Special Issue

Induction of autologous human cytotoxic T lymphocytes (CTL) from peripheral blood against tumor cells

Tadao Ohno

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

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Introduction

T lymphocytes such as natural killer (NK) cells, lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TIL) and cytotoxic T lymphocytes (CTL) have been considered to be useful for adoptive immunotherapy of human tumors (Rosenberg *et al.*, 1985; Sato *et al.*, 1986; Rosenberg *et al.*, 1988). Lymphokine-activated killer (LAK) cells were once thought to be promising from animal experiments (Takahashi *et al.*, 1993a; Takahashi *et al.*, 1993b), but were not so effective in clinical studies (Fisher *et al.*, 1988; Rosenberg *et al.*, 1987).

Cytotoxic T lymphocytes (CTL) have higher cytotoxicity (Miyatake *et al.*, 1988) than LAK cells on specific tumors that have been used as antigen-like stimulators during induction of lymphocyte proliferation. If autologous CTL could be generated, they should be better than LAK and TIL for adoptive immunotherapy (Crowley *et al.*, 1991; Wolfel *et al.*, 1993) since CTL are known to kill tumor cells more efficiently than LAK and TIL (Slingluff *et al.*, 1994). This led us to expect that CTL might be useful clinically for adoptive immunotherapy of tumors.

There are many reports of establishment and characterization of tumor-specific murine CTL lines and possible therapeutic effects of CTL (Ibayashi *et al.*, 1985; Kedar *et al.*, 1984; Plata, 1982; Ryser *et al.*, 1978; Yamasaki *et al.*, 1984). However, although human autologous CTL are required for clinical treat-

ment of tumors, induction of human CTL is not easy. As Gillis *et al.* (1979) succeeded in induction of murine CTL, De Vries and Spits (1984) used a similar mixed lymphocyte reaction for initial activation of human lymphocytes. Abo and Balch (1982), Mazumder *et al.* (1982 and 1983), and Mukherji *et al.* (1989) tried continuous stimulation of lymphocytes with lectins together with inactivated target tumor cells. Nakashima *et al.* (1994) used tumor-involved lymphnodes as a source of candidate CTL and Rosenberg *et al.* (1986) cultured TIL as CTL.

However, practical barriers do exist for induction of CTL. First, stimulation of T lymphocyte with a sufficient amount of target tumor cells is essential to induce CTL *in vitro*, but primary culture and subsequent establishment of human tumor cell lines is generally time-consuming, tedious processes and not practical for all patients (Hay *et al.*, 1994). Second, autologous CTL are the most suitable to avoid unnecessary side effects of lymphocyte reactions, but at present, routine induction of autologous CTL from lymphocytes is a hard technique except when the CTL are recruited from TIL (Rosenberg *et al.*, 1986), and the small pieces of tumors removed surgically are usually insufficient to yield a number of TIL. Therefore, induction of autologous CTL against small brain tumors from peripheral blood lymphocytes is usually unsuccessful except special cases (Letessier *et al.*, 1991; Miyatake *et al.*, 1988).

In the present mini-review article, I will describe shortly on induction of CTL from peripheral blood mononuclear cells (PBMC), first on allogeneic established cell lines together with serum/plasma-free culture of the induced CTL, second on formalin-fixed paraffin-embedded specimens of the gastric cancer tissue, and third on autologous brain tumor tissue fragments. Finally, I will introduce recent technique to induce CTL on antigenic peptide-loaded cells.

Induction of allogeneic CTL against human lung cancer cells

To generate CTL from PBMC, we utilized the culture medium RHAM α which has originally been developed for serum-free culture of human lymphocytes and applied to LAK cell culture supplemented with 5% plasma (Kawai, *et al.*, 1992). By combining this novel enriched medium supplemented with an interleukin cocktail of IL-1, -2, -4, and -6 (Nakashima *et al.*, 1994) and 5% autologous plasma of the PBMC donor, we could induce reproducibly allogeneic CTL against the human lung squamous carcinoma cell line SQ-5 from PBMC of healthy volunteers (Liu *et al.*, 1994). Briefly, the PBMC were cocultured on X-ray irradiated live SQ-5 cells. This coculture resulted in detachment and killing of almost all SQ-5 cells within 5 days. After this induction period and subsequent subculture for 2 weeks, allogeneic CTL grown from the PBMC killed SQ-5 cells but not other human cell lines such as the lung squamous carcinoma cell line AOI, the lung adenocarcinoma cell line A549, the NK cell-sensitive cell line K562, and the LAK cell-sensitive cell line Daudi (Figure 1). The CTL (named SQ-5-CTL) could be routinely subcultured on unirradiated target SQ-5 cells at an E/T ratio of 10, since SQ-5 cells disappeared within 1 day after addition of the SQ-5-CTL. Phenotypic analysis by flow cytometry showed that the SQ-5-CTL culture contained 98% CD3⁺, 95% CD8⁺, and 5% CD4⁺ after cultured for over one month.

Serum/plasma-free culture of the allogeneic CTL

In typical cases of lung cancer, treatment with LAK cells required 10^{10} – 10^{11} effector cells for treatment of one patient, indicating that 10–30 liter-scale culture (cell concentration: 10^6 – 3×10^6 ml⁻¹) is necessary, with use of 1–3 liters of human serum. Considering the difficulties in safe and efficient preparation, continuous

Figure 1. Killing activity of the allogeneic SQ-5-CTL.

low cost supply, and relatively complicated handling of human serum or plasma, serum/plasma-free culture of CTL seems desirable. The dependencies of CTL growth on serum/plasma and target cells were tested in the RHAM α medium containing the IL-cocktail (Liu *et al.*, 1994).

Growth of SQ-5-CTL depended on target cell supplementation at each subculture at 5 day intervals, without autologous plasma, when human serum albumin (HSA) was added at 0.5%. During the first 5 day period of subculture, the number of CTL increased 4.4-fold in medium with 5% autologous plasma and 3-fold in medium with 0.5% HSA. After initial induction of CTL for 5 days, long-term serum/plasma-free culture was carried out with bovine serum albumin (BSA). During 6 subcultures after the 5-day induction culture, the mean doubling times of CTL were 40 and 50 h in media containing plasma and BSA, respectively. Although CTL grew less in BSA medium than in plasma medium, the cumulative number of CTL cultured in serum/plasma-free conditions reached 10^{11} in 35 days.

Induction of CTL on live and formalin-fixed paraffin-embedded gastric cancer cells

If possible, more stable sources of the tumor-specific antigen are desirable than established cell lines or operationally removed fresh tumor tissues for induction of CTL. After removal of tumor tissues by operation, formalin-fixed paraffin-embedded blocks of the tissue are always prepared and stored for subsequent diag-

nosis. As formalin fixation preserves the specific antigenicity of tumor cells (Pollard and Holgate, 1987), these preparations should be an alternative source for CTL induction. After established a cancer cell line, we tried to generate CTL from peripheral blood on both the live target cells and the corresponding fixed paraffin-embedded sections (Liu *et al.*, 1995). The gastric adenocarcinoma cell line GT3TKB was established by extensive passage of cells in ascites of a 53-year-old patient. Based on reported techniques for CTL generation (Crowley *et al.*, 1991; Wolfel *et al.*, 1993; Liu *et al.*, 1994), selective growth of the CTL was observed on repeated stimulation of PBMC with X-ray-irradiated live GT3TKB [Figure 2, defined as CTL(live)].

Paraffin-embedded thin sections of the original gastric mucosa containing the cancer and surrounding normal mucosal tissue were washed sequentially with toluene and ethanol. They were then added to PBMC in culture medium RHAM α containing 5% autologous plasma and the IL-cocktail. Microscopic examination showed that the lymphocytes that attached to the tissue sections grew as aggregates, while most of the unattached lymphocytes died within the first 2 weeks. Once the cells began to grow [Figure 2, defined as CTL(fix), the lag phase of growth of CTL(fix) was 4 times that of CTL(live) in the induction culture], the two types of CTL showed almost the same cumulative growth curves when they entered the log-phase. As the sections gradually became fragmented and disappeared during dilution culture of the lymphocytes and further sections were not added, the growth of CTL(fix) rapidly slowed down after 56 days. But on use of a culture surface precoated with anti-CD3 monoclonal antibody for stimulation, CTL(fix) continued to grow as in the presence of cancerous tissue sections (Figure 2, dotted line) for 4 months.

Both types of CTL showed strong killing activity on live target GT3TKB cells assayed by staining with crystal violet (CV) after removal of the non-adherent effector lymphocytes (Saotome *et al.*, 1989). CTL(live) and CTL(fix) killed over 85% of the GT3TKB cells in 24 h at an effector/target (E/T) ratio of 1. On incubation for 24 h at this low E/T ratio, LAK and NK did not kill GT3TKB cells efficiently.

In 48 h incubation for specificity assay, CTL(fix) showed highly specific killing activity of the GT3TKB cells, but not of the other gastric tumor cell line GCIY, normal kidney cells HPT12N, or NK-sensitive K562 cells. They caused partial killing of LAK-sensitive Daudi cells. These activities were almost the same

Figure 2. Cumulative growth curves of CTL(fix) and CTL(live).

as those of CTL(live). Phenotype analyses by flow cytometry indicated that both types of CTL were CD3⁺CD16⁻CD8⁺, while LAK were a mixture of CD4⁺ cells (28.1%) and CD8⁺ cells (34.0%). We confirmed that the target GT3TKB cells were expressing MHC-class I molecules on their surface by cytofluorometry.

Induction of autologous CTL against brain tumor cells

We tried to induce autologous CTL from PBMC by coculture directly on minced tissue fragments of glioblastoma multiforme (GM) (Tsurushima *et al.*, 1996), since in a common primary culture medium, DMEM containing 10% FBS, human GM cells from the patient did not proliferate, although small numbers of astrocyte-like cells and fibroblasts grew out from the tumor fragments for at most 5–7 days. Since reported CTLs are usually restricted by major histocompatibility complex (MHC) class I molecules expressed on the target cell surface (Roberts *et al.*, 1987; Crowley *et al.*, 1991) and the MHC class I molecules are known to be induced with interferon- γ (IFN γ) (Heron *et al.*, 1978; Wallach *et al.*, 1982; Carrel *et al.*, 1985) we also added human IFN γ to the medium for the first 4 days. After this period, IFN γ was omitted from the culture medium. As a control, LAK cells were also induced from the same PBMC by the method reported previously (Grimm *et al.*, 1982). After cultured for 14 days, the CTL (CTL-IFN γ) killed almost all tumor cells at an E/T ratio of 1.67 on 48 h incubation (Figure 3). The CTL induced

Figure 3. Comparison of activities of autologous LAK, CTL, and CTL-IFN γ on the freshly dissociated glioblastoma cells.

without IFN γ addition killed 82% of the target cells, while LAK cells killed only 33%.

Both of CTL and CTL-IFN γ had similar cytotoxicity on allogeneic GM cell line U87-MG to LAK cells and no substantial cytotoxicity on allogeneic GM cell line KG-1-C co-cultured for 24 h at higher E/T ratios. Both of the CTLs showed no cytotoxicity against LAK-sensitive Daudi cells, NK-sensitive K562 cells or autologous fibroblasts outgrown from the brain tumor, although they showed slight cytotoxicities against allogeneic GM cell lines. These results suggest that human autologous CTL can be induced from PBMC depending on brain tumor fragments but not on established tumor cell lines or on long-term primary cultured tumor cells. These are advantageous to established cell lines as the target, since we could save time for long-term primary culture and the tumor cells are thought to be minimally deviated from their state *in vivo*. These findings should contribute to a simple method of CTL generation and subsequent adoptive immunotherapy of brain tumors.

Antigenic peptides for CTL induction

In a T-cell receptor-mediated and MHC-restricted manner, CTL recognize antigenic epitope (peptides) presented by MHC-class I molecules on the surface of target cells (reviewed by Knuth *et al.*, 1992). A number of peptides have been identified recognized by

CTL/TIL against melanoma. These are octa-, nona-, or deca-peptides identified as a part of MAGE-1, -3 antigens (van der Bruggen *et al.*, 1991; Gaugler *et al.*, 1994), melanocyte-specific tyrosinase (Brichard *et al.*, 1993), gp100 melanocyte specific protein (Bakker *et al.*, 1994), melanocyte differentiation antigen Melan A/MART-1 and gp75 (Coulie *et al.*, 1994; Kawakami *et al.*, 1995; Wang *et al.*, 1995). Although the original idea was derived from viral-encoded peptides eluted from MHC-class I molecules (Falk *et al.*, 1991; Jardetzky *et al.*, 1991), researchers adapted a peptide stimulation strategy for the induction of a CTL response not only to synthetic viral peptides of influenza matrix protein (Gotch *et al.*, 1987) and hepatitis B and C viruses (Cerny *et al.*, 1995; Bertoletti *et al.*, 1991) but also to MAGE-2 and -3 gene peptides (Celis *et al.*, 1994), HLA-A0201-binding tyrosinase peptide (Visseren *et al.*, 1995), and peptides from myelin basic protein, proteolipid protein, and myelin-associated glycoprotein (Tsuchida *et al.*, 1994).

However, for induction of CTL, the approach of peptide-loading on target cells requires repetitive long-term stimulation or a large quantity of the peptides for bulk cultures containing large numbers of naive PBMC (Cerny *et al.*, 1995). Successful activation of CTL is critically dependent on adequate presentation of the epitopes involved. Since dendritic cells express high levels of MHC-class I and II and costimulatory molecules, Ossevoort *et al.* (1995) demonstrated the effectiveness of highly purified spleen den-

dritic cells that efficiently induce primary peptide-specific CTL responses *in vitro* to human papilloma virus 16 E7(49–57) peptide. Independently, Bakker *et al.* (1995) also successfully generated anti-melanoma CTL from healthy donors after presentation of the epitope-peptides by dendritic cells *in vitro*. In the *in vivo* trials, cultured autologous antigen presenting cells pulsed with the synthetic nonapeptide of MAGE-1 gene product were capable of inducing autologous melanoma-reactive and the nonapeptide-specific CTLs in patients with advanced stage IV melanoma (Mukherji *et al.*, 1995).

Conclusion

Induction and large scale culture of specific CTL against tumor cells are important for adoptive immunotherapy. Tumor cell killing activity of the allogeneic SQ-5-CTL could be maintained for over a month without apparent decrease of target specificity in serum/plasma-free culture conditions. With an autologous set of PBMC and target tumor cells, it should also be possible to maintain autologous CTL under serum/plasma-free conditions once the CTL have been induced in the presence of serum or plasma and an appropriate interleukin cocktail.

Moreover, our study suggests that tumor-specific CTL can be generated from peripheral blood on formalin-fixed paraffin-embedded tumor tissue sections as on live tumor cells. On the culture on an anti-CD3 monoclonal antibody-coated surface, the cumulative number of CTL(fix) was calculated to reach over 6×10^{13} for 121 days. This growth potency of the CTL implies that it is possible to treat a patient for more than 100 therapeutic courses.

Whenever we find epitope-peptides presented on the surface of tumor cells, successful induction of CTL responses with peptide-pulsed dendritic cells illustrates the potential use of antigen-presenting cells for vaccination protocols in human tumor.

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References

- Abo T, Balch CM (1982) Characterization of HNK-1+ (Leu-7) human lymphocytes. II. Distinguishing phenotypic and functional properties of natural killer cells from activated NK-like cells. *J Immunol* 129: 1758–1761.
- Bakker ABH, Schreurs MWJ, De Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG (1994) Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J Exp Med* 179: 1005–1009.
- Bakker ABH, Marland G, de Boer AJ, Huijbens RJF, Danen EHJ, Adema GJ, Figdor CG (1995) Generation of antimelanoma cytotoxic T lymphocytes from healthy donors after presentation of melanoma-associated antigen-derived epitopes by dendritic cells *in vitro*. *Cancer Res* 55: 5330–5334.
- Bertoletti A, Ferrari C, Fiaccadori F, Penna A, Margolske R, Schlicht HJ, Fowler P, Guillehot S, Chisari FV (1991) HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. *Proc Natl Acad Sci USA* 88: 10445–10449.
- Brichard V, Van Pel A, Wolfel T, Wolfel C, de Plaen E, Lethe B, Coulie P, Boon T (1993) The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 178: 489–495.
- Carrel WJ, Schmidt-Kessen A, Giuffrè L (1985) Recombinant interferon- γ can induce the expression of HLA-DR and -DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumor associated antigens. *Eur J Immunol* 15: 118–123.
- Celis E, Tsai V, Crimi C, DeMars R, Wentworth PA, Chesnut RW, Grey HM, Sette A, Serra HM (1994) Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. *Proc Natl Acad Sci USA* 91: 2105–2109.
- Cerny A, Fowler P, Brothers MA, Houghton M, Schlicht HJ, Chisari FV (1995) Induction *in vitro* of a primary human antiviral cytotoxic T cell response. *Eur J Immunol* 25: 627–630.
- Coulie PG, Brichard V, Vanpel A, Wolfel T, Schneider J, Traversari C, Mattei S, Deplaen E, Lurquin C, Szikora JP, Renauld JC, Boon T (1994) A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 180: 35–42, 1994.
- Crowley NJ, Darrow TL, Quinn-Allen MA and Seigler HF (1991) MHC-restricted recognition of autologous melanoma by tumor-specific cytotoxic T cells - evidence for restriction by a dominant HLA-A allele. *J Immunol* 146: 1692–1699.
- De Vries JE, Spits H (1984) Cloned human cytotoxic T lymphocyte (CTL) lines reactive with autologous melanoma cells. I. *In vitro* generation, isolation and analysis of phenotype and specificity. *J Immunol* 132: 510–519.
- Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee H-G (1991) Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351: 290.
- Fisher RI, Coltman CA Jr, Doroshow JH, Rayner AA, Hawkins MJ, Mier JW, Wiernik P, McMannis JD, Weiss GR, Margolin KA, Gemlo BT, Hoth DF, Parkinson DR, Paietta E (1988) Metastatic renal cancer treated with interleukin-2 and lymphokine-activated killer cells: a phase II clinical trial. *Annals Internal Med* 108: 518–523.
- Gaugler B, Vandeneuynde B, Vanderbruggen P, Romero P, Gaforio JJ, Deplaen E, Lethe B, Brasseur F, Boon T (1994) Human gene Mage-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J Exp Med* 179: 921–930.

- Gillis S, Union NA, Baker PE, Smith KA (1979) The *in vitro* generation and sustained culture of nude mouse cytotoxic T-lymphocytes. *J Exp Med* 149: 1460–1476.
- Gotch F, Rothbard J, Howland K, Townsend A, McMichael AJ (1987) Cytotoxic T lymphocytes recognize a fragment of influenza virus matrix protein in association with HLA-A2. *Nature* 326: 881–885.
- Grimm EA, Mazumder A, Zhang HS and Rosenberg SA (1982) Lymphokine-activated killer cell phenomenon ; Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J Exp Med* 155: 1823–1841.
- Hay RJ, Park J-G, Gazar A (Edited) (1994), Atlas of human tumor cell lines. Academic Press, Inc., CA, USA.
- Heron I, Hokland M, Berg K (1978) Enhanced expression of beta 2-microglobulin and HLA antigens on human lymphoid cells by interferon. *Proc Natl Acad Sci USA* 75: 6215–6219.
- Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA (1995) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc Natl Acad Sci USA*, 91: 3515–3519.
- Kawai K, Sasaki T, Saijo-Kurita K, Akaza H, Koiso K, Ohno T (1992) Additive effects of antitumor drugs and lymphokine-activated killer cell cytotoxic activity in tumor cell killing determined by lactate-dehydrogenase-release assay. *Cancer Immunol Immunother* 35: 225–229.
- Kedar E, Chriqui-Zeira E, Mitelman S (1984) Methods for amplifying the induction and expression of cytotoxic response *in vitro* to syngeneic and autologous freshly-isolated solid tumors of mice. *Cancer Immunol Immunother* 18: 126–134.
- Knuth A, Wolfel T, Meyer zum Buschenfelde KH (1992) T cell responses to human malignant tumours. *Cancer Surv* 13: 39–52.
- Ibayashi Y, Uede T, Uede T and Kikuchi K (1985) Functional analysis of mononuclear cells infiltrating into tumors: differential cytotoxicity of mononuclear cells from tumors of immune and non-immune rats. *J Immunol* 134: 648–653.
- Jardetzky TS, Lane WS, Robinson RA, Madden DR, Wiley DC (1991) Identification of self peptides bound to purified HLA-B27. *Nature* 353: 326–329.
- Letessier EM, Heo DS, Okarma T, Johnson JT, Herberman RB (1991) Enrichment in tumor-reactive CD8+ T-lymphocytes by positive selection from the blood and lymphnodes of patients with head and neck cancer. *Cancer Res* 51: 3891–3899.
- Liu SQ, Shiba R, Kim BS, Saijo K, Ohno T (1994) Long-term serum/plasma-free culture of human cytotoxic T lymphocytes induced from peripheral blood. *Cancer Immunol Immunother* 39: 279–285.
- Liu SQ, Saijo K, Todoroki T, Ohno T (1995) Induction of human autologous cytotoxic T lymphocytes on formalin-fixed and paraffin-embedded tumour sections. *Nature Med* 1: 267–271 (Correction from 'autologous' to 'allogeneic' will appear in *Nature Med.* 2: April, 1996).
- Mazumder A, Grimm EA, Zhang HZ, Rosenberg SA (1982) Lysis of fresh human solid tumors by autologous lymphocytes activated *in vitro* with lectins. *Cancer Res* 42: 913–918.
- Mazumder A, Grimm EA, Zhang HZ, Rosenberg SA (1983) Characterization of the lysis of fresh human solid tumors by autologous lymphocytes activated *in vitro* with phytohemagglutinin. *J Immunol* 130: 958–964.
- Miyatake SI, Kikuchi H, Iwasaki K, Yamashita J, Li ZY, Namba Y, Hanaoka M (1988) Specific cytotoxic activity of T lymphocyte clones derived from a patient with gliosarcoma. *J Neurosurg* 69: 751–759.
- Mukherji B, Guha A, Chakraborty NG, Sivanandham M, Nashed AL, Sporn JR, Ergin M (1989) Clonal analysis of cytotoxic and regulatory T cell responses against human melanoma. *J Exp Med* 169: 1961–1976.
- Mukherji B, Chakraborty NG, Yamasaki S, Okino T, Yamase H, Sporn JR, Kurtzman SK, Ergin MT, Ozoles J, Meehan J, Mauri F (1995) Induction of antigen-specific cytotoxic T cells *in situ* in human melanoma by immunization with synthetic peptide-pulsed autologous antigen presenting cells. *Proc Natl Acad Sci USA* 92: 8078–8082.
- Nakashima M, Watanabe T, Koprowski H, Schuchter L, Steplewski Z (1994) *In vitro* expression of tumor specific, HLA-restricted human CD8+ cytolytic T lymphocytes. *Cell Immunol* 155: 53–61.
- Ossevoort MA, Feltkamp MCW, van Veen KJH, Melief CJM, Kast WM (1995) Dendritic cells as carriers for a cytotoxic T-lymphocyte epitope-based peptide vaccine in protection against a human papillomavirus type 16-induced tumor. *J Immunother* 18: 86–94.
- Plata F (1982) Specificity studies of cytolytic T lymphocytes directed against murine leukemia virus-induced tumors: analysis by monoclonal cytolytic T lymphocytes. *J Exp Med* 155: 1050–1062.
- Pollard K, Holgate CS (1987) Surface antigen preservation by PLP and formalin fixation and the effects of tissue processing. *J Immunol Methods* 96: 145–145.
- Roberts TE, Shipton U, Moore M (1987) Role of MHC class-I antigen and the CD3 complex in the lysis of autologous human tumors by T-cell clones. *Int J Cancer* 39: 436–441.
- Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, Seipp CA, Simpson C, and Reichert CM (1985) Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* 313: 1485–1492.
- Rosenberg SA, Spiess P, Lafreniere R (1986) A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 233: 1318–1323.
- Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan M, Robertson CN, Lee R, Rubin JT, Seipp CA, Simpson CG, White DE (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 316: 889–897.
- Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP and White DE (1988) Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *N Engl J Med* 319: 1676–1680.
- Ryser JE, Cerottini JC, Brunner KT (1978) Generation of cytolytic T lymphocytes *in vitro*. IX. Induction of secondary CTL responses in primary long-term MLC by supernatants from secondary MLC. *J Immunol* 120: 370–377.
- Saotome K, Morita H, Umeda M (1989) Cytotoxicity test with simplified crystalviolet staining method using microtitre plates and its application to injection drugs. *Toxicol in Vitro* 3: 317–321.
- Sato T, Sato N, Takahashi S, Koshiba H and Kikuchi K (1986) Specific cytotoxicity of a long-term cultured T-cell clone on human autologous mammary cancer cells. *Cancer Res* 46: 4384–4389.
- Slingluff CL, Cox AL, Stover JMJ, Moore MM, Hunt DF and Engelhard VH (1994) Cytotoxic T-lymphocyte response to autologous human squamous cell cancer of the lung: epitope reconstitution

- with peptides extracted from HLA-Aw68. *Cancer Res* 54: 2731–2737.
- Takahashi H, Nakada T and Puisieux I (1993a) Inhibition of human colon cancer growth by antibody-directed human LAK cells in SCID mice. *Science* 259: 1460–1463.
- Takahashi T, Ishikura H, Iwai K, Takahashi C, Kato H, Tanabe T, Yoshiki T (1993b) Cytokine regulation of cell-to-cell interactions in lymphokine-activated killer cell cytotoxicity in vitro. *Cancer Immunol Immunother* 36: 76–82.
- Tsuchida T, Parker KC, Turner RV, McFarland HF, Coligan JE, Bid-dison WE (1994) Autoreactive CD8⁺ T-cell responses to human myelin protein-derived peptides. *Proc Natl Acad Sci USA* 91: 10859–10863.
- Tsurushima H, Liu SQ, Tsuboi K, Yoshii Y, Nose T, Ohno T (1996) Induction of human autologous cytotoxic T lymphocytes against minced tissues of glioblastoma multiforme. *J Neurosurg* 84: 258–263.
- van der Bruggen P, Traversari C, Chomez P, Lurquin C, de Plaen E, van den Eynde B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254: 1643–1647.
- Visseren MJW, van Elsas A, van der Voort EIH, Rensing ME, Kast WM, Schrier PI, Melief CJM (1995) *J Immunol* 154: 3991–3998.
- Wallach D, Fellous M, Revel M (1982) Preferential effect of interferon-gamma on the synthesis of HLA antigens and their mRNAs in human cells. *Nature* 299: 833–836.
- Wang X-F, Robbins PF, Kawakami Y, Kang X-Q, Rosenberg SA (1995) Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. *J Exp Med* 181: 799–804.
- Wolfel T, Herr W, Coulie P, Schmitt U, Buschenfelde K-HM and Knuth A (1993) Lysis of human pancreatic adenocarcinoma cells by autologous HLA-class I-restricted cytolytic T-lymphocyte (CTL) clones. *Int J Cancer* 54: 636–644.
- Yamasaki T, Handa H, Yamashita J, Watanabe Y, Namba Y and Hanaoka M (1984) Specific adoptive immunotherapy with tumor-specific cytotoxic T-lymphocyte clone for murine malignant gliomas. *Cancer Res* 44: 1776–1783.

Address for Correspondence: Tadao Ohno, RIKEN Cell Bank, The Institute of Physical and Chemical Research (RIKEN), Koyadai 3–1–1, Tsukuba Science City, 305, Japan.