Therapeutic use of a long-term cytotoxic T cell line recognizing a common tumour-associated antigen: the pattern of in vitro reactivity predicts the in vivo effect on different tumours

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Summary. A long-term cultured cytotoxic T lymphocyte (CTL) line (E/88) was obtained from splenic lymphocytes of BALB/c (H-2d) mice bearing the weakly immunogenic colonic carcinoma C26. This line was shown to be α/βTCR+Vβ6+CD3+CD8+CD4- and to recognize a common tumour-associated antigen on syngeneic carcinomas and sarcomas in a major-histocompatibility-complex-restricted and T-cell-receptor (TCR)-mediated fashion. The assessment of cytotoxic activity on a panel of 30 normal and neoplastic target cells of differing etiology and histotype showed that E/88 CTL lysed syngeneic colon carcinomas and some fibrosarcomas but not leukemias, lymphomas or mammary carcinomas. Clones derived from the E/88 line exhibited the same lytic pattern. Moreover, anti-T3, anti-Lyt2.2, anti-α/βTCR and anti-Vβ6 mAbs as well as anti-H-2d antisera abolished cytotoxicity when used in blocking experiments. The therapeutic activity of E/88 CTL upon in vivo transfer was assessed in mice bearing either experimental or spontaneous metastases of C26. In both models therapy with E/88 lymphocytes in combination or not with interleukin-2 was highly effective. Adoptive immunotherapy carried out with two clones obtained from line E/88 showed comparable therapeutic effects. In addition, treatment of syngeneic mice bearing experimental metastases of in vitro E/88-lysable or E/88-resistant tumours, showed that E/88 CTL can eradicate metastases of the former but not of the latter neoplasms. These data indicate that long-term CTL lines recognizing common tumour-associated antigens can be derived from tumour-bearing animals and used in adoptive immunotherapy of tumours previously shown to be lysed in vitro by these effectors.

Key words: Adoptive immunotherapy – Cytotoxic T cell line – Common tumour-associated antigens

Introduction

The therapeutic potential of T-cell-mediated adoptive immunotherapy has been demonstrated in a variety of experimental models with highly immunogenic tumours. In those studies the adoptive transfer of a sufficient number of immune lymphocytes, obtained from tumour-immunized syngeneic animals, was shown to mediate the regression of established primary and metastatic tumours (see [5, 11]).

Recently, the focus of experimental immunotherapy has been moved to model systems, which can provide adequate guidelines for clinical strategies. As human tumours may not be sufficiently immunogenic, poorly immunogenic tumours or tumours lacking detectable tumour-specific transplantation antigens have been used. In addition, anti-tumour effector cells have been activated in vitro by procedures that can be feasible also for the treatment of human neoplasms, and interleukin-2 (IL-2)-activated killer cells (LAK), tumour-infiltrating lymphocytes (TIL) or T cells obtained from mice bearing progressive tumours and sensitized in vitro with autologous tumour cells have been tested for therapeutic capacity [16–18, 21]. In these studies adoptive immunotherapy with activated T cells obtained from the tumour or spleen of tumour-bearing mice, in combination with IL-2, proved to be far more effective than that with LAKs [16, 18]. TIL were shown not to be effective or impossible to derive from non-immunogenic sarcomas [23]. However, it has been shown that T cells capable of reducing metastases upon in vivo transfer can be obtained after in vitro activation of cells from tumour-draining lymph nodes of mice bearing a sarcoma that was non-immunogenic in transplantation assays [3]. This work indicated that tumour-associated antigens (TAA) other than tumour-specific transplantation antigens may be expressed by overtly non-immunogenic tumours and serve as target molecules for T-cell-mediated adoptive immunotherapy.

Common TAA have been detected by transplantation studies and by specific cytotoxic T lymphocytes (CTL) in mouse melanomas [6], chemically induced sarcomas [7–9], radiation-induced leukemias [10] and colonic carci-
nomas [14, 20]. Common TAA of human tumours, as defined by T cell clones that specifically lyse the autologous tumour as well as allogeneic tumours that express the same restricting human leucocyte antigen A, have been found among melanomas and soft tissue and bone sarcomas [4, 22]. The presence of shared TAA may therefore be exploited for immunotherapeutic protocols, when T cells recognizing such TAA are used for adoptive immunotherapy in combination or not with cytokines.

To see whether common TAA can be target of CTL with therapeutic capacity in adoptive immunotherapy, we have used a CTL line (E/88) obtained from splenic lymphocytes of BALB/c mice bearing the poorly immunogenic syngeneic colon carcinoma C26. The cytototoxicity of such lines was shown to be major-histocompatibility-complex (MHC)-restricted, mediated by the T cell receptor for antigen and to be directed against a common TAA expressed by syngeneic carcinomas and sarcomas but not by mammary carcinomas and lymphomas. The present data also show that the in vivo adoptive transfer of E/88 CTL alone or in combination with a low dose of IL-2, can eradicate metastases of those tumours that were recognized and killed in vitro by E/88 lymphocytes. The therapeutic potential of these T cells was also confirmed in an adjuvant treatment of mice bearing post-surgical metastases of C26.

Materials and methods

Mice. Adult, 3-month-old BALB/cAnNCiBR, C57Bl/6NCrBR, DBA/2NCrBR, C3H/HeNCrBR and BALB/B/Ola/Hsd female mice, obtained from Charles River Co. (Calco, Italy) and from Nossan (Corregzana, Italy) were used. They were kept and handled according to the guidelines for the care and use of animals of our Institute.

Tumours and cell lines. C26 adenocarcinoma, originally induced by N-methyl-N-nitrosourea [1], was maintained in vivo by subcutaneous (s.c.) transplantation of tumour fragments in syngeneic BALB/c mice.

Tumour cell lines used in this study were the following: C26, MPC26, MEC26, and ML26 established from s.c. nodules and from lung, hepatic and lymph nodal metastases of C26 respectively [14]; the BALB/c 1,2-dimethylhydrazine-induced colon adenocarcinoma CS1 [1]; the fibrosarcomas CA-2, GL17, QA-1-2e12l and QA-1-2e122 of BALB/c, DB1 and DB3 of DBA/2, B6-1 of the C57Bl/L6, C3H-3 and C3H-7 of the C3H/He, and MB4 of the BALB.B strain, induced in our laboratory by s.c. application of 3-methylcholanthrene. WEHI/164 (originally provided by Professor G. Forini, University of Turin, Italy) and solid and ascitic variants of MethA (from Dr. L. J. Old, MSKCC, New York, USA) were also sarcomas of the BALB/c strain. Two clones of spontaneous BALB/c mammary adenocarcinoma TSA c115 and TSA c1E1 were obtained from Dr. P. Lohlini (University of Bologna, Italy); the Moloney-virus-induced YC8 BALB/c, YAC-1 (A/Sn) lymphomas, and the LSTRA and MBL2 leukemias, of the BALB/c and C57Bl6 strains, respectively, were originally obtained from Professor L. Chieco Bianchi (University of Padua, Italy). The DBA/2 P815 mastocytoma was kindly provided by Dr. T. Boon (Ludwig Institute, Brussels, Belgium); the spontaneous B16 melanoma and the ovarian reticulum cell sarcoma M3076, both of the C57Bl6 strain, were obtained from Dr. M. D’Incalci (Mario Negri Institute, Milan, Italy). All cell lines were maintained in continuous in vitro culture and were shown to be mycoplasma-free by electron microscopy analysis.

Antibodies. Monoclonal antibodies (mAbs) to the following molecules were used for the phenotypic characterization of line E/88, or in experiments designed to block cytoxicity: T3e (145-2c11); NEN, Bosson, Mass., USA), Lyt2.2 (2.43; ATCC, Rockville, Md., USA), L3T4 ( GK1.5; ATCC), IL-2 receptor (7D4; ATCC), LFA-1a (M17/4.2; ATCC), MAC1b (M17/15.11.HL; ATCC), homing receptor for endothelial venules (MEL14; ATCC), CD45/T200 (30F11.1; PhaRmigen, San Diego, Calif., USA), Ly5.2/2 (RA3-682; PhaRmigen), Fc receptor (24G2; NEN), Thy1.2 (Becton Dickinson, Sunnyvale, Calif., USA), asialoGM1 (ASGM1; Wako Chemicals, Neuss, FRG). Anti-TCR mAbs were provided by PhaRmigen and recognized the following T cell receptor chains: αβ (H157-597), VB3 (KJ-25), VB5 (MR9.4), VB6 (RR4-7), VB7 (TR310), VB9 (MR10-2), VB11 (RR3-15), VB13 (MR12.4), VB17a (KJ-23), Vc3.2 (RR3-16), and Vc11 (RR8-1).

For indirect immunofluorescence, specific fluorescein-isothiocyanate-coated anti-rat Ig (PhaRmigen), anti-mouse Ig (Technogenetics, S. Margherita, Torinese, Italy), anti-hamster Ig (Southern Biotechnology Associates, Birmingham, Ala., USA) or anti-rabbit Ig (Vector, Burlinghame, Calif., USA) secondary sera were used; fluorescence was analysed by an EPICS flow cytometer.

Origin, generation, maintenance and cloning of long-term CTL line E/88. Line E/88 was obtained from pooled spleen lymphocytes of two BALB/c mice bearing s.c. C26 nodules 4 weeks after tumour excision. Red cells were removed from splenocytes by treatment with buffer ammonium chloride solution and seeded (5 × 10^6 cells/well) in 2 ml in 24-well plates (Costar, Cambridge, Mass., USA) with stimulating irradiated (150 Gy) C26 tumour cells (5 × 10^5/well) and irradiated (30 Gy) syngeneic splenocytes (5 × 10^6/well) as feeder in RPMI-1640 medium supplemented with 10% fetal calf serum, antibiotics, glutamine, vitamins, non-essential amino acids, sodium pyruvate, HEPES buffer and 2-mercaptoethanol. After 10 days, 2.5 × 10^6 lymphocytes/well were re-stimulated and 100 U/ml IL-2 (EuroCetus, Amsterdam, The Netherlands) were added to the culture. Lymphocytes were then stimulated once a week and every 5 days their lytic activity was tested on C26 and the allogeneic tumour B16; after the fourth stimulation, lymphocytes lysed C26 but not B16 cells. The E/88 line was kept in culture with irradiated C26 cells, irradiated splenocytes as feeder and 25 U/ml IL-2, and was maintained in culture for several months; exogenous IL-2 is essential for its in vitro growth. Clones were derived either by micromanipulation or by limiting dilution. A single cell, or 0.3 cell, was placed into round-bottomed 96-well Costar plates with stimulating and feeder cells and 100 U/ml IL-2. Single colonies were visible 1 week later in some wells and after 12 days they were expanded after reducing IL-2 to 50 U/ml and then to 25 U/ml; at this time cells were tested after expansion.

Cell-mediated cytolysis. The specificity of line E/88 was determined in standard 3^1Cr-release assays as previously described [15]. Briefly, labelled target cells (2 × 10^5 cells/well) were incubated with lymphocyte effectors at different ratios in a total volume of 0.2 ml in 24-well plates for 4 h at 37°C. Supernatants were harvested and counted in a gamma counter. Percentage lysis was calculated as follows:

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\text{Percentage lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100
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Inhibition of cytoxicity was assessed by preincubating effector cells or targets with mAbs for 30 min at 37°C in 96-well V-bottomed microtiter plates (PB International, Milan, Italy). Lymphokine-activated killer cells (LAK) and allogeneic lymphocytes from mixed lymphocyte cultures, used as control effectors in the cytotoxic assays were obtained as described [14].

Proliferation assays. Proliferative responses of line E/88 (10^6 cells/well) in the presence of 150 Gy-inactivated tumour cells were measured at an effector/tumour ratio of 10/1, in the presence or absence of IL-2 (25 U/ml), in triplicate samples in round-bottomed 96-well microtiter plates. After 5 days of incubation at 37°C, each well was pulsed overnight with 1 μCi [H]thymidine. The cells were then collected onto glass fibres using a cell harvester (Skatron, Sterling, Va., USA) and the radioactivity incorporated was determined with a beta counter.

Immunotherapy experiments. Adoptive immunotherapy with the E/88 CTL line was carried out in mice bearing spontaneous post-surgical metastases of C26, and in mice bearing experimental metastases induced