stony Brook, NY); the XhoI fragment from 2F, cDNA clone by Dr. R. Baserga (Department of Pathology, Temple University School of Medicine, Philadelphia, PA); the ferritin cDNA clone 36.8 by Dr. F. Costanzo (Department of Biochemistry and Medical Biotechnology, II Medical School, Naples, Italy); the PstI fragment from human IL-2 cDNA clone p3-16 by Dr. S.Y. Yang (Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY); the EcoRI fragment from human Tac clone pII-2R2 by Dr. W.C. Greene (Howard Hughes Medical Institute, Duke University School of Medicine, Durham, NC); and the PstI fragment from human interferon gamma (IFN-7) cDNA clone SWIF by Dr. G. Trinchieri (Wistar Institute of Anatomy and Biology, Philadelphia, PA). cDNA probes were labeled with [a-32P]dCTP (3,000 Ci/mmol; Amersham Co, Arlington Heights, IL) by random priming26 to a specific activity of 50 µCi/ µg.

Proliferation assays. Proliferation assays were performed as previously described.23 Briefly, cells (2 x 10^5/mL) were incubated in 96-well microtiter plates (Falcon Labware, Becton Dickinson, Oxnard, CA) in the presence of the indicated reagents in triplicates, at 37°C in a 5% CO2 atmosphere for the indicated times. Then, 0.5 µCi of tritiated thymidine (3H-dT, specific activity 47 Ci/mmole; Amersham Co, Milan, Italy) were added to each well. After 15 hours of incubation, cultures were harvested on glass fiber strips with a Phd harvester (Cambridge Technology, Inc, Cambridge, MA) and 'H-dT incorporation was measured in a gamma counter.

Immunofluorescence (IF). One hundred microliters of cell suspension (4 x 10^5 cells/mL) were incubated with a saturating amount of FITC-conjugated MoAb at 4°C for 30 minutes, washed twice with PBS, and analyzed with a cytofluorograph Spectrum III (Ortho Diagnostic Systems). Results are expressed as percent of positive cells and mean fluorescence intensity. The specific cell fluorescence intensity was expressed as channel numbers on a log scale. The mean fluorescence intensity was calculated by integrating the fluorescence histograms for the positive cells as compared with control unstained cells. Negative controls were less than 1% stained cells and 50% of mean fluorescence intensity.

RNA hybridization analysis. Cell RNA was extracted according to Chomczynski et al.22 RNA (10 µg) was electrophoresed through an agarose gel containing formaldehyde and transferred to nitrocellulose filters (Amersham Int plc) using 20X SSC (1X SSC: 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate, pH 7.0) as described by Thomas.28 Blots were baked at 80°C under vacuum for 2 hours and prehybridized at 42°C for 12 to 16 hours using the prehybridization buffer. The latter consists of 50% formamide, 5X SSC, 5X Denhard's reagent, 0.2 mol/L sodium phosphate, pH 7.0, and denatured salmon sperm DNA (250 µg/mL). Filters were hybridized for 12 to 16 hours at 42°C in the prehybridization buffer containing 32P-labeled cDNA probes. After three washings at room temperature for 5 minutes each time with 2X SSC containing 0.1% SDS, blots were dried and autoradiographed at -70°C for at least 12 hours using XAR.5 X-ray film (Eastman Kodak, Rochester, NY) and an intensifying screen (Dupont Co, Wilmington, DE).

Lectin-dependent cytotoxic assays. Cytotoxicity assays were performed as previously described.28 Briefly, PBMC were cultured with the indicated reagents for the indicated time intervals. K562 cells were radiolabeled by incubation with sodium chromate (5Cr, specific activity 555.99 mCi/mg; New England Nuclear Research Product, Boston, MA) for 1 hour at 37°C. Effector and target cells were incubated together at a 10:1 ratio for 4 hours at 37°C in the presence of 1% PHA-P. The cell suspension was then centrifuged at 800g for 5 minutes and 50 µL of supernatant was harvested and analyzed for released radioactivity in a gamma counter. Spontaneous release was determined by incubating target cells in medium alone. Maximum release was determined by lysing cells with 0.5% Nonidet P-40. Specific lysis was calculated according to the following formula:

\[
\text{Specific lysis} = \frac{\text{Experimental cpm} - \text{Background cpm}}{\text{Total Release cpm} - \text{Background cpm}} \times 100
\]

RESULTS

Mitogenic effect of anti-HLA class I MoAbs on T-prolymphocytic leukemia (T-PLL) and T-CLL cells. T-PLL cells from patient TRT were 94% CD2+, 94% CD3+, 86% CD4+, 94% CD8+, and 3% HLA class II+. T-CLL cells from patient MC were 90% CD2+, 86% CD3+, 1% CD4+, 75% CD8+, and 2% HLA class II+. Less than 3% of both cell types were stained with anti-CD14 MoAb anti-Leu-M3, and anti-HLA class I MoAbs was specific, because addition of anti-CD2 MoAb 9.6 or of a control mouse IgG did not affect 'H-dT incorporation of both types of cells; the proliferative response was maximal between 94 and 118 hours of incubation. The mitogenic effect of anti-HLA class I MoAbs was specific, because addition of anti-CD2 MoAb 9.6 or of a control mouse IgG did not affect 'H-dT incorporation by leukemic cells. Furthermore, the anti-HLA class I MoAb Q6/64, TP25.99, and W6/32 did not induce proliferation of PBMC from normal donors. The latter proliferated when incubated with PHA-P. Representative results are shown in Fig 1.

Two lines of evidence indicate that the mitogenic effect of anti-HLA class I MoAbs reflects a direct interaction with HLA class I antigens expressed by leukemic cells and is not mediated by binding to Fc receptors on accessory cells. First, anti-HLA class I MoAbs stimulate proliferation of leukemic cell preparations that contain less than 0.3% of cells stained by anti-CD14 MoAb anti-Leu-M3 after sequential incubation with either anti-CD14 MoAb anti-Leu-M3 or anti-HLA class I MoAb Q5/13 and with rabbit comple-
HLA CLASS I ANTIGENS AND T-CLL

Fig 1. Induction by anti-HLA class I MoAbs of T-CLL and T-PLL cell proliferation. PBMC from a healthy donor (A), from patient MC (CD1-CD2-CD3-CD4-CD8+)(B), and from patient TRT (CD1-CD2-CD3-CD4-CD8+) (C) (2 x 10^6/200 μL RPMI 1640 medium supplemented with 10% FCS) were incubated at 37°C with anti-HLA class I MoAb Q6/64 (●), TP25.99 (▲), and W6/32 (●) (10 μg/mL). Cells incubated in medium (○) or with anti-CD2 MoAb 9.6 (●) were used as negative controls. Cells incubated with PHA-P (□) were used as positive controls. At the indicated times 1H-TdR (0.5 μCi) was added to each well. After an additional 15 hours of incubation, 1H-dT incorporation in cell DNA was measured. Results are the means of triplicate determinations. Values obtained by analyzing normal PBMC incubated in medium were similar to those obtained by analyzing PBMC incubated in medium supplemented with anti-HLA class I MoAb Q6/64, TP25.99, and W6/32 (A).

Fig 2. Induction of 1-PU cell proliferation by whole Ig and Fab' fragments of anti-HLA class I MoAbs. PBMC from patient TRT were incubated for 96 hours at 37°C with whole Ig (●) or Fab' fragments (▲) of anti–HLA-B MoAb Q6/64. Cultures were then added with 1H-dT and incubation was prolonged for an additional 15 hours. 1H-dT incorporation was then measured. Results are means of triplicate determinations.

Induction of gene expression in T-PLL and T-CLL cells by anti-HLA class I MoAbs. Activated T lymphocytes express specific genes in different steps of G1 phase. 29,30 To investigate whether stimulation of DNA synthesis in T-PLL and T-CLL cells by anti-HLA class I MoAbs resulted from cell progression through cell cycle, we analyzed induction of genes expressed in early, mid, and late G1 phase. c-myc messenger RNA (mRNA), a marker of early G1 phase, was induced in T-CLL cells cultured with anti-HLA class I MoAbs (Fig 3, upper band) and was increased in T-PLL cells (Fig 4). Furthermore, the level of 2F1 mRNA, a marker of early to mid G1 phase, was increased in T-CLL cells cultured with anti-HLA class I MoAbs (Fig 3, middle band). The effect of anti-HLA class I MoAbs on gene expression was specific, because they did not modulate the levels of ferritin mRNA (Fig 3, lower band). Lastly, after
we tested the effect of anti-HLA class I MoAbs on the proliferation of T-CLL cells stimulated with anti-CD3 or anti-CD2 MoAbs. These experiments could not be performed with T-PLL cells, because cells from patient TRT were not available. MoAb OKT3 induced proliferation of T-CLL cell preparations that contained about 2% of HLA class II-positive cells or that contained less than 0.3% of cells stained by anti-CD14 MoAb anti-Leu-M3 after sequential incubation with anti-HLA class II MoAb Q5/13 and with rabbit complement. Anti-HLA class I MoAbs inhibited T-CLL cell proliferation induced by MoAb OKT3 in

in incubation with anti-HLA class I MoAbs, T-CLL cells expressed higher amounts of Tac and IFN-γ mRNAs, two markers of mid to late G1 phase (Figs 5 and 6).

**Inhibition by a protein kinase C inhibitor of the mitogenic effect of anti-HLA class I MoAbs on T-CLL cells.** Activation of protein kinase C is one of the possible mechanisms by which cell surface receptors generate intracellular signals. To assess the role of protein kinase C in the transduction of HLA class I antigen-mediated mitogenic signal, we tested the effect of the protein kinase C inhibitor H7 on the proliferation of T-CLL cells stimulated with anti-HLA class I MoAbs. To test the inhibitory effect on initial steps of cell activation, 3H-thymidine incorporation was measured after 72 hours of incubation of cells with anti-HLA class I MoAbs in the presence of H7. These experiments could not be performed with T-PLL cells, because cells from patient TRT were not available. As shown in Fig 7, H7 inhibited in a dose-dependent fashion the proliferation of T-CLL cells stimulated with anti-HLA class I MoAbs. The inhibitory effect of H7 was more marked than that of HA1004, a calcium antagonist with a low affinity for protein kinase C. These results indicate that activation of protein kinase C is required for the mitogenic effect of anti-HLA class I MoAbs.

**Effect of anti-HLA class I MoAbs on the proliferation of T-CLL cells stimulated via the CD3/Ti or the CD2 pathway.** Anti-HLA class I MoAbs inhibit the proliferation of normal T lymphocytes induced via the CD3/Ti pathway, but enhance that induced via the CD2 pathway. Therefore,

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**Fig 5. Induction of IFN-γ mRNA by anti-HLA class I MoAbs in T-CLL cells.** PBMC (1 × 10⁶) were isolated from patient MC (lane 1) and incubated with anti-HLA-B MoAb 06/64 (10 μg/mL) for 1 (lane 2), 2 (lane 3), 4 (lane 4), and 7 (lane 5) days. PBMC from a healthy donor were incubated with PHA-P (1 μg/mL) and PMA (20 ng/mL) for 22 hours (lane 6). At the end of the incubation period, RNA was extracted, electrophoresed through a formaldehyde-containing agarose gel, transferred to a nitrocellulose filter, and hybridized with a 32P-labeled Pst I fragment from IFN-γ cDNA clone SWIF (upper band) and with a 32P-labeled Pst I fragment from IL-2 cDNA clone p3-16 (lower band). Filter was autoradiographed at −70°C for 48 hours.

**Fig 6. Induction of Tac mRNA by anti-HLA class I MoAbs in T-CLL cells.** PBMC (1 × 10⁶) were isolated from patient MC (lane 1) and incubated with anti-HLA-B MoAb 06/64 (10 μg/mL) for 1 (lane 2), 2 (lane 3), 4 (lane 4), and 7 (lane 5) days. PBMC from a healthy donor were incubated with PHA-P (1 μg/mL) and PMA (20 ng/mL) for 22 hours (lane 6). At the end of the incubation period, RNA was extracted, electrophoresed through a formaldehyde-containing agarose gel, transferred to a nitrocellulose filter, and hybridized with a 32P-labeled EcoRI fragment from Tac cDNA clone pL-2R2. Filter was autoradiographed at −70°C for 48 hours.
Fig 7. Effect of the protein kinase inhibitors H7 on the proliferation of T-CLL cells stimulated with anti-HLA class I MoAbs. PBMC from the patient MC were cultured with MoAb 06/64 (10 pg/mL) in the presence of the indicated concentrations of inhibitors H7 (□) or HA1004 (●) for 72 hours. ³H-dT was then added and following 15 hours of incubation its incorporation was measured.

Cell preparations containing about 2% of HLA class II-positive cells (Table 1). The inhibition occurred also with T-CLL cell preparations containing less than 0.3% of cells stained by anti-CD14 MoAb anti-Leu-M3 after sequential incubation with either anti-CD14 MoAb anti-Leu-M3 or anti-HLA class II MoAb Q5/13 and with rabbit complement (Fig 8). The latter finding suggests that the inhibitory effect of anti-HLA class I MoAbs is not caused by a displacement of MoAb OKT3 from Fc receptors of accessory cells. This conclusion is corroborated by the ability of Fab' fragments of anti-HLA class I MoAbs to inhibit MoAb OKT3-induced T-CLL cell proliferation to an extent similar to that of whole IgGs (Fig 8).

Anti-HLA class I MoAbs enhanced proliferation of T-CLL cells induced by the combination of anti-CD2 MoAb 9.6 and 9-1 and by the individual anti-CD2 MoAbs. The ³H-dT incorporation induced by MoAb Q6/64 with the anti-CD2 MoAbs is slightly higher than the sum of ³H-dT incorporation stimulated by each MoAb (Table 2).

Table 1. Effect of Anti-HLA Class I MoAbs on T-CLL Cell Proliferation Induced Via the CD3/Ti Pathway

<table>
<thead>
<tr>
<th>MoAb OKT3 (20 ng/mL)</th>
<th>MoAb O6/64 (10 μg/mL)</th>
<th>³H-dT Incorporation (cpm)</th>
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<tr>
<td>-</td>
<td>-</td>
<td>968* 1,528</td>
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<tr>
<td>-</td>
<td>+</td>
<td>6,410 1,222</td>
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<td>33,866 81,242</td>
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<tr>
<td>+</td>
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<td>6,956 8,237</td>
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</table>

PBMC from patient MC and from a normal donor were incubated in the presence of the indicated reagents for 96 hours. At that time, cells stimulated with the MoAb OKT3 displayed their maximal proliferation. ³H-dT was then added and after 16 hours of incubation its incorporation was measured.

RESULTS

The present study has shown for the first time that anti-HLA class I MoAbs are mitogenic for T-leukemic cells. The mitogenic activity does not appear to be mediated by accessory cells, because anti-HLA class I MoAbs induced proliferation of T-leukemic cell preparations that contained less than 0.3% of cells stained by anti-CD14 MoAb anti-Leu-M3. This concentration of monocytes is at least one order of magnitude lower than that found by Dasgupta et al to be required to mediate the modulatory effect of anti-HLA class I MoAbs on cell proliferation. The proliferation of leukemic T cells stimulated by anti-HLA class I MoAbs is likely to reflect their interaction with HLA class I antigens expressed by T-leukemic cells and not with a

Induction of cytotoxic activity in T-CLL cells by anti-HLA class I MoAbs. Because mitogenic stimuli can also induce functional maturation in cytotoxic lymphocytes, we analyzed whether anti-HLA class I MoAbs could induce cytotoxic activity in T-CLL cells. For this purpose, we tested the lectin-dependent cytotoxic activity of T-CLL cells before and after incubation with anti-HLA class I MoAbs. K562 cells were used as targets. These experiments could not be performed with T-PLL cells because cells from patient TRT were not available. Cells freshly isolated from the PB of the patient MC displayed a cytotoxic activity that was lost after 5 days of culture in control medium. On the other hand, when incubated with anti-HLA class I MoAbs, cells from patient MC retained their cytotoxic activity by 68% (Table 3).

DISCUSSION

The present study has shown for the first time that anti-HLA class I MoAbs are mitogenic for T-leukemic cells. The mitogenic activity does not appear to be mediated by accessory cells, because anti-HLA class I MoAbs induced proliferation of T-leukemic cell preparations that contained less than 0.3% of cells stained by anti-CD14 MoAb anti-Leu-M3. This concentration of monocytes is at least one order of magnitude lower than that found by Dasgupta et al to be required to mediate the modulatory effect of anti-HLA class I MoAbs on cell proliferation. The proliferation of leukemic T cells stimulated by anti-HLA class I MoAbs is likely to reflect their interaction with HLA class I antigens expressed by T-leukemic cells and not with a
Table 2. Effect of Anti-HLA Class I MoAbs on T-CLL Cell Proliferation Induced Via the CD2 Pathway

<table>
<thead>
<tr>
<th>MoAb 9.6 (10 μg/mL)</th>
<th>MoAb 9-1 (10 ng/mL)</th>
<th>MoAb Q6/64 (10 μg/mL)</th>
<th>MC Cells</th>
<th>Normal PBMC</th>
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<tr>
<td>cpm</td>
<td>cpm-Control</td>
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</table>

PBMC from patient MC and from a normal donor were incubated in the presence of the indicated reagents for 144 hours. At that time, cells stimulated with the MoAb 9.6 + 9-1 displayed their maximal proliferation. [H]-dT was then added and after 15 hours of incubation incorporation was measured.

*Results are means of triplicate determinations; standard deviations were lower than 15%.

cross-reacting unrelated structure, because the three anti-HLA class I MoAbs tested recognize distinct antigenic determinants: MoAb Q6/64 reacts with a determinant unrelated to HLA class I antigens. Furthermore, stimulated with anti-CD3 MoAbs is similar to that on CD3/Ti and the CD2 pathway.** The effect of anti-HLA class I MoAbs on the proliferation of T-leukemic cells stimulated normal T lymphocytes, because they inhibit the proliferation of both types of cells stimulated via the CD3/Ti pathway. On the other hand, the effect of anti-HLA class I MoAbs is not mediated by cell aggregation, because Fab' fragments are as antigenic as whole IgG. These results suggest that HLA class I molecules can transduce a signal that results in a proliferative stimulus for T-leukemic cells.

Anti-HLA class I MoAbs do not trigger the proliferation of normal T cells, but modulate that induced via the CD3/Ti and the CD2 pathway.** The effect of anti-HLA class I MoAbs on the proliferation of T-leukemic cells stimulated with anti-CD3 MoAbs is similar to that on normal T lymphocytes, because they inhibit the proliferation of both types of cells stimulated via the CD3/Ti pathway. On the other hand, the effect of anti-HLA class I MoAbs on the proliferation of T-leukemic cells stimulated with anti-CD3 MoAbs is somehow different from that on normal T lymphocytes. Although anti-HLA class I MoAbs enhance the proliferation of both types of cells stimulated via the CD2 pathway, the effect is detectable in the presence of MoAb 9.6 or MoAb 9-1 alone, in neoplastic but not in normal T lymphocytes. These results suggest that anti-HLA class I MoAbs mediate the same signal in normal and leukemic T lymphocytes; the differences in the proliferative response of the two types of cells may reflect abnormalities in the regulatory mechanisms that control the proliferation of T-leukemic cells and/or an activation state of these cells. These possibilities are consistent with the proliferative response of T-leukemic cells incubated with soluble anti-CD3 MoAbs in the absence of accessory cells, because anti-CD3 MoAbs have been shown to induce proliferation of preactivated T cells and of T-cell lines.**

Although T-CLL and T-PLL cells from patients MC and TRT, respectively, have many features of resting T lymphocytes (such as low levels of thymidine incorporation, proliferative response to anti-CD2 and anti-CD3 MoAbs, absence of activation antigens, ie, Tac, Tfr, HLA class II antigens), they display some characteristics of activated T cells, such as proliferative response to IL-2 and to soluble MoAb OKT3. Furthermore, T-CLL and T-PLL cells express, although at low levels, 2F, and c-myc mRNA, respectively. Therefore, some steps of G1 phase appear deregulated in these cells. One or more of them might be responsible for the mitogenic effect exerted by anti-HLA class I MoAbs in the absence of detectable cell stimulation via the CD2 pathway.

Activation of leukemic T cells by anti-HLA class I MoAbs follows the scheme usually observed in the activation of normal T lymphocytes by mitogens:** activation of protein kinase C, induction of genes expressed in G1 phase, S phase. Nevertheless, induction of IL-2, a factor required by normal T lymphocytes to proliferate, was not detected by Northern blot (Fig 4) and in biologic tests of the supernatants (data not shown). Therefore, it appears that leukemic T-cell proliferation can be obtained in the absence of IL-2, even when other steps of the cell cycle appear to be normally regulated. These results are in agreement with recent data obtained with normal T lymphocytes that factors different from IL-2, such as IL-4, activation of protein kinase C, induction of genes expressed in G1 phase, S phase. Nevertheless, induction of IL-2, a factor required by normal T lymphocytes to proliferate, was not detected by Northern blot (Fig 4) and in biologic tests of the supernatants (data not shown). Therefore, it appears that leukemic T-cell proliferation can be obtained in the absence of IL-2, even when other steps of the cell cycle appear to be normally regulated. These results are in agreement with recent data obtained with normal T lymphocytes that factors different from IL-2, such as IL-4 (which, however, does not stimulate the proliferation of T-CLL cells from patient MC) or IL-7, can induce S phase in activated T lymphocytes. Furthermore, T-CLL and T-PLL cells from patients MC and TRT, respectively, proliferate when incubated with IFN-γ (unpublished results). The latter cytokine is induced in T-CLL cells from patient MC by incubation with anti-HLA class I MoAbs.

In conclusion, the present results identify a deregulated pathway of leukemic T-cell proliferation, mediated by triggering of surface HLA class I molecules. Because triggering of HLA class I antigens might occur in vivo, for instance, by interaction with CD8 normal T lymphocytes activated in the course of an immune response to antigens expressed by leukemic cells, this pathway has a potential role in induction and/or maintenance of neoplastic cell growth. Furthermore, the ability of anti-HLA class I MoAbs to restore in cultured T-CLL cells the cytolytic activity of T-CLL cells is induced in T-CLL cells from patient MC by incubation with anti-HLA class I MoAbs.

Table 3. Effect of Anti-HLA Class I MoAbs on the Cytotoxic Activity of T-CLL Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>51Cr Release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC from a healthy donor</td>
<td>9</td>
</tr>
<tr>
<td>Freshly isolated MC cells</td>
<td>85</td>
</tr>
<tr>
<td>MC cells cultured with control medium</td>
<td>14</td>
</tr>
<tr>
<td>MC cell cultured with MoAb TP25.99 (10 μg/mL)</td>
<td>58</td>
</tr>
</tbody>
</table>

PBMC from patient MC were tested for their ability to lyse 51Cr-labeled K562 cells before and after 5 days of incubation with the indicated reagents. PBMC from a normal individual were tested as a control.
activity of freshly isolated cells suggests that HLA class I antigens may mediate a signal contributing to the activated state of the cell in vivo.

The HLA class I antigen-mediated pathway of cell proliferation is active in cells from a patient with T-PLL and one with T-CLL. In both malignancies, cells have “mature” phenotypes, and are considered the neoplastic counterparts of differentiated T lymphocytes or late thymocytes. On the other hand, anti-HLA class I MoAbs have not induced proliferation of ATLL and B-CLL cells (data not shown). Therefore, the proliferative response to HLA class I antigen-mediated stimulation may be a feature of specific T-cell disorders, characterized by differentiated phenotypes and proliferative patterns. Investigations in a large number of patients are required to test the validity of this hypothesis.

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T-cell malignancies with mature phenotypes: altered cell cycle regulation by HLA class I molecules

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