HUMAN CYTOTOXIC T LYMPHOCYTES FOR TUMOR THERAPY

Induction on formalin-fixed tumor tissues and expansion on immobilized lectins

T. OHNO
RIKEN Cell Bank, The Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba Science City, 305, Japan

Introduction

Cytotoxic T lymphocytes (CTL) exhibit strong killing activity against tumor cells and, therefore, are expected to be employed in adoptive immunotherapy for human tumors. Generation of CTL requires autologous/syngeneic target tumor cells or tumor-derived antigenic peptides presented on antigen-presenting cells. However, successful generation of CTL has been limited to cases where sufficient tumor cells (Crowley et al., 1991; Kawakami et al., 1994; Wolfel et al., 1994) or tumor-derived antigenic peptides (Slingluff et al., 1994; Tjoa et al., 1994) were available for repeated stimulation of CTL growth for a prolonged culture period. The supply of target tumor cells is a key problem due to the difficulty of establishing a tumor cell line from every patient (Hay et al., 1994). Therefore, an alternative and stable source of tumor-specific antigens is desirable.

We have reported that, when cultured on formalin-fixed paraffin-embedded tumor sections as a substitute for live tumor cells, human autologous CTL with high specificity and strong killing activity against the target tumor cells were induced (Liu et al., 1996a), since formalin fixation preserved the specific antigenicity of the target (Pollard and Holgate, 1987). The supply of tumor sections, however, was insufficient when the CTL grew to a considerably large number, and therefore, we needed to restimulate the CTL with anti-CD3 monoclonal antibody to maintain the culture (Liu et al., 1995). Repeated stimulation with this antibody sometimes results in loss of cytotoxic activity in the CTL population (Muul et al., 1987). In clinical practice, conditioning of CTL by timely restimulation in prolonged culture is essential for repeated administration to a patient.

To solve this timing problem, two approaches were examined in the present study. One approach is to employ formalin-fixed primary target cells, such as primary cultured (but not yet completely established) tumor cells, tumor cells dissociated from tumor tissues, or tumor cells in ascites, as a stable source of tumor antigens, available any time, for further stimulation of CTL. Another approach is to activate the CTL by means of non-specific stimulatory molecules immobilized on an insoluble matrix.

Restimulation by formalin-fixed tumor cells

TKB-1p-CTL are known to kill the autologous target TKB-1p cells, but not allogeneic renal carcinoma cells or gastric carcinoma cells (Tsurushima et al., submitted for publication).

We attempted to stimulate the growth of these CTL using live TKB-1p cells previously irradiated with X-rays or formalin-fixed TKB-1p cells as target cells at E/T...
ratios of 1 and 10. As shown in Fig. 1, the number of control CTL gradually decreased after culturing for 2 days. This control culture consisted of all the components except the target cells. With the formalin-fixed TKB-1p cells as target cells, the CTL grew continuously at E/T ratios of 1 and 10 with mean doubling times of 95 hr and 119 hr, respectively. With the X-ray-irradiated live TKB-1p cells as target cells, the growth rates of the CTL were nearly the same at both E/T ratios of 1 and 10; mean doubling times were 138 hr and 106 hr, respectively. These values were similar to the mean doubling time observed for CTL stimulated using formalin-fixed tumor cells as target cells at the E/T ratio of 10 and were greater than that observed using these fixed target cells at the E/T ratio of 1.

![Graph showing continuous stimulation of CTL growth with formalin-fixed tumor cells](image)

**Fig. 1 Continuous stimulation of CTL growth with formalin-fixed tumor cells**

Arrows indicate restimulation with X-ray-irradiated live or formalin-fixed TKB-1p cells at the E/T ratio indicated. Each point is the mean value of duplicate observations. The control culture consisted of all the components except target cells.

The results suggest that CTL are continuously expandable when cultured on formalin-fixed autologous tumor cells as well as when cultured on live cells of an autologous established tumor cell line. Even though we have failed to establish an autologous tumor cell line, many investigators may have target tumor cells available found in small pieces of dissected tumor tissue, in the fluid of the thoracic cavity and ascites, or in quiescent primary cultures. If fixed and supplied properly, these cells could also serve as a source of tumor antigens available any time for further stimulation of CTL.

**Restimulation by immobilized Con A**

The plant-derived lectin, concanavalin A (Con A) (Baker et al., 1979; Quentmeier et al., 1992; Shinomura et al., 1986) is able to induce cytokine production in peripheral blood mononuclear cells. Considering the cost of long term culture for expansion of CTL, Con A is one of the candidates for use as a non-specific stimulator of CTL growth. However, since Con A itself has a toxic effect on lymphocytes at high concentrations,