

GENERATION OF AN HLA-RESTRICTED CYTOTOXIC
T CELL LINE REACTIVE AGAINST CULTURED TUMOR
CELLS FROM A PATIENT INFECTED WITH HUMAN T
CELL LEUKEMIA/LYMPHOMA VIRUS

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The term human T cell leukemia/lymphoma virus (HTLV) denotes a unique family of T cell tropic human type C retroviruses (1, 2).¹ We studied the generation of cytotoxic T lymphocytes (CTL) derived from a patient who had an unusually long survival after therapy for an HTLV-associated T cell lymphoma. The purpose of this paper is to report the characteristics of a T cell growth factor-dependent line of CTL from this patient which lyses autologous tumor cells infected with HTLV. The data suggest a form of cytotoxic activity restricted by products of the major histocompatibility complex (MHC).

Materials and Methods

The Patient. MJ is a 53-yr-old white male who has circulating antibodies against internal structural proteins of HTLV. In 1977, he was diagnosed as having cutaneous T cell lymphoma, and he eventually became a complete responder to a regimen of chemotherapy, whole-body electron beam irradiation, and topical nitrogen mustard. The patient's disease was in clinical remission when these studies were initiated in January 1983.

Target Cells. MJ tumor is an HTLV-producing neoplastic T cell line derived from patient MJ and now propagated in the absence of exogenous T cell growth factor. HUT-102-B2 and WA tumor are HTLV-producing T cell lines derived from CR and WA, patients who had T cell lymphomas and evidence of infection with HTLV in vivo. MJ-B and CR-B are Epstein-Barr virus (EBV)-transformed B cell lines derived from patients MJ and CR, respectively. C1/MJ and C5/MJ are cell lines that resulted from the in vitro infection of two unrelated normal human cord cell populations using HTLV from the index patient MJ. C91/PL is a cell line that resulted from the in vitro infection of unrelated normal human cord cells with HTLV derived from PL, another HTLV-infected patient who had a T cell lymphoma. Other cell lines used included Molt 4, a long-term T cell line; Daudi, an EBV-transformed B cell line; K562, an erythroid line; Chang, a human hepatoma cell line; and NS-1, a mouse plasma cell line. We also used EBV-transformed B cell lines, GT-B and MM-B, derived from normal individuals who were partially HLA-matched with the index patient, MJ.

Cell Separation and Generation of CTL in Long-term Culture. Peripheral blood mononu-

¹ The strains of HTLV studied in this report belong to the subgroup HTLV-I.

clear cells (PBM) were prepared from heparinized blood of patient MJ and normal individuals by Ficoll-Isopaque gradient centrifugation. Aliquots of PBM (10^7 cells) from patient MJ or a normal individual were co-cultured with 10^6 irradiated (12,000 rad) MJ tumor cells in upright flasks (3050; Costar, Cambridge, MA) at 37°C in 5% CO_2 -containing humidified air in 10 ml of RPMI 1640 supplemented with 10% heat-inactivated human AB serum, 4 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 50 U/ml penicillin, and 50 $\mu\text{g}/\text{ml}$ streptomycin. After day 6, these cultured cells were continuously exposed to 20% lectin-depleted T cell growth factor (*ld*-TCGF) containing RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) without further use of human serum. The cultures were repeatedly stimulated with irradiated MJ tumor cells at 7–14-d intervals.

Cytotoxicity Assay. A 4-h ^{51}Cr -release assay was carried out in round-bottomed microtiter plates (Linbro Chemical Co., Hamden, CT). 10,000 ^{51}Cr -labeled target cells were mixed with various numbers of effector cells in 150 μl RPMI 1640 supplemented with 4% FCS. After the plates were incubated for 4 h at 37°C in 5% CO_2 -containing humidified air, supernatants were harvested with the Titertek Supernatant Collection System and measured for radioactivity. The percentage specific ^{51}Cr release was determined by the following formula: $100 \times [(\text{release in test} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Spontaneous release was always $<20\%$ of maximum release for all target cells.

Competitive Cold-Target Inhibition Assays. The effectors were dispersed in 100 μl RPMI 1640 supplemented with 4% FCS and various numbers of unlabeled target cells were added in 50 μl medium. Then ^{51}Cr -labeled MJ tumor cells (10^4 cells/well) were added in 50 μl of medium. The ratios of effector/labeled target cells were 5:1 or 10:1. The plates were then incubated and processed using the usual cytotoxic assay to determine specific ^{51}Cr release.

Analysis of Surface Antigens by Monoclonal Antibodies. The reactivity of cells with OKT3, OKT4, OKT8 (Ortho Diagnostic Systems Inc., Westwood, MA), HLA-DR (Becton, Dickinson & Co., Sunnyvale, CA), and anti-Tac (3) monoclonal antibodies was determined by indirect immunofluorescent microscopy or by fluorescence-activated cell sorter analysis (BD FACS Systems, Sunnyvale, CA).

Results

Generation of a Specific CTL Line from the Patient. Fresh PBM from patient MJ did not mediate specific cytotoxicity (data not shown). However, on day 20 of culture the patient's cells mediated a substantial level of specific cytotoxicity against MJ tumor cells (Fig. 1A-1). By contrast, a normal individual's PBM exposed to MJ tumor cells and cultured under the same conditions did not mediate specific cytotoxicity and killed a variety of target cells (Fig. 1B).

After repeated cycles of stimulation with MJ tumor cells and expansion with *ld*-TCGF, the cytotoxicity of the cultured cells from the patient against various tumor targets other than his autologous tumor cells was progressively reduced. On day 27 and beyond, the patient's cultured cells were cytotoxic specifically for his own tumor cells in effector/target ratios of 5:1 or less compared with a variety of unrelated HTLV-infected and uninfected target cells (Fig. 1A-2 and A-3). The specificity was confirmed by competitive cold-target inhibition studies, which showed a linear inhibition of cytotoxic effector activity by the addition of unlabeled MJ tumor cells (Fig. 2). The CTL line has now been maintained for >150 d.

Almost all cells in the CTL line after 22 d in culture reacted with OKT3 (96–100%) and OKT8 (98–100%) antibodies; 43 and 92% reacted with anti-Tac and HLA-DR antibodies, respectively. There were $<3\%$ OKT4 reactive cells. How-

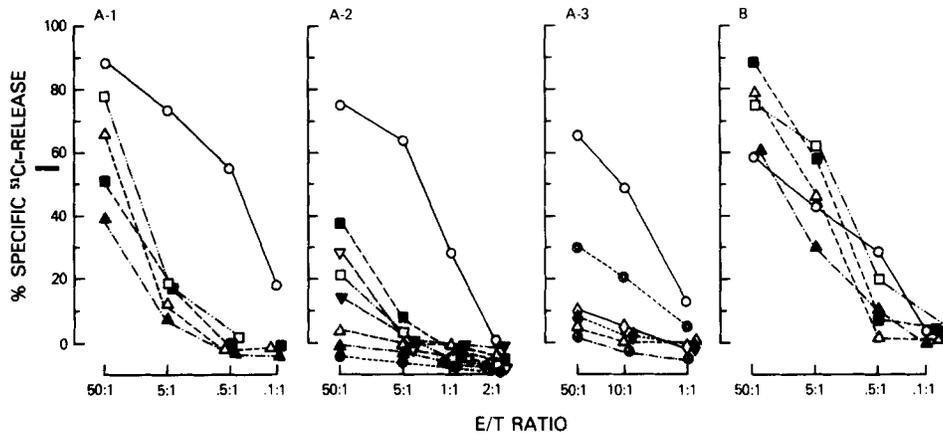


FIGURE 1. Generation of a CTL line (MJ CTL) reactive with autologous HTLV-infected tumor cells. MJ CTL were generated by co-culturing 10^7 PBL from the patient MJ with 10^6 irradiated MJ tumor cells in vitro. On day 6, these cells were exposed to *ld*-TCGF. PBM from a normal individual were treated in the same manner. MJ CTL were tested on day 20 (A-1), day 27 (A-2), and day 47 (A-3). Comparably cultured cells from a normal individual were tested on day 21 (B). Target cells: MJ tumor (○), HUT-102-B2 (△), CR-B (▲), Chang (□), Molt4 (■), K562 (▽), Daudi (▼), NS-1 (●), WA tumor (⊙), Cl/MJ (⊕), C5/MJ (◇), and C91/PL (◆). Cytotoxicity was measured in a 4-h ⁵¹Cr-release assay. The specificity of the cytotoxic activity progressively increased with time in culture.

ever, in the MJ tumor cell population, we observed a different phenotypic profile: 0–17% of the cells were reactive with OKT3, 61–68% were reactive with OKT4, and <1% were reactive with OKT8.

HLA Restriction of Cytotoxic Activity. The specific T cell-mediated lysis of virally infected target cells requires an associative recognition of MHC-encoded antigens in a variety of settings (4). Consequently, we tested the CTL from patient MJ (MJ CTL), MJ tumor, and various cell lines for expression of HLA alloantigens (Table I) using the microcytotoxicity technique (5).

MJ CTL failed to kill various HTLV-infected target cells that did not share HLA antigens expressed by MJ CTL. However, they were cytotoxic against MJ tumor cells and WA tumor cells, which did share A1, B8, Cw7, and DR3 (Fig. 1A-3), suggesting an HLA-restricted form of cytotoxicity. To confirm and extend these observations, cold-target competition assays were performed (Fig. 2B and C). The cytotoxic activity of MJ CTL against MJ tumor cells was selectively blocked by unlabeled MJ and WA tumor cells but not by any other HLA-unmatched cells tested.

To further characterize the specificity of MJ CTL, we tested EBV-transformed cells from normal individuals who shared A1, B8, and DR antigens with patient MJ. Moreover, we tested an EBV-transformed line derived from the index patient himself. MJ CTL did not kill these various HLA-matched control target cells (Table II). The cytotoxicity of MJ CTL against autologous MJ tumor cells was not inhibited by the partially HLA-matched MM-B and GT-B cells as cold targets (Fig. 2C). These data suggest that the cytotoxicity of MJ CTL is directed against HTLV-related antigen(s) and that HTLV-specific cytolytic activity is restricted by products linked to the MHC.

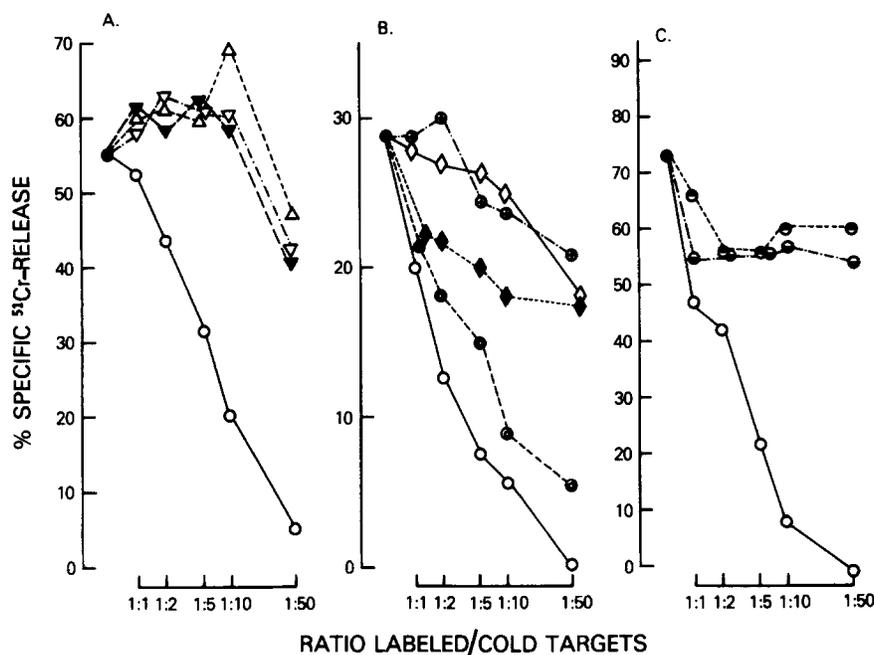


FIGURE 2. Competitive cold-target inhibition of MJ CTL activity. In these studies, labeled MJ tumor cells were used as targets in the presence or absence (●) of various competitive cold-target cells. The effector/labeled target cells ratios were 5:1, 5:1, and 10:1 in A, B, and C, respectively. Cold-target cells: MJ tumor (○), HUT-102-B2 (△), K562 (▽), Daudi (▼), WA-tumor (●), Cl/MJ (⊕), C5/MJ (◇), C91/PL (◆), GT-B (⊖), and MM-B (⊙). The values represent the mean percentage specific ^{51}Cr release in a 4-h assay done in triplicate. MJ or WA tumor cells (known to share certain histocompatibility antigens) could competitively inhibit the cytotoxic activity.

Discussion

This initial characterization of a cytotoxic T cell response to a human retrovirus-associated tumor establishes a parallel with the well-studied animal models of immune responses to retrovirus-induced tumors (6). This is of interest from several perspectives. The first involves the relationship between cell-mediated immunity to type C retroviruses and leukemogenesis. There are data suggesting that chronic immunostimulation and the profound general amplification of the immune system resulting from persistent viremia eventually leads to somatic errors, bringing about a neoplastic transformation of proliferating T cells (7).

In a related point of view, T cells "transformed" by retroviruses might represent precisely those lymphocytes whose antigen specificity is directed against viral determinants (receptor-mediated leukemogenesis theory) (8), and continuous exposure to the stimulating antigen would be necessary in maintaining the T cell-transformed state. The receptor-mediated leukemogenesis theory per se requires no event beyond the development of the matching receptor and ligand in an appropriate microenvironment. Accordingly, in certain settings, susceptibility to viral leukemogenesis could paradoxically correlate with various cell-mediated immune responses (including the generation of cytotoxic T cells). If this hypothesis is correct, the induction of immune tolerance to virus-associated antigen(s) would decrease the likelihood of virally induced transformation.

TABLE I
The HLA-Phenotype* of Cells from Patient MJ and HTLV-infected Target Cells

Cells	HLA-A	HLA-B	HLA-C	HLA-DR
MJ PBM [‡]	A1	B8, B27	Cw6, Cw7	DR3, DR7
MJ CTL	A1	B8, B27	Cw6, Cw7	DR3, DR7
MJ Tumor	A1	B8, B27 (B17)	Cw6, Cw7	DR3, DR7
WA Tumor	A1, Aw24 (A11)	B8, Bw22 (B15)	Cw3, Cw7	DR3
HUT-102-B2	Aw30, Aw31 (A10) (A11) (A29)	B17, B18 (Bw35) (Bw50) (B12)		DR2, DRw6
C1/MJ	A10, A29	B12, Bw35 (Bw53)	Cw1	DR2
C5/MJ	Aw32 (Aw30)	B5, Bw35 (Bw21)	Cw4	DR2
C91/PL	A3	Bw51, Bw52	Cw4, Cw6	DR2, DR4
GT-B [§]	A1, A3	B8	Cw7	DR3
MM-B [¶]	A1, A3	B8, Bw35	Cw4, Cw6	DR3, DR7

* It has been previously noted that cells infected with HTLV appear to frequently express extra (alien) class I histocompatibility antigens (2, 12) When appropriate uninfected control cells from the same individuals were available for HLA typing, alien histocompatibility markers are listed in parentheses.

[‡] Freshly harvested PBM cells from patient MJ.

[¶] EBV-infected B cells obtained from normal control individuals.

TABLE II
Lack of Cytotoxicity of MJ CTL Against Autologous and Partially HLA-matched EBV-transformed B Cell Line Targets

Effectors	Target cells							
	MJ tumor		MJ-B		GT-B		MM-B	
	10:1*	1:1	10:1	1:1	10:1	1:1	10:1	1:1
MJ CTL [‡]	63.8 ± 3.0 [§]	24.6 ± 5.2	6.3 ± 1.8	5.3 ± 1.3	-4.5 ± 0.1	-10.7 ± 1.9	-1.9 ± 0.3	-1.3 ± 0.8
TO CTL [†]	36.2 ± 4.2	17.9 ± 4.1	22.7 ± 6.0	15.9 ± 2.7	29.9 ± 1.3	8.7 ± 2.4		
MI CTL [†]	20.3 ± 2.9	4.4 ± 1.5					9.9 ± 2.4	0.4 ± 1.2

* Effector/target ratio.

[‡] MJ CTL were tested on day 51 of culture.

[§] All determinations were made in triplicate and data are expressed as the mean ± 1 SD.

[†] TO CTL and MI CTL were derived from normal individuals' PBM, continuously exposed to *ld*-TCGF for 14 and 52 d, respectively, after stimulation with irradiated WA tumor and irradiated MJ tumor cells, respectively.

An additional perspective from which the current results may be viewed relates to the role of the host cell-mediated immune response in the control of tumor growth. Several studies have shown that the emergence of specific cell-mediated immunity in the context of a regressing tumor confers resistance to subsequent challenge with the same tumor (9). Such immunity results from the generation of regulatory and cytotoxic effector T cell responder populations, and this immunity can be adoptively transferred to unimmunized syngeneic hosts.

Of perhaps greater clinical relevance are reports involving murine models of adoptive immunotherapy or chemoimmunotherapy in which fresh or cultured immune T cells specific for retrovirus-induced tumors were able to effect complete tumor regression upon transfer to syngeneic hosts already bearing otherwise lethal, disseminated tumors (10, 11). The potential clinical applications of cytotoxic T cells propagated in vitro are topics for future research.

Summary

Lymphocytes from a patient who had an unusually long survival after therapy for a human T cell leukemia/lymphoma virus (HTLV)-associated T cell lymphoma were stimulated in vitro with an autologous tumor cell line, and the

generation of cytotoxic T lymphocytes (CTL) was studied. CTL generated were directed against autologous HTLV-associated tumor cells. These propagated CTL were OKT3+, OKT4-, and OKT8+. The cytotoxic activity required target tumor cells that were infected with HTLV and also expressed histocompatibility antigens in common with the patient, suggesting a major histocompatibility complex-restricted associative recognition of target antigens expressed on the tumor cell membrane.

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