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Requirement of $CD4^+$ T cells and antigen-presenting cells for primary *in vitro* generation of $CD8^+$ cytotoxic T cells against L^d-binding self-peptide p2Ca

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SUMMARY

We investigated the cellular requirement for primary *in vitro* generation of cytotoxic T-lymphocytes (CTL) in BALB/c spleen cells against L^d-binding self-peptide p2Ca. Depletion of $CD4^+$ T-cells *in vitro* by pretreatment with anti-CD4 monoclonal antibody (mAb) and complement or *in vivo* by administration of anti-CD4 mAb abrogated generation of CTL. Depletion of adherent cells by passing spleen cells through a nylon wool (NW) column also abrogated generation of CTL. Addition of peritoneal exudate cells (PEC) to spleen cells passed through the NW column restored CTL generation. These findings indicate that both $CD4^+$ T-cells and antigen-presenting cells (APC) were necessary for CTL generation. Treatment of PEC with paraformaldehyde (PFA), but not mitomycin-C (MMC) abrogated their ability to restore CTL generation when mixed with spleen cells from the NW column, suggesting that an endocytic pathway could be involved in presentation of p2Ca on APC.

INTRODUCTION

$CD8^+$ cytotoxic T-cells (CTL) obtained from mice after infection with virus recognize a peptide fragment in association with a major histocompatibility complex (MHC) class I molecule on infected cells.[1,2] A synthetic short peptide could, thus, serve efficiently as a target antigen for CTL. On the other hand, generation of CTL responses by sensitization either *in vivo*[3] or *in vitro*[4] with a short peptide could not easily be achieved. However, it was demonstrated that with high cell densities of responding cells and high concentrations of peptides, CTL responses were generated with some peptides of either endogenous or exogenous origin.[4–6] These findings suggest that the general failure to generate CTL responses *in vitro* is due to difficulty in achieving a sufficient density of antigenic determinant with the peptide.[5]

CTL clone 2C was derived from BALB.B spleen cells stimulated with the P815 (DBA/2) tumour and BALB/c spleen cells.[7] 2C cells are specific for allogeneic L^d antigen and lyse only L^d-bearing target cells. Some fractions of acid extract of BALB/c thymocytes and spleen cells separated by reversed-phase high-performance liquid chromatography (HPLC)-induced target sensitivity of B2 cells that were produced by transfecting the L^d gene into low L^d-expressing SP2/0 BALB/c myeloma cells against 2C CTL. The amino acid sequence of the active fraction was determined by Edman degradation. p2Ca was thus defined as an octamer peptide.[8,9] In this study, we showed that $CD4^+$ T cells and antigen-presenting cells (APC) were required for generation of CTL in BALB/c spleen cells in an *in vitro* primary response against endogenous peptide p2Ca.

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Abbreviations: NW, nylon wool; PFA, paraformaldehyde.

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MATERIALS AND METHODS

Mice

BALB/c (H-2^d) and C57BL/6 (B6) (H-2^b) mice were purchased from Japan SLC (Shizuoka, Japan). These mice were bred in our laboratory animal centre.

Tumours

P815 is a methylcholanthrene-induced mastocytoma in a DBA/2 mouse.[10] RL♂4 and RL♀8 are radiation-induced leukemias in BALB/c mice.[11] RVA, RVC and RVD are leukemias induced by injection of radiation-leukemia virus (RadLV) into neonatal BALB/c mice.[12] EL4 is a chemically induced leukemia of B6 origin.[13] These tumours were maintained in tissue culture.

Monoclonal antibody

Anti-L3T4 (CD4) monoclonal antibody (mAb), a rat antibody of the IgG2b immunoglobulin class, produced by hybridoma GK1.5,[14] was provided by Dr F. Fitch (University of Chicago,

Chicago, IL). Anti-Lyt-2.2 (CD8) mAb, a mouse antibody of the IgG2a class, produced by hybridoma 19/178[15] was provided by Dr U. Hämmerling (Memorial Sloan–Kettering Cancer Center, New York, NY).[15] Anti-Lyt-2.1 mAb, a mouse antibody of the IgG2b class has been described previously.[15] Anti-H-2K^d and anti-H-2D^d mAbs are mouse IgG2a antibodies produced by hybridomas HB159 and HB102, respectively.[16] Both mAbs were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Anti-H-2L^d mAb is a mouse IgG2a antibody produced by a hybridoma 30-5-7.[16] Anti-IA^d mAb is a mouse IgG2b antibody produced by hybridoma MK-D6.[17] Anti-IE^{k,d} mAb is a mouse IgG antibody produced by hybridoma ISCR3.[18] These mAbs are provided by Dr N. Shinohara (Mitsubishi Kasei Institute of Life Science, Machida, Japan). Anti-mouse IgD mAb, a rat antibody of the IgG2b immunoglobulin class, produced by hybridoma R12.256, was provided by Dr N. Tada (Tokai University School of Medicine, Isehara, Japan).

Peptides

The amino acid sequence of the p2Ca peptide (LSPFPFDL) is derived from mouse 2-oxoglutarate dehydrogenase.[9] The amino acid sequence of the tum$^-$ peptide corresponds to residues 12–24 (ISTQNHRALDLVA) of the mutant protein P91A$^-$ (exon 4) from the tum$^-$ P815 variant.[19] The amino acid sequence of the murine cytomegalovirus (MCMV) peptide corresponds to residues 168–176 (YPHFMPTNL) of the MCMV immediate early protein pp89.[20] These peptides were synthesized by the standard solid-phase method using Fmoc chemistry in an Applied Biosystems 430A peptide synthesizer (Foster City, CA). Cleavage of the peptide from the resin and removal of the side-chain protecting groups were carried out using trifluoroacetic acid (TFA). The peptides were purified by reversed-phase HPLC on a Vydac C4 column in 0·05% trifluoroacetic acid (TFA) with an acetonitrile gradient.

Paraformaldehyde fixation

Peritoneal exudate cells (PEC) were fixed in 0·1% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for 30 min on ice, and washed three times before use.

Generation of concanavalin A blasts

A sample of 2×10^7 spleen cells was cultured with concanavalin A (Con A) at a concentration of 5 μg/ml for 3 days to generate Con A blasts.

Generation of primary CTL responses in vitro *on stimulation with p2Ca peptide and allogeneic stimulator cells*

BALB/c spleen cells were cultured with various concentrations of the p2Ca peptide or mitomycin C (MMC)-treated B6 spleen cells in 24-well tissue culture plates in 2 ml of RPMI-1640 (Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml of streptomycin and 5×10^{-5} M 2-mercaptoethanol (2ME) for 5 days at 37° under 5% CO_2 in air. Cells were treated with MMC at a concentration of 50 μg/ml at 37° for 30 min.

Cell-mediated cytotoxicity assay

Cell-mediated cytotoxicity was tested by conventional 4 hr chromium-release assay as described.[11] Briefly, P815 cells pulsed with or without peptide, tumour cells and Con A blasts were labelled with 1·85 MBq of Na_2CrO_4 (New England Nuclear, Boston, MA) for 1 hr at 37° under 5% CO_2 in air. Then they were washed and used as target cells. For pulsing target cells with peptide, P815 cells (5×10^5) were incubated with various concentrations of peptide for 16 hr at 37° under 5% CO_2 in air. The mean radioactivity of duplicate samples was calculated and the percentage of specific lysis was determined by the following equation:

$$\text{percent } ^{51}\text{Cr release} = 100 \times \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}},$$

spontaneous release being measured as counts from target cells incubated in medium alone, and maximum release as counts from target cells exposed to 1% Triton-X.

Elimination of CD4$^+$ T cells in vitro

A sample of 5×10^7 spleen cells was incubated with anti-L3T4 (CD4) mAb (diluted 1 : 20 with MEM) in a volume of 200 μl for 30 min on ice. The cells were then washed once and resuspended in 750 μl of preselected rabbit serum (diluted 1 : 12 with MEM) as a source of complement (C). After incubation for 30 min at 37°, the cells were washed three times before use.

Depletion of CD4$^+$ T cells in vivo

BALB/c mice were treated intravenously with anti-L3T4 (CD4) mAb (diluted 1 : 4 with MEM) in a volume of 200 μl on days −7 and −3 before removal of the spleens for culture.

RESULTS

Generation of cytotoxicity in BALB/c spleen cells by primary *in vitro* stimulation with p2Ca peptide

BALB/c spleen cells ($5–7·5 \times 10^6$) were cultured with p2Ca peptide for 5 days and cytotoxicity was determined on ^{51}Cr-labelled P815 (DBA/2) target cells pulsed with p2Ca by

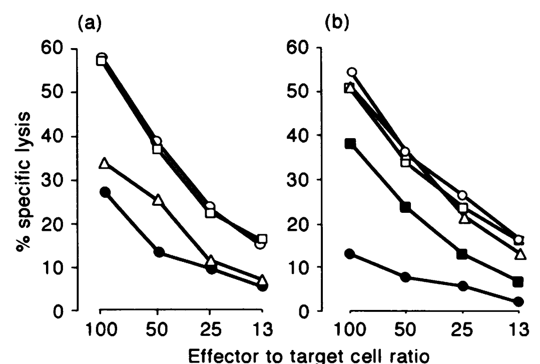

Figure 1. Generation of cytotoxicity in BALB/c spleen cells by primary *in vitro* stimulation with p2Ca peptide. For (a), samples of $7·5 \times 10^6$ responding BALB/c spleen cells were cultured for 5 days in the presence of 10^{-4} (●), 10^{-5} (○), 10^{-6} (□) and 10^{-7} M (△) p2Ca peptide. Effector cells were assayed on P815 target cells that had been incubated for 16 hr with 10^{-4} M p2Ca peptide. For (b), P815 target cells were incubated for 16 hr with 10^{-2} (○), 10^{-3} (□), 10^{-4} (△) and 10^{-5} M (■) p2Ca peptide. (●), untreated P815 target cells.

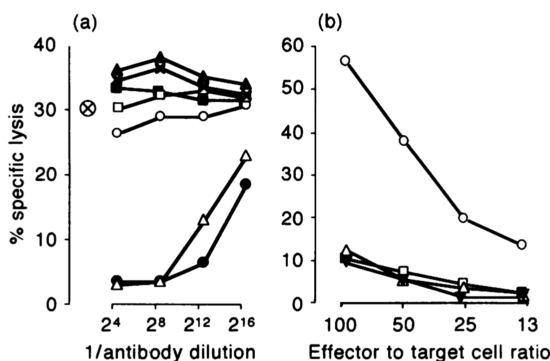

Figure 2. Antibody blocking (a) and direct cytotoxicity (b) of BALB/c anti-p2Ca CTL. For (a), antibody was present throughout the 4-hr ^{51}Cr-release assay. The effector cell to target cell ratio in these tests was 75. The antisera added were anti-Lyt-2.2 (CD8) mAb (●), anti-L3T4 (CD4) mAb (▲), anti-K^d mAb (○), anti-I-A^d mAb (×), anti-I-E^d mAb (□), anti-D^d mAb (■) or anti-L^d mAb (△). (⊗), none. P815 cells incubated for 16 hr with 10^{-4} M p2Ca peptide were used for targets. For (b), the target cells were P815 cells pulsed with MCMV (△), tum$^-$ (□) and p2Ca (○) peptide. (▼), P815 cells alone.

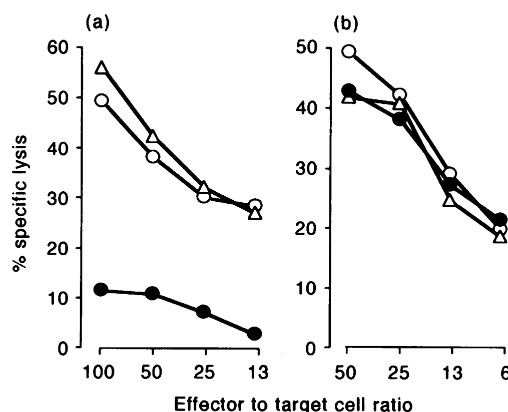

Figure 4. Effect of *in vivo* depletion of CD4$^+$ cells on generation of p2Ca CTL. Spleen cells from BALB/c mice that had been treated *in vivo* with anti-L3T4 (CD4) mAb (●), R12.256 mAb (control) (△) or MEM (○) 7 and 3 days previously, were cultured in the presence of p2Ca (a) or with MMC-treated B6 spleen cells (b) as described in the legend of Fig. 3. The target cells were P815 pulsed with p2Ca (a) and EL4 (b).

overnight incubation. As shown in Fig. 1, the optimal concentration of p2Ca was 10^{-5}–10^{-6} M for *in vitro* sensitization and 10^{-2}–10^{-4} M for pulsing P815 target cells. From these results, a concentration of 10^{-5} M p2Ca was used for *in vitro* sensitization of BALB/c spleen cells and of 10^{-4} M for pulsing ^{51}Cr labelled P815 target cells in subsequent analyses.

Antibody blocking of cytotoxicity

Blocking of cytotoxicity by addition of mAbs to assay cultures was investigated in the absence of exogenously added complement. As shown in Fig. 2a, cytotoxicity was blocked by anti-Lyt-2.2 (CD8) mAb and anti-L^d mAb, but not by anti-L3T4 (CD4) mAb, anti-K^d mAb, anti-D^d mAb, anti-IA^d mAb or anti-IE^d mAb.

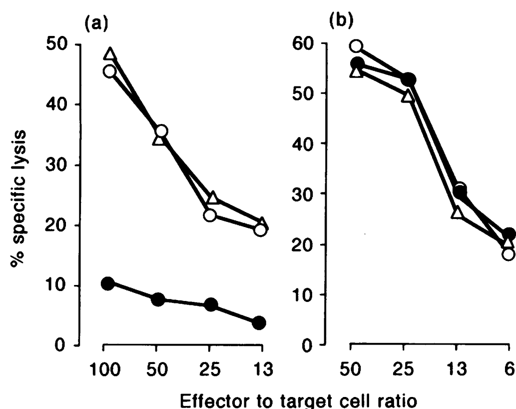

Figure 3. Effect of *in vitro* elimination of CD4$^+$ cells on generation of p2Ca CTL. BALB/c spleen cells were treated with anti-L3T4 (CD4) mAb (●) or R12.256 mAb (control) (△) and complement (C). (○), C alone. For (a), remaining spleen cells ($7{\cdot}5 \times 10^6$) were cultured for 5 days in the presence of 10^{-5} M p2Ca, and cytotoxicity was measured by 4 hr ^{51}Cr-release assay. The target cells were P815 pulsed with p2Ca. For (b), remaining spleen cells (3×10^6) were cultured with 2×10^6 MMC-treated B6 spleen cells for 5 days and cytotoxicity was assayed similarly. EL4 cells were used as targets.

Analysis of specificity of BALB/c anti-p2Ca CTL

Direct cytotoxicity was examined with BALB/c anti-p2Ca CTL against a panel of targets. P815 pulsed with peptides that bind to L^d, BALB/c leukemias RL♂4, RL♀8, RVA, RVC and RVD, and BALB/c spleen Con A blasts. No cytotoxicity was observed on these cells (Fig. 2b).

Requirement of CD4$^+$ T cells in BALB/c spleen cells for primary *in vitro* generation of CTL against p2Ca

The requirement of CD4$^+$ T cells in BALB/c spleen cells for primary *in vitro* generation of CTL against p2Ca was investigated. As shown in Fig. 3, elimination of CD4$^+$ T cells from spleen cells before culture by treatment with anti-L3T4 (CD4) mAb and complement-diminished CTL generation on stimulation with p2Ca, but not on stimulation with allogeneic B6 cells. We further examined the requirement of CD4$^+$ T cells for generation of CTL by *in vivo* depletion experiments. Treatment of BALB/c mice with anti-L3T4 (CD4) mAb abrogated the *in vitro* generation of CTL in spleen cells on stimulation with p2Ca, but not on stimulation with allogeneic B6 cells (Fig. 4).

Requirement of APC in BALB/c spleen cells for primary *in vitro* generation of CTC against p2Ca

The requirement of APC in BALB/c spleen cells for primary *in vitro* generation of CTL against p2Ca was investigated. As shown in Fig. 5, passage of spleen cells through a nylon wool (NW) column before culture abrogated the generation of CTL on stimulation with p2Ca. Addition of PEC to spleen cells from an NW-column restored CTL generation. Treatment of PEC with PFA, but not MMC, abrogated the effect of PEC in restoring generation of CTL by spleen cells from the NW column.

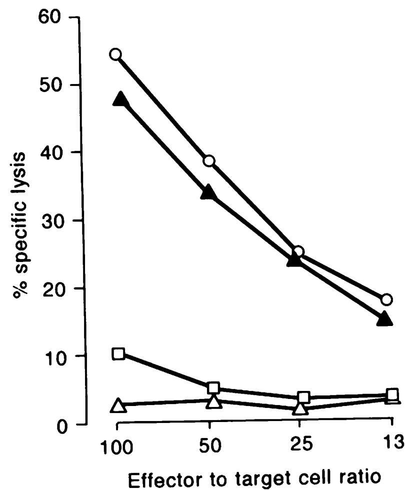

Figure 5. Effect of depletion of adherent cells from BALB/c spleen cells in generation of p2Ca CTL. Untreated BALB/c spleen cells (○), BALB/c spleen cells passed through an NW column (△), BALB/c spleen cells passed through an NW column admixed with PEC that had been treated with MMC (▲) or PFA (□) were cultured in the presence of 10^{-5} M p2Ca for 5 days. $CD3^{+}$ TCR-$\alpha\beta$ T cells in spleen cells were enriched to 78·5–90·5% by passage through the NW column.

Table 1. Effects of mAbs on *in vitro* sensitization with p2Ca of cultured BALB/c spleen cells

mAb	% ^{51}Cr release 0·5%*	0·25%*
none	71	–
anti-I-A^{d}	64	70
anti-I-E^{d}	60	69
anti-I-A^{d} + anti-I-E^{d}	37	42
anti-Lyt-2.1 (control)	59	68

*Concentration of mAb added to cultures. BALB/c spleen cells (5×10^{6}) were sensitized *in vitro* with p2Ca (1×10^{-5} M) in 24-well plastic plates in the presence of mAbs. Effector to target cell ratio was 100.

Effects of addition of anti-I-A^{d} and/or anti-I-E^{d} mAb to cultures for *in vitro* sensitization

Anti-I-A^{d} mAb and/or anti-I-E^{d} mAb was added to cultures for *in vitro* sensitization with p2Ca and effects on CTL generation were examined. As shown in Table 1, generation of cytotoxicity was inhibited by the addition of both anti-I-A^{d} and anti-I-E^{d} mAb, but not of either alone.

DISCUSSION

In this study, we investigated the cellular requirements for primary *in vitro* generation of CTL in BALB/c spleen cells against L^{d}-binding self-peptide p2Ca and demonstrated that both $CD4^{+}$ T cells and APC were necessary. Cytotoxic effector cells generated in BALB/c spleen cells on stimulation with p2Ca efficiently lysed P815 targets pulsed with p2Ca, but lysed unpulsed P815 cells only slightly, consistent with previous findings by Tjoa & Kranz.[21] Elimination and antibody blocking of cytotoxicity indicated that CTL generated against p2Ca were mainly $CD8^{+}$ T cells and their recognition was restricted to L^{d}. Depletion of $CD4^{+}$ T cells *in vitro* by pretreatment with anti-CD4 mAb and complement or *in vivo* by administration of anti-CD4 mAb abrogated the generation of CTL. Depletion of adherent cells by passing spleen cells through an NW column also abrogated generation of CTL.

It is generally difficult to generate CTL by primary *in vitro* stimulation with MHC class I binding peptides.[22] Recently, however, several peptides[5,21,23] have been shown to be capable of generating CTL using rather high concentration of the peptides for priming. The MHC class I binding peptides added to the culture bind to the cognate class I molecules directly with empty molecules or by peptide exchange[1,2,24] on APC, and the complex of the peptide and the MHC class I molecule is recognized by pCTL through T-cell receptor (TCR). The requirement of $CD4^{+}$ T cells for generation of $CD8^{+}$ CTL against L^{d}-binding p2Ca peptide appears contradictory. The cellular mechanisms involved are unknown, but the following possibilities may be considered. In experiments, the concentrations of the peptides required for generation of *in vitro* CTL responses were rather high (10^{-5}–10^{-6} M). The peptides bound to the cognate MHC class I molecules at high concentration somehow stimulated $CD4^{+}$ T cells to help in generating CTL. There are several reports showing a requirement of $CD4^{+}$ T cells for generation of a $CD8^{+}$ CTL response in *in vivo* priming with peptides,[25–27] although other reports showed that $CD4^{+}$ cell and MHC class II^{+} cell-independent generations of primary CTL responses specific for peptide were induced by peptide-loaded RMA-S cells.[28] Alternatively, the peptides could bind to MHC class II as well as class I molecules when present in cultures at high concentrations and serve as helper and target epitopes, respectively. The finding that peptides isolated from class II molecules were heterogeneous[29] is consistent with this notion, although there are some specific motifs for particular class II molecules.[30] Binding of class I peptides to class II molecules could occur directly or by an endosomal pathway. However, treatment of APC with PFA, but not MMC, abrogated generation of CTL. Furthermore, anti-I-A^{d} mAb plus anti-I-E^{d} mAb present in the culture of spleen cells for *in vitro* sensitization with p2Ca diminished CTL generation. This finding suggests that both I-A and -E molecules are involved in presentation of p2Ca on APC through the endocytic pathway. Thus, it is likely that the peptides bound to MHC class II antigen on APC stimulated $CD4^{+}$ T cells and helped $CD8^{+}$ CTL to respond to the peptide bound to MHC class I molecules.

APC such as macrophages and dendritic cells have been shown to have potent stimulatory effects on T cells.[31] The present finding that NW-adherent cells were necessary for CTL generation suggests that presentation of the peptide on MHC class I (and class II, see above) molecules on APC and co-stimulation are necessary for primary *in vitro* generation of $CD8^{+}$ CTL in stimulation with L^{d}-binding p2Ca peptide.

p2Ca is a naturally occurring peptide in BALB/c thymocytes and spleen cells. The finding that CTL were generated

in BALB/c spleen cells on stimulation with p2Ca suggests that T cells reactive with p2Ca are present and not clonally eliminated in BALB/c mice. pCTL could not be stimulated to differentiate into functional CTL because of the physiological level of the peptide expressed in the mouse.[6] The findings that CTL generated against p2Ca did not lyse BALB/c leukemias and spleen Con A blasts are consistent with this notion.

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REFERENCES

1. Townsend A. & Bodmer H. (1989) Antigen recognition by class I-restricted T lymphocytes. *Ann Rev Immunol* **7**, 601.
2. McMichael A.J., Gotch F.M. & Rothbard J. (1986) HLA B37 determines an influenza A virus nucleoprotein epitope recognized by cytotoxic T lymphocytes. *J Exp Med* **164**, 1397.
3. Carbone F.R. & Bevan M.J. (1989) Induction of ovalbumin-specific cytotoxic T cells by *in vivo* peptide immunization. *J Exp Med* **169**, 603.
4. Carbone F.R., Moore M.W., Sheil J.M. & Bevan M.J. (1988) Induction of cytotoxic T lymphocytes by primary *in vitro* stimulation with peptides. *J Exp Med* **167**, 1767.
5. Alexander M.A., Damico C.A., Wieties K.M., Hansen T.H. & Connolly J.M. (1991) Correlation between CD8 dependency and determinant density using peptide-induced, L^d-restricted cytotoxic T lymphocytes. *J Exp Med* **173**, 849.
6. Schild H., Rötzschke O., Kalbacher H. & Rammensee H.G. (1990) Limit of T cell tolerance to self proteins by peptide presentation. *Science* **247**, 1587.
7. Kranz D.M., Sherman D.H., Sitkovsky M.V., Pasternack M.S. & Eisen H.N. (1984) Immunoprecipitation of cell surface structures of cloned cytotoxic T lymphocytes by clone-specific antisera. *Proc Natl Acad Sci USA* **81**, 573.
8. Udaka K., Tsomides T.J. & Eisen H.N. (1992) A naturally occurring peptide recognized by alloreactive $CD8^+$ cytotoxic T lymphocytes in association with a class I MHC protein. *Cell* **69**, 989.
9. Udaka K., Tsomides T.J., Walden P., Fukusen N. & Eisen H.N. (1993) A ubiquitous protein is the source of naturally occurring peptides that are recognized by a $CD8^+$ T-cell clone. *Proc Natl Acad Sci USA* **90**, 11272.
10. Uyttenhove C., Van Snick J. & Boon T. (1980) Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. I. Rejection by syngeneic mice. *J Exp Med* **152**, 1175.
11. Nakayama E., Shiku H., Takahashi T., Oettgen H.F. & Old L.J. (1979) Definition of a unique cell surface antigen of mouse leukemia RL♂1 by cell-mediated cytotoxicity. *Proc Natl Acad Sci USA* **76**, 3486.
12. Stockert E., DeLeo A.B., O'Donell P.V., Obata Y. & Old L.J. (1979) G(AKSL2): a new cell surface antigen of the mouse related to the dualtropic mink cell focus-inducing class of murine leukemia virus detected by naturally occurring antibody. *J Exp Med* **149**, 200.
13. Gorer P.A. (1950) Studies in antibody response of mice to tumour inoculation. *Br J Cancer* **4**, 372.
14. Dialynas D.P., Quan Z.S., Wall K.A. *et al.* (1983) Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J Immunol* **131**, 2445.
15. Nakayama E. (1982) Blocking of effector cell cytotoxicity and T-cell proliferation by Lyt antisera. *Immunol Rev* **68**, 117.
16. Ozato K., Hansen T.H. & Sachs D.H. (1980) Monoclonal antibodies to mouse MHC antigens. II. Antibodies to the $H\text{-}2L^d$ antigen, the products of a third polymorphic locus of the mouse major histocompatibility complex. *J Immunol* **125**, 2473.
17. Kappler J.W., Skidmore B., White, J. & Marrack P. (1981) Antigen-inducible, *H-2*-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. *J Exp Med* **153**, 1198.
18. Watanabe M., Suzuki T., Taniguchi M. & Shinohara N. (1983) Monoclonal anti-Ia murine alloantibodies crossreactive with the Ia-homologues of other mammalian species including humans. *Transplantation* **36**, 712.
19. Lurquin C., Van Pel A., Mariamé B. *et al.* (1989) Structure of the gene of Tum^- transplantation antigen P91A: the mutated exon encodes a peptide recognized with L^d by cytolytic T cells. *Cell* **58**, 293.
20. Reddehase M.J., Rothbard J.B. & Koszinowski U.H. (1989) A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes. *Nature* **337**, 651.
21. Tjoa B.A. & Kranz D.M. (1994) Generation of cytotoxic T-lymphocytes to a self-peptide/class I complex: A model for peptide-mediated tumor rejection. *Cancer Res* **54**, 204.
22. Kos F.J. & Müllbacher A. (1992) Induction of primary anti-viral cytotoxic T cells by *in vitro* stimulation with short synthetic peptide and interleukin-7. *Eur J Immunol* **22**, 3183.
23. Celis E., Tsai V., Crimi C. *et al.* (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.
24. Maryanski J.L., Pala P., Corradin G., Jordan B.R. & Cerottini J.C. (1986) H-2-restricted cytotoxic T cells specific for HLA can recognize a synthetic HLA peptide. *Nature* **324**, 578.
25. Fayolle C., Deriaud E. & Leclerc C. (1991) *In vivo* induction of cytotoxic T cell response by a free synthetic peptide requires $CD4^+$ T cell help. *J Immunol* **147**, 4069.
26. Lasarte J.J., Sarobe P., Gullón A., Prieto J. & Borrás-Cuesta F. (1992) Induction of cytotoxic T lymphocytes in mice against the principal neutralizing domain of HIV-1 by immunization with an engineered T-cytotoxic-T-helper synthetic peptide construct. *Cellular Immunol* **141**, 211.
27. Tindle R.W., Fernando G.J.P., Sterling J.C. & Frazer I.H. (1991) A 'public' T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes. *Proc Natl Acad Sci USA* **88**, f587.
28. De Bruijn M.L.H., Schumacher T.N.M., Nieland J.D., Ploegh H.L., Kast W.M. & Melief C.J.M. (1991) Peptide loading of empty major histocompatibility complex molecules on RMA-S cells allows the induction of primary cytotoxic T lymphocyte responses. *Eur J Immunol* **21**, 2963.
29. Rammensee H.-G., Falk K. & Rötzschke O. (1993) MHC molecules as peptide receptors. *Curr Opin Immunol* **5**, 35.
30. Rudensky A.Y., Preston-Hurlburt P., Al-Ramadi B.K., Rothbard J. & Janeway C.A. Jr (1992) Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs. *Nature* **359**, 429.
31. Rock K.L., Rothstein L., Gamble S. & Fleischacker C. (1993) Characterization of antigen-presenting cells that present exogenous antigens in association with class I MHC molecules. *J Immunol* **150**, 438.