

CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR SELF TUMOR IMMUNOGLOBULIN EXPRESS T CELL RECEPTOR δ CHAIN

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One goal of cancer research is to exploit the exquisite specificity of immune recognition to eliminate tumors *in vivo*. Although their role in immune surveillance of tumor cells remains unclear, CTL are possible candidates for adoptive immunotherapy. The effectiveness of this therapy depends on CTL recognition of specific tumor antigen. Ig on B cell lymphomas is an ideal tumor-specific antigen since it has a variable region (idiotype) that is tumor specific (1, 2). We report here that human CTL specific for the idiotype on autologous B cell tumors could be selectively expanded *in vitro*. The phenotype of these CTL is CD3⁺,CD4⁻,CD8⁻ and they express the δ chain of the TCR. Such CTL could be grown *in vitro* for the treatment of B cell lymphoma.

Materials and Methods

Cell Lines. Tumor cell lines were established as described (3) from the primary tumors of three patients with Burkitt's lymphoma. The SUP-B8 cell line was derived from tumor isolated from the bone marrow of a 15-yr-old female with Burkitt's lymphoma with extensive bone marrow involvement (B cell ALL). The patient achieved a complete remission after treatment with chemotherapy but suffered an isolated central nervous system relapse. She received an autologous bone marrow transplant, depleted of malignant cells with anti-B1 mAb, following preparation with total body irradiation and high dose cyclophosphamide. Recurrent B cell acute lymphoblastic leukemia was documented 5 wk after transplant, and 9 mo after initial diagnosis. Peripheral blood was obtained from the patient at the time of leukemic relapse. The SUP-B12 cell line was derived from tumor isolated from a 7-yr-old male with Burkitt's lymphoma with extensive bone marrow involvement (B cell ALL). He was treated with intensive chemotherapy and complete remission was documented 4 wk after the start of treatment. He received intensive maintenance chemotherapy, which was electively discontinued 18 mo later. He remains in continuous complete remission. PBL were obtained from this patient both during and after chemotherapy while in remission. The SUP-B17 cell line was derived from the malignant ascites fluid from a 3 and 3/12-yr-old male who presented with a primary Burkitt's lymphoma in the right lower quadrant of the abdomen. Bone marrow and cerebrospinal fluid examinations were normal and he was designated stage III. Complete remission was obtained with chemotherapy, which was electively discontinued after 15

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mo. This patient remains in continuous complete remission off of therapy. For all three patients, EBV-transformed B lymphocyte lines were established from PBL (4). T cell lines were generated by coculture of patient's PBL with an irradiated (10,000 rad) mixture of autologous tumor and EBV-transformed B lymphoblasts (5:1). The CTL-A2 line was derived by coculture of the PBL from a normal donor (HLA-A3; B7, w38; DR6) with the irradiated B cell line JY (HLA-A2; B7; DR4,6) (5, 6). Tumor and EBV-transformed cell lines were maintained in RPMI 1640 supplemented with 10% calf serum. T cell lines were maintained in RPMI 1640 supplemented with 10% FCS and T cell growth factors (7) and were stimulated weekly with the irradiated mixture designated above. Cells were cloned by limiting dilution (8).

⁵¹Cr-release Assays. Assays were carried out for 4 h as previously described (9). Antibodies were added at the beginning of the assay and were present throughout.

Monoclonal Antibodies. The following mAbs specific for the indicated molecules were used for cell surface staining and/or inhibition of cytotoxicity: Leu-1/anti-CD1, OKT4/anti-CD4, OKT8/anti-CD8; PA2.6/anti-HLA class I (10); OKT3/anti-CD3; TS1.16/anti-HLA class II (11); TS1.18/anti-LFA-1 (CDw18) (9); WT31/anti-TCR- α/β (12); TCR δ 1/anti-TCR- γ/δ (13). The antiidiotype, μ , λ , and κ mAbs were prepared and characterized as described (14-16).

Results and Discussion

Antitumor CTL were derived by in vitro coculture of the PBL from three separate patients with an irradiated mixture of their autologous Burkitt's lymphoma tumor cells plus an autologous B lymphoblastoid cell line derived from EBV-transformed PBL. Cell lines generated under these conditions were stable for at least 6 mo in continuous culture. Antitumor CTL could not be generated by culturing PBL from two of these patients with only their autologous tumor cell lines (SUP-B12, SUP-B17) without the EBV-transformed lines. This may reflect the lack of expression of the lymphocyte function-associated antigen 1 (LFA-1) on the SUP-B12 and SUP-B17 tumor cells (4). The SUP-B8 cell line expresses normal levels of LFA-1 (4).

After the cells were in culture for 6 wk their specificity was ascertained with a panel of 40 target cells (Fig. 1, with 16 representative lines). Cell lines were gener-

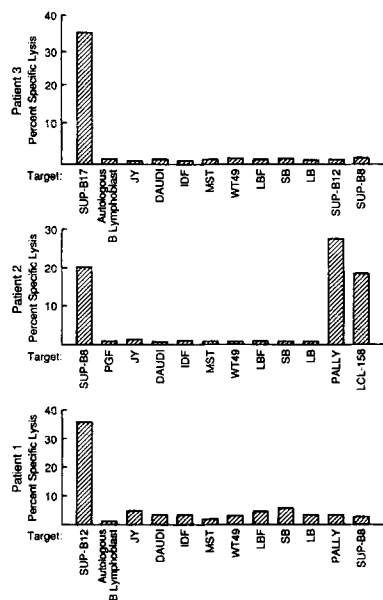


FIGURE 1. Specificity of cytotoxicity by antitumor CTL. The antitumor CTL from three patients were tested for cytotoxic activity in a standard ⁵¹Cr-release assay (6) using an E/T ratio of 1. These target cells represent the spectrum of different HLA specificities, cell types, and Ig idiotypes from the 40 total target cells tested. HLA types were determined by microcytotoxicity assays using both PBL and tumor cell lines for each patient at the HLA typing laboratory, Stanford University Blood Center, Stanford, CA. SUP-B12 is the autologous tumor line derived from patient 1, SUP-B8 is the autologous tumor line derived from patient 2, and SUP-B17 is the autologous tumor line derived from patient 3. The HLA types of the target cells used are: JY (HLA-A2; B7; DR4,6), Daudi (HLA-A⁻; B⁻; DR6), IDF (HLA-A26,w69; B18,38; DR5), MST (HLA-A3; B7; DR2), WT49 (HLA-A2; B17; DR3), LBF (HLA-A30; B12; DR7), SB (HLA-A1,2; B44,17; DR2,6), LB (HLA-Aw68; B40; DR6), PALLY (HLA-A26,28.2; B14,38; DR1,4), PGF (HLA-A3; B7; DR2), LCL-158 (HLA-A32,w68; B51,62; DR1), SUP-B8 (HLA-A1,2; B8,27; DR1,3), SUP-B12 (HLA-A2,26; B7,35; DR4,6), SUP-B17 (HLA-A2,25; B18,45; DR2,4). No autologous EBV-transformed B cell line was available for patient 2.

ated from each patient on at least six separate occasions, and the specificity of lysis by each line was similar to the results shown here. The CTL lines generated from patients 1 and 3 lysed only their autologous tumor cell lines and not the autologous EBV lines. The CTL line generated from patient 2 lysed not only the autologous tumor cell line SUP-B8 but also all targets expressing HLA-DR1 (Pally, LCL-158, SUP-B8 shown). Treatment of this heterogeneous CTL line with mAb to CD4 and complement showed that only CD4⁺ CTL lysed the HLA-DR1⁺ targets whereas CD4⁻ CTL specifically lysed the autologous SUP-B17 tumor (data not shown).

To localize the epitopes of the tumor-specific antigens recognized by these CTL, we tested mAb to idiotype, μ , and λ or κ for inhibition of cytolysis. The isotype of the Ig expressed by the SUP-B8 and SUP-B12 cell lines is μ/κ ; that of the SUP-B17 cell line is μ/λ . Our specificity controls were alloreactive CTL derived from normal donors and specific for HLA-A2 (5, 6). In all three cases, mAbs to Ig specifically inhibited tumor directed cytolysis (Fig. 2). Lysis of SUP-B12 cells by the autologous CTL line was specifically inhibited by anti-SUP-B12 idiotype and anti- μ . Similarly, lysis of SUP-B8 cells by the autologous antitumor CTL line was inhibited by anti-SUP-B8 idiotype and anti- μ . Finally, lysis of SUP-B17 cells was inhibited by anti- λ and antiidiotype, but not by anti- μ . In each case, lysis was inhibited only by the mAb to the specific tumor idiotype and not by mAb to either of the other two tumor idiotypes. Thus, Ig determinants can serve as target antigens in an autologous system.

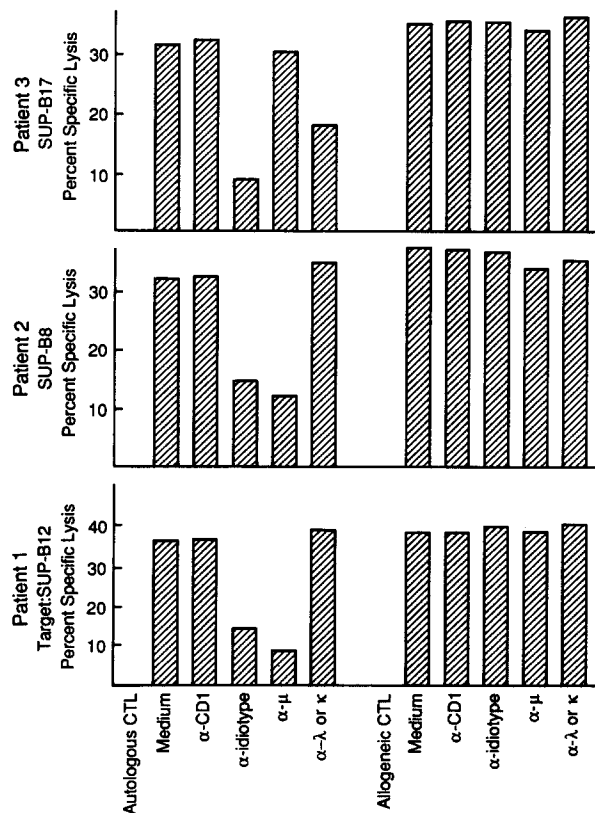


FIGURE 2. mAbs to Ig inhibit cytolysis by tumor-specific CTL. Lysis of tumor cells by the autologous antitumor CTL line or the HLA-A2-specific CTL CTL-A2 was tested using an E/T ratio of 1:1. The targets were SUP-B12, SUP-B8, and SUP-B17 for patients 1, 2, and 3, respectively. Similar inhibition was observed at E/T ratios of 2:1 and 5:1. These E/T ratios were chosen because they fall on the linear portion of the lysis curve. Purified antibodies were added at a final concentration of 25 μ g/ml to ⁵¹Cr-labeled targets for 20 min at room temperature before the addition of CTL. Anti- κ chain was used for SUP-B8 and SUP-B12 and anti- λ chain was used for SUP-B17. Results shown are from one of five similar experiments. In experiments not shown, mAb were titrated from 50 μ g/ml down to 1 μ g/ml. Inhibition was observed at all concentrations and the percent inhibition decreased proportionately with the concentration of mAb.

Since cytotoxicity is tumor cell specific, the target determinants are presumably the Ig idiotype.

To confirm that Ig was the target antigen and to establish the phenotype of these tumor specific CTL, we cloned CTL by limiting dilution. Clones were obtained only from patient 3. Clones that lysed the autologous tumor cell line SUP-B17 were expanded. Four T cell clones lysed the autologous tumor cell line but not the autologous EBV B cell line or any of 40 other target cells tested, including SUP-B8, SUP-B12, and five other Burkitt's cell lines (data not shown). Lysis was inhibited by mAb to λ , but not by mAbs to μ or the idiotype (Fig. 3). Thus, this particular CTL clone may recognize an idiotypic determinant associated with the λ light chain but distinct from the epitope recognized by the antiidiotypic mAb. The polyclonal CTL line from which this CTL was cloned contained cells which could be inhibited by mAb to λ and idiotype (Fig. 2).

To identify other molecules involved in the lysis of the SUP-B17 tumor cells, we tested mAb to various cell surface molecules for inhibition of cytolysis (Fig. 3). The results for the heterogeneous CTL cultures and all four CTL clones were similar. Lysis of SUP-B17 by clone 18 was inhibited only by the mAb to CD3 (OKT3) and a mAb to the β subunit of LFA-1 (TS1/18); mAbs specific for CD4 (OKT4), CD8

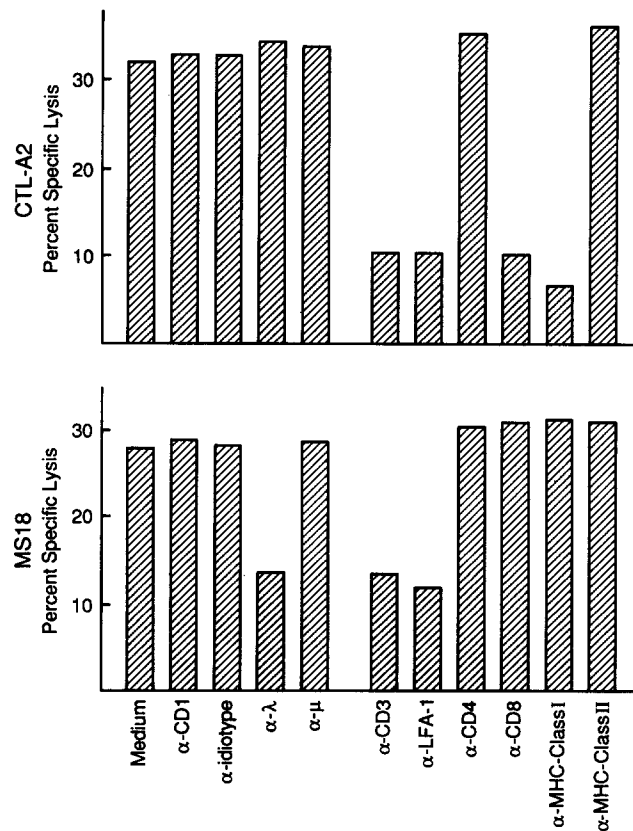


FIGURE 3. Effect of mAbs specific for various CTL or target cell molecules on cytolysis. Lysis of SUP-B17 by the tumor-specific clone 18 or the HLA-A2-specific cell line CTL-A2 was tested with an E/T ratio of 1:1. mAbs to Ig and to CD1 (Leu-1), CD3 (OKT3), LFA-1 (TS1/18), CD4 (OKT4), CD8 (OKT8), MHC class I (PA2.6), and class II (TS1/16) were added at a final concentration of 25 μ g/ml to 51 Cr-labeled targets for 20 min at room temperature before the addition of CTL.

(OKT8), MHC class I (PA2.6), and MHC class II (TS1/16) antigens had no inhibitory effect.

In contrast, lysis of both SUP-B17 and JY cells by the HLA-A2-specific CTL line CTL-A2 (5, 6) was inhibited by mAbs to HLA-class I, CD3, CD8, and LFA-1, as has been shown previously for CTL that are HLA class I restricted and express CD3 and CD8 (5, 6, 9).

Since mAbs to CD4, CD8, or MHC molecules did not affect lysis by the tumor-specific CTL clones, we examined the cell surface phenotype of the clones and the heterogeneous CTL line from which they were derived (Fig. 4). All four clones (represented by clone 18, panel A) and the parental anti-SUP-B17 T cell line were strongly positive for CD3 expression. Approximately two-thirds of the parental T cell line (panel B) expressed the CD8 molecule, but no CD4-expressing cells were detected. None of the four clones expressed either CD4 or CD8 molecules. Since the lack of expression of both CD4 and CD8 molecules has been associated with expression of the TCR- γ/δ (15-17), the molecular nature of the TCR on these cells was investigated with two mAbs to the TCR (Fig. 4 E-H). WT31, a mAb to a framework determinant on the TCR- α/β -CD3 complex (12) reacted strongly with the Jurkat cell line (Fig. 4 H). TCR δ 1 is a mAb to the TCR δ chain that was produced by immunization with the purified TCR- γ/δ complex from the leukemic cell line PEER (13). Most CTL in the parental anti-SUP-B17 cell line were stained with the TCR δ 1 mAb, while a minor population was positive for WT31 (Fig. 4 F). A representative clone (clone 18) was positive for TCR δ 1; no binding of WT31 was detected (Fig. 4 E). Since, as far as is known, the TCR δ chain is invariably expressed as part of

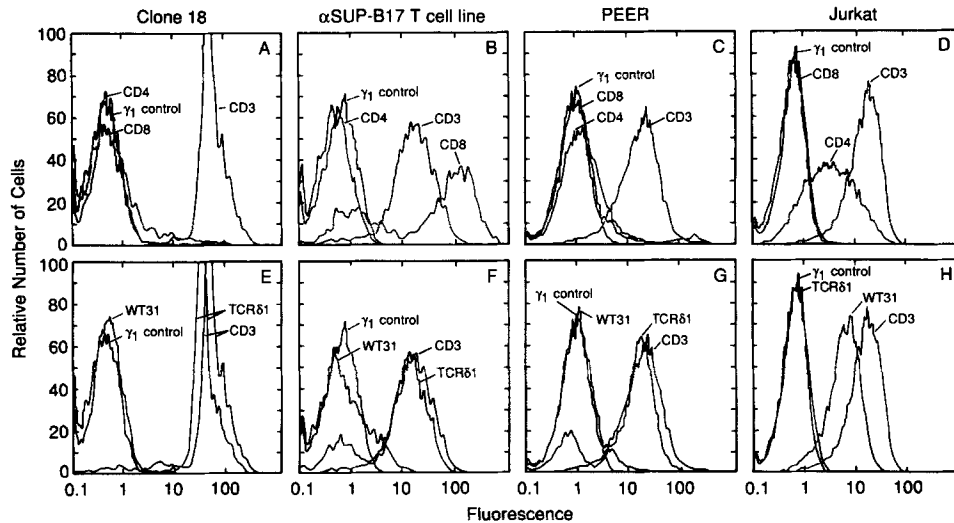


FIGURE 4. FACS analysis of T cell differentiation antigens and TCR structures on T cell lines. Clone 18 (A and E), the parental anti-SUP-B17 T cell line (B and F), PEER (C and G), and Jurkat (D and H) were stained with antibodies recognizing CD3 (Leu-4), CD4 (Leu-3), CD8 (Leu-2), the CD3- α/β molecular complex (WT31), the δ chain of the CD3- γ/δ complex (anti-TCR δ 1), and an isotype matched negative control (2). Cells were treated sequentially with unconjugated mouse mAbs followed by FITC-conjugated goat antibodies to mouse Ig. Cells were analyzed on a multiparameter FACS II.

the TCR- γ/δ -CD3 complex (18), we assume that these cells also express the TCR γ chain.

Although TCR- δ genes are rearranged and expressed early in thymic ontogeny (19-22), there are very few cells with TCR- γ/δ heterodimers detectable in the thymus or periphery at birth. In human adults, only 3-10% of peripheral T cells express a TCR- γ/δ (23-25). Many IL-2-dependent T cell lines and clones that express TCR- γ/δ exhibit cytotoxicity against a broad panel of tumor targets (16, 17, 27). Recognition and cytotoxicity are neither restricted by nor directed against MHC antigens, so that it is possible that T cells expressing TCR- γ/δ mediate MHC-unrestricted cytotoxicity. However, it is unclear whether such nonspecific cytotoxicity is a result of in vitro culture or reflects the in vivo activity of these cells.

CTL lines with a similar specificity for autologous tumor targets were established from these patients on >20 separate occasions over the past 2 yr. During this period, patient 3 was on continuation chemotherapy and the percentage of CD3⁺,CD4⁻, CD8⁻ cells in his peripheral blood ranged from 15 to 50% (data not shown). We cannot establish whether these CD4⁻,CD8⁻, γ/δ ⁺ cells arose as a result of host factors, such as malignancy or chemotherapy, or because of the stimulatory antigens present on the autologous tumor. Nonetheless, our findings suggest a physiologic role for T cells with TCR- γ/δ in MHC-unrestricted autologous tumor cell recognition and raise the intriguing possibility that some but not all (16, 28, 29) γ/δ T cells may be restricted by Ig determinants in much the same manner as α/β T cells are restricted by MHC molecules.

Summary

CTL are thought to play a role in the elimination of transformed cells in vivo. The effectiveness of such CTL is in part dependent on recognition of tumor specific antigens. Among the best characterized tumor-specific antigens are the unique or idiotypic determinants on the Ig of B cell lymphomas. Here we describe the generation and properties of human CTL specific for the idiotype on autologous B cell tumors. These cells are CD3⁺,CD4⁻,CD8⁻ and express the δ chain of the TCR. Such cells may prove useful in tumor-specific adoptive therapy.

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