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Tumor-specific autologous cytotoxic T lymphocytes from tissue sections

To the editor — For the generation of human tumor-specific autologous cytotoxic T lymphocytes (CTLs), we have previously shown that formalin-fixed paraffin-embedded sections of a gastric cancer patient are a stable tumor-antigen source[1], although subsequent study revealed that the CTLs were allogeneic[2]. Here we show genuine autologous CTLs generated on the pathological sections.

Renal carcinoma cells (Pt.46C) and the corresponding normal renal epithelial cells (Pt. 46N) were derived from a 66-year-old woman. By coculturing autologous peripheral blood mononuclear cells with Pt.46C in RHAMα medium containing 5% autologous plasma and interleukin-1, -2, -4 and -6, CTLs were induced and proliferated [CTL(live)]. CTLs were also induced on formalin-fixed paraffin-embedded sections of the carcinoma tissue after removal of paraffin with xylene and ethanol [CTL(fix)][1]. The individual identity of Pt.46C, Pt.46N, the pathological sections, and the CTL(fix) was confirmed by DNA fingerprinting using the polymerase chain reaction (PCR) with three pairs of DNA primers, *D1S80* (ref. 3), *pYNZ22* (ref. 4) and *DXS52* (ref. 5), to amplify the single-locus variable number of tandem repeats in genomic DNA. All bands in each of the three PCR products showed identical patterns — sufficient to confirm the CTL(fix) to be autologous.

We confirmed the cytotoxic activity of CTL(fix) against live Pt.46C at the E/T ratio of 10 (Fig. 1*a*). CTL(fix) did kill Pt.46C but not the normal Pt. 46N. Human leukocyte antigen (HLA)-typing[6] revealed that Pt.46C was HLA-A2402. CTL(fix) did not lyse an HLA-A2402 gastric adenocarcinoma cell line (GT3TKB), HLA-A2402 renal tumor cell lines (TUHR3TKB and TUHR4TKB) or HLA type-unmatched renal tumor OS-RC-2 cells. CTL(fix) killed half of the other HLA-A2402 renal carcinoma Hpt.10. The cytotoxicity of CTL(fix) against Pt.46C and Hpt.10 was inhibited by antibodies against MHC-class I, CD8 and CD3 (Fig. 1*b*). These characteristics of CTL(fix) were observed in CTL(live).

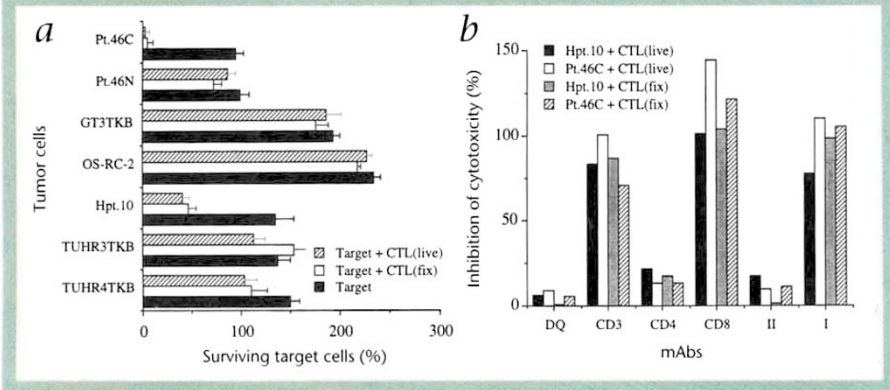

Fig. 1 Characteristics of the autologous CTLs of Pt.46 with a renal carcinoma. *a*, Specific cytotoxicities of CTL(fix) (13-day culture) at an effector/target (E/T) ratio of 10 and CTL(live) (35-day culture) at an E/T ratio of 4. One-hundred percent surviving target cells refers to the number of cells present at the initiation of the coculture with CTLs for the 24-h killing assay. Surviving target cells were stained with crystal violet and quantified[1]. Note that both CTLs killed autologous carcinoma Pt.46C but not the corresponding normal cells, Pt.46N. *b*, Inhibition of the CTL activities by monoclonal antibodies (mAbs) against cell surface molecules[1]. DQ, anti-human MHC DQ; CD3, anti-human CD3; CD4, anti-human CD4; CD8, anti-human CD8; II, anti-human MHC class-II; I, anti-human MHC class-I.

In another case, a 60-year-old man with renal carcinoma (Pt.49), CTL(fix) were also generated on autologous pathological sections. As described[1], the CTL(fix) showed growth response to the carcinoma tissue part, but not to the normal tissue part, of the sections during a 10-day incubation. Autologous CTLs were also generated on the sections from an endometrial carcinoma tissue of a 56-year-old patient (MIN-2) and her live carcinoma cell line (HLA-type was A1). Both the CTL(fix) and the CTL(live) of MIN-2 killed HLA-A1-expressing OMC-1 (cervix carcinoma) and T24 (bladder carcinoma) but not the HLA type-unmatched GT3TKB.

These results imply that this technique of CTL generation is applicable to many kinds of tumors and to adoptive immunotherapy of tumors. (Partly supported by the Science and Technology Agency of Japan.)

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