Lysis of Ras Oncogene-transformed Cells by Specific Cytotoxic T Lymphocytes Elicited by Primary In Vitro Immunization with Mutated Ras Peptide

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Summary

Ras protooncogenes are activated by characteristic point mutations in a wide variety of malignancies. The expressed p21w proteins are oncogenic by virtue of single substituted amino acids, usually at position 12 or 61 of the 189-residue p21w protein. In the current study, the ability of class I major histocompatibility complex (MHC)-restricted T cells to recognize the altered segment of a transforming p21w protein and to lyse cells transformed by the corresponding ras oncogene was examined. Synthetic ras peptides encompassing the common activating substitution of leucine for glutamine at position 61 were constructed with an amino acid motif appropriate for binding to the H-2Kb murine class I MHC molecule. Cytotoxic T lymphocytes (CTL) specific for bound ras leucine 61 peptide were elicited by in vitro immunization of normal lymphocytes with synthetic peptides. The ras peptide-induced CTL specifically lysed syngeneic fibroblasts transformed by an activated ras gene encoding oncogenic p21w protein containing the same single amino acid substitution. Thus, in some circumstances, mutated p21w protein can serve as a tumor-specific antigen.

Tumor-specific CTL can eradicate antigenic tumors in experimental models (1). However, the use of specific CTL for cancer therapy has been limited by a paucity of defined tumor-specific antigens. In general, CTL recognize peptide fragments derived from endogenous cellular proteins, processed internally, and presented at the cell surface bound to class I MHC molecules (2–4). The appropriateness of any particular protein to serve as a target for CTL depends upon whether the protein has antigenic segments with the proper molecular configuration or amino acid “motif” to bind to class I MHC molecules; whether the resultant peptide–MHC molecular complex is presented at the cell surface in a concentration high enough to stimulate the antigen-specific TCR; and whether the peptide–MHC molecular complex is within the host T cell repertoire. Many tumor cells have been shown to harbor mutated protooncogenes with transforming activity. Theoretically, the abnormal proteins encoded by mutated protooncogene may contain peptide sequences as the result of activating mutations which are uniquely expressed in tumor cells and which potentially satisfy the necessary conditions for specific CTL recognition.

Mutated ras protooncogenes are particularly prevalent among human tumors (5). Activating point mutations of ras predominantly occur within codons 12 and 61 and result in the expression of a limited number of oncogenic p21w proteins bearing single amino acid substitutions (5). The mutations disrupt the normal signaling function of p21w and contribute to malignant transformation (6, 7). Oncogenic p21w protein bearing a single amino acid substitution represents a tumor-specific protein present only in transformed cells. The current study elicited CTL specific for the mutated segment of a transforming p21w protein by primary in vitro immunization with corresponding synthetic ras peptides and examined the ability of the mutated p21w protein expressed by transformed cells to serve as a target for specific CTL lysis, i.e., to serve as a tumor-specific antigen.

Materials and Methods

Mice. Female C57Bl/6 (B6)1 mice, 6–12-wk-old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in the animal care facilities of the University of Washington.

1 Abbreviations used in this paper: BLKSVHD, BLKSVHD.213; B6, C57Bl/6; CM, culture medium; Dp, H-2Dp; Kp, H-2Kp; T-OVA, trypsinized OVA.
Tumor Cell Lines. BLKSVHD.213 (BLKSVHD), a SV40-transformed fibroblast line of B6 origin (8), was obtained from the American Type Culture Collection (Rockville, MD). FBL-3, a Friend virus–induced leukemia, and EL4, a chemically-induced thymoma, both of B6 origin, were subcloned and maintained in our laboratory (9). L cells transfected with H-2K (K) were a gift from Dr. S. Nathenson (Albert Einstein School of Medicine, New York) (10). The TAP-2–deficient cell line, RMA-S (derived from RBL-5 after mutagenization and selection with anti-H-2 Streisand plus complement for loss of MHC class I expression), was a gift of Dr. C. Ohlen (Karolinska Institute, Stockholm, Sweden) (11). All cell lines were grown in culture medium (CM) consisting of RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 2.5 × 10⁻⁵ M 2-ME, 200 U/ml penicillin, 200 U/ml streptomycin, 10 mM l-glutamine, and 10% FCS. Cells were kept in 25-ml flasks at 37°C, in a humidified atmosphere with 5% CO₂.

Peptides. Synthetic ras peptides were synthesized on a synthesizer (model 430A; Applied Biosystems, Foster City, CA) using BOC/benzyl based chemistry. Individual peptides were cleaved from the resin by reaction with hydrogen fluoride/anisole and purified by reversed phase chromatography. Characterization of the purified peptides was by amino acid analysis, analytical HPLC, and mass spectrometric determination of molecular weight. A mixture of 36 overlapping ras peptides was synthesized in a single multiplex procedure. The amino acid sequence of p21res was from residue 53 through 69 is LLIDILGTAAGLEEYSAMR. The peptide mixture consisted of p53-61, -63, -64, -65, -66, -67, -68, -69; p54-61, -62, -63, -64, -65, -66, -67, -68, -69; p55-61, -62, -63, -64, -65, -66, -67, -68, -69; p56-61, -62, -63, -64, -65, -66, -67, -68, -69. To construct the peptide mixture arginine (corresponding to residue 68 of p21res) was coupled to aspartyl (residue 69) resin, then arginyl resin was introduced into the reaction vessel and methionine (residue 67) was coupled to the mixture of the two resins. This strategy of alternate coupling of an amino acid followed by the addition of the same amino acid resin to the mixture was continued through residues 61. Residues G60, A59, T58, and D57 were coupled conventionally. Then residues L55, I58, D54, and L53 were successively coupled with resin being removed after each cycle. These four resulting resins with NH₂-terminal L56, I55, D54, and L53 were combined, cleaved with hydrogen fluoride, and the peptides were dissolved in aqueous acetic acid and chromatographed on Sephadex G-10. Synthetic OVA peptide p257-264 was a gift from Dr. S. Jameson (University of Washington, Seattle, WA). Peptide fragments of trypsinized OVA (T-OVA) were generated by trypsin digestion according to the method of Moore et al. (12).

Plasmids and Cell Transfection. The plasmid, pRSVL61, containing a synthetic ras gene which encodes p21res with the single amino acid substitution of leucine for glutamine at residue 61 was kindly provided by Dr. S. Nishimura (National Cancer Center Institute, Tokyo, Japan) (13). The plasmid utilizes the LTR of Rous sarcoma virus to enable expression of the synthetic ras gene in eucaryotic cells. The Homer 6 plasmid containing the aphasis enzyme phosphotransferase was obtained from Dr. O. Finn (Pittsburgh Cancer Institute, Pittsburgh, PA) (14). Plasmids were expanded in Escherichia coli strain HB101 and purified by CsCl centrifugation. BLKSVHD fibroblasts were cotransfected with pRSVL61 and Homer 6 using the calcium phosphate precipitation method of Sambrook et al. (15). Transformed cells were cloned and expanded in selection medium containing G418 at 1 mg/ml. A total of 5 × 10⁷ spleen cells (vol. 10 ml) supplemented with sensitizing peptide (25 μg/ml) were incubated in up to 25 ml flasks for 7 d. The cultured cells were washed and tested in 4-h ⁵¹Cr-release assays for the ability to lyse EL4 cells that had been preincubated for 2 h with designated targeting peptide (25 μg/ml).

Generation of T Cell Lines and Clones. T cell lines and clones were generated from in vitro–sensitized B6 spleen cells by repetitive stimulation of the primed spleen cells with synthetic ras peptides followed by rIL-2 as previously described (17). Primed spleen cells were transferred to 24-well plates at 4 × 10⁶ cells per well with irradiated syngeneic spleen cells (3,000 rad) at 2 × 10⁶ cells per well and the sensitizing peptide (25 μg/ml). The lymphocytes were cultured for 5 d then split at 1:2 into fresh CM. On day 10, lymphocytes were restimulated with Ag. 2 d later cultures were split 1:2 and supplemented with human rIL-2 (5 U/ml), generously provided by Hoffmann-La Roche, Inc. (Nutley, NJ). Thereafter, T cells were maintained by periodic restimulation with Ag (5 μg/ml) and irradiated syngeneic spleen cells, followed 2 d later by rIL-2 (5 U/ml). Cells were cloned by limiting dilution as previously described (17).

Cytotoxicity Assays. The cytotoxic activity of elicited T cells was measured by 4-h ⁵¹Cr-release assays. Assays were performed as previously described (9). Where indicated, target cells were preincubated with the designated targeting or blocking peptides before use in ⁵¹Cr-release assays.

Results and Discussion

Peptide binding with any particular MHC molecule is predicated on the component amino acids of the peptide being correctly aligned to the unique binding pockets of the MHC cleft (18). Key “anchoring” residues of antigenic peptides have been elucidated for a limited number of MHC molecules, including murine class I MHC molecules of the H-2b haplotype (19). The region of p21res protein encompassing the residue 61 activation site contains a putative K b-binding motif (19), consisting of Y64 and M67. One of the most common mutations of residue 61 consists of the substitution of leucine for glutamine. To determine whether, in fact, fragments of aberrant p21res, which contain an altered residue 61, can bind to class I MHC molecules—the sine qua non of CTL generation—synthetic peptides corresponding to the altered segment of p21res were assayed for binding and stabilization of K b class I MHC molecules on the mutated cell line, RMA-S (H-2b) (11).

Surface class I MHC molecules normally exist as trimolecular structures composed of class I H chain, bound peptide and β₂-microglobulin (20, 21). Both peptide and β₂-
microglobulin are necessary for stable expression of class I MHC molecules at the cell surface. RMA-S cells express low levels of surface class I MHC molecules, attributable to the impaired transport of endogenously processed peptides for association with class I H chains (22, 23). However, provision of exogenous peptides that can bind to the unoccupied antigen binding groove, stabilize, and enhance the expression of cell surface class I MHC molecules (24). Accordingly, RMA-S cells were incubated with a variety of synthetic peptides corresponding to the amino acid sequence of p21\(^{L61}\). Multiple experiments to elicit CTL with individual Ras L61 peptide ranging from 8 to 12 mer in size proved unsuccessful.

The specificity of the ras L61 peptide-induced CTL was further examined with individual ras L61 target peptides. A CTL line was established from in vitro-sensitized lymphocytes that expressed the CD4\(^+\)CD8\(^+\) cell surface phenotype and that lysed EL4 tumor cells in the presence of selected ras L61 peptides (Table 2). Homologous peptides corresponding to normal p21\(^{wt}\) protein, were unable to target lysis by the CTL line (Table 2). Moreover, irrelevant peptides with high affinity to K\(^b\) did not sensitize targets for lysis by the CTL line (data not shown). Thus, the ras L61 peptide-induced CTL specifically recognized only L61-substituted ras peptides.

The generation of peptide-specific CTL response requires both peptide binding to cell I MHC molecules and the presence of T cells with antigen-specific TCRs capable of recognizing that particular peptide–MHC complex. “Holes” in the T cell repertoire may exist for particular peptides bound in particular configurations. To circumvent the possibility that the ras L61 peptides used in initial experiments were not of the appropriate size or configuration for the induction of CTL, a panel of 36 overlapping ras L61 peptides was synthesized and used in combination to immunize lymphocytes in vitro. The peptides ranged from 6 to 17 residues in length and were systematically staggered from the NH\(_2\) and COOH termini (see Materials and Methods). The presumption was that multiple CTL specific for several different peptide/K\(^b\) configurations would be elicited. Lymphocytes were cultured in vitro with the ras L61 peptide mixture and assayed for cytotoxicity. Ras L61 peptide–specific CTL were induced as evidenced by augmented lysis of syngeneic tumor cells (EL4) preincubated with the ras L61 peptide mixture compared to tumor cells alone (Table 1). The ras L61 peptide–specific CTL mediated enhanced lysis of tumor cells incubated with the sensitizing ras L61 peptide mixture but not with irrelevant peptide fragments of T-OVA. Conversely, CTL elicited by in vitro immunization to T-OVA peptides mediated enhanced lysis of tumor cells incubated with T-OVA peptides but not with the ras L61 peptide mixture (Table 1).

The specificity of the ras L61 peptide-induced CTL was strongly lysed targets pulsed with individual ras L61 peptides that stabilized K\(^b\) expression, but not with ras L61 peptides that did not stabilize K\(^b\) (Table 2). However, the degree of cytotoxicity directed against individual K\(^b\)-binding ras L61 peptide was not directly proportionate to the ability of the peptide to stabilize K\(^b\) on RMA-S. Smaller ras L61 peptides (7 and 8 mer), which were less effective than larger peptides in stabilizing K\(^b\) (Fig. 1), to be possible to prime CTL in vivo by immunization with peptides that exhibit appropriate class I MHC binding motifs (28–30), thereby circumventing the need to immunize with viable cells synthesizing the nominated antigen. In a few instances, it has been possible to elicit CTL by priming with similar peptides in vitro, although CTL generated by peptide priming often fail to lyse cells that synthesize the parental target protein. In initial experiments, individual K\(^b\)-binding ras L61 peptides were tested for the ability to immunize B6 spleen cells in vitro. Multiple experiments to elicit CTL with individual K\(^b\)-binding ras L61 peptides ranging from 8 to 12 mer in size proved unsuccessful.
Table 1. Cytolytic Activity of Lymphocytes Immunized In Vitro with ras L61 Peptides

| Sensitizing peptide | Targeting peptide | Specific lysis |
|---------------------|-------------------|---------------|
|                     |                   | 80:1  | 20:1  | 5:1  |
| ras L61*            | None*             | 36s   | 15    | 3    |
| ras L61             | ras L61           | 50    | 26    | 8    |
| ras L61             | T-OVA             | 37    | 15    | 3    |
| T-OVA               | None*             | 37    | 14    | 4    |
| T-OVA               | ras L61           | 37    | 12    | 4    |
| T-OVA               | T-OVA             | 52    | 29    | 8    |

* B6 spleen cells were incubated for 7 d with the indicated peptides at 25 μg/ml. ras L61 denotes the ras L61 peptide mixture and T-OVA denotes trypsinized OVA, described in Materials and Methods.
† 32Cr-labeled EL4 cells were preincubated for 2 h with the indicated peptide at 25 μg/ml, then used as targets.
§ E/T ratio.

Cytolytic activity of target cells with a synthetic OVA peptide (p257–264) with high affinity for Kb (25), markedly reduced the ability of ras L61 peptides to sensitize syngeneic target cells for lysis by ras L61-specific CTL (Fig. 2). By contrast, normal ras peptides, p56–67 Q61 and p60–67 Q61, did not block the ability of homologous ras L61 peptides to sensitize targets for lysis, providing additional evidence that normal ras Q61 peptides do not bind to Kβ with high affinity.

Under normal circumstances, antigenic class I MHC-restricted peptide determinants are proteolytically excised from endogenously produced proteins (32). Potentially antigenic determinants nested within an intact protein may be destroyed by inappropriate cleavage of proteins by proteases during antigen processing. Furthermore, competing or suppressive determinants at other sites within a protein may mask CTL recognition of a potentially antigenic peptide segment (33).

Table 2. Lysis of EL4 Tumor Cells Incubated with Various Synthetic ras Peptides by ras L61 Peptide-induced CTL

| Targeting ras peptide | Amino acid sequence | Specific lysis |
|-----------------------|---------------------|---------------|
|                       |                     | 40:1  | 20:1  | 10:1 |
| ras L61               |                     | 76*   | 70    | 62   |
| p60–66 L61            | G L E E Y S A       | 7     | 2     | 2    |
| p61–67 L61            | G L E E Y S A M     | 81    | 76    | 67   |
| p60–67 L61            | G L E E Y S A M     | 72    | 59    | 55   |
| p59–67 L61            | A G L E E Y S A     | 61    | 49    | 44   |
| p56–67 L61            | L D T A G L E E Y S A | 58   | 47    | 31   |
| p60–67 Q61            | G Q E E Y S A       | 5     | 4     | 2    |

* The arrow denotes residue 61.
† 32Cr-labeled EL4 cells were preincubated for 2 h with the indicated peptide (25 μg/ml) then used as targets.
§ E/T ratio.
Table 3. Lysis of H-2Kb Transfected Cells Incubated with Various Synthetic ras Peptides by ras L61 Peptide-induced CTL

| Targeting ras peptide | Amino acid sequence          | Specific lysis |
|------------------------|------------------------------|---------------|
|                        |                              | 40:1 | 20:1 | 10:1 | 5:1 |
| ras L61                |                              | 55%  | 49%  | 45%  | 37% |
| p60-66 L61             | G L E E Y S A                 | 4%   | 3%   | 3%   | 2%  |
| p61-67 L61             | L E E Y S A M                 | 56%  | 51%  | 50%  | 47% |
| p60-67 L61             | G L E E Y S A M               | 49%  | 39%  | 30%  | 26% |
| p59-67 L61             | A G L E E Y S A M             | 41%  | 33%  | 31%  | 27% |
| p56-67 L61             | L D T A G L E E Y S A M       | 46%  | 34%  | 22%  | 14% |
| p60-67 Q61             | G Q E E Y S A M               | 5%   | 4%   | 2%   | 2%  |

* The arrow denotes residue 61.
† 51Cr-labeled L cells transfected with Kb were preincubated for 2 h with the indicated peptide (50 μg/ml), then used as targets.
‡ E:T ratio.

the peptide mixture, have not been experimentally determined. However, it is most likely that the ras L61 peptide mixture concurrently primed CD4+ T cells to provide requisite help for the elicitation of CD8+ CTL responses. Previous studies have shown that longer ras L61 peptides are capable of eliciting Th cell responses (17), and many studies have emphasized the role of CD4+ T cells in the generation of CTL (34).

In vivo immunization with similar ras peptides should engender similar CTL responses, with the caveat that little is known concerning the optimal methods to prime in vivo with class I MHC-binding peptides. CTL specific for the mutated region of p53 have been generated by in vivo priming (35). It might be possible to generate ras-specific CTL by the use of recombinant vaccinia virus encoding relevant ras proteins (36). However, antivaccinia responses may predominate, especially in previously primed individuals. Alternatively immunization with soluble protein has in some circumstances been reported to predispose to the generation of primed CTL to proteins, including p21<sup>W</sup> (37). However, the use of in vitro priming, especially if adaptable to humans, may allow a more precise determination of antigenic peptide epitopes and a more rapid determination of epitopes that are actually processed and presented by malignant cells. The further development of primary in vitro immunization may greatly hasten the realization of specific T cell therapy for malignancy.

The current study confirms that recognition of oncogenic ras proteins by mammalian CTL is possible. The ability of CTL to lyse transformed cells on the basis of the expression of an abnormal ras oncogene suggests that it might be feasible to define circumstances for eliciting and utilizing specific CTL

Figure 2. Inhibition of ras L61 peptide-specific cytotoxicity by the Kb-binding ovalbumin peptide, OVA p257-264. 51Cr-labeled EL4 cells were incubated in serial dilutions of either ras p60-67 L61 (open symbols) or ras p56-67 L61 (closed symbols) peptides plus the indicated competing peptide (5 μg/ml) for 1 h, then used as targets in 4-h chromium release assays. Competing peptides included: ras p60-67 Q61 (8 mer), ras p56-67 Q61 (12 mer), and OVA p257-264. The data represent the percent specific lysis of the target cells by the ras L61-specific CTL line.

Figure 3. Lysis of p21<sup>W</sup>transformed cells by syngeneic ras L61-specific CTL. The ras L61 peptide-induced CTL line was assayed for the ability to lyse the indicated target cells in 4-h chromium release assays. (BLKSVHD + ras L61) BLKSVHD cells transformed with the c-Ha-ras L61 oncogene.

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to treat ras oncogene-transformed malignancies. The determination of whether ras-positive tumors elicit ras-specific immune responses and whether elicited immune responses affect tumor progression and/or the expression of p21\textsuperscript{ras} protein may allow the elucidation of the concept of immune surveillance and/or immunological escape at a defined molecular level.

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References

1. Greenberg, P.D. 1991. Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. Adv. Immunol. 49:281.

2. Lamb, J.R., D.D. Eckels, P. Lake, J.N. Woody, and N. Green. 1982. Human T-cell clones recognize chemically synthesized peptides of influenza haemagglutinin. Nature (Lond.). 300:66.

3. Townsend, A.R.M., R.M. Gotch, and J. Davely. 1985. Cytotoxic T cells recognize fragments of the influenza nucleoprotein. Cell. 42:457.

4. Townsend, A.R.M., J. Bastin, K. Gould, and G.G. Brownlee. 1986. Cytotoxic T lymphocytes recognize influenza haemagglutinin that lacks a signal sequence. Nature (Lond.). 324:575.

5. Barbacid, M. 1987. Ras genes. Annu. Rev Biochem. 56:779.

6. Seeburg, P.H., WW. Colby, D.J. Capon, D.V. Goeddel, and A.D. Levinson. 1984. Biological properties of human c-Ha-ras\textsuperscript{1} genes mutated at codon 12. Nature (Lond.). 312:71.

7. Der, C.J., T. Finkel, and G.M. Cooper. 1985. Biological and biochemical properties of human ras\textsuperscript{H} genes mutated at codon 61. Cell. 44:467.

8. Patek, P.Q., J.L. Collins, and M. Cohn. 1978. Transformed cell lines susceptible or resistant to in vivo surveillance against tumorigenesis. Nature (Lond.). 276:510.

9. Peace, D.J., D.E. Kern, K.R. Schultz, P.D. Greenberg, and M.A. Cheever. 1988. Interleukin-4-induced lymphokine-activated killer cells: lytic activity is mediated by phenotypically distinct NK-like and T cell-like large granular lymphocytes. J. Immunol. 140:3679.

10. Schulze, D.H., L.R. Pease, Y. Obata, S.G. Nathenson, A.A. Reyes, S. Ikuta, and R.B. Wallace. 1983. Identification of the cloned gene for the murine transplantation antigen H-2\textsuperscript{K}\textsuperscript{p} by hybridization with synthetic oligonucleotides. Mol. Cell. Biol. 3:750.

11. Ljunggren, H.-G., and K. Kärre. 1985. Host resistance directed selectively against H-2-deficient variants. Analysis of the mechanism. J. Exp. Med. 162:1745.

12. Moore, M.W., F.R. Carbone, and M.J. Bevan. 1988. Introduction of soluble protein into the class I pathway of antigen processing and presentation. Cell. 54:777.

13. Kamiya, H., K. Miura, N. Ohtomo, T. Koda, M. Kakinuma, S. Nishimura, and E. Ohtsuka. 1989. Transformation of NIH3T3 cells with synthetic c-Ha-ras genes. JPN J. Cancer Res. 80:200.

14. Spandidos, D.A., and N.M. Wilkie. 1984. Malignant transformation of early passage rodent cells by a single mutated human oncogene. Nature (Lond.). 310:469.

15. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Vol. III. Ch. 16, 30–37.

16. Carbone, F.R., M.W. Moore, J.M. Sheil, and M.J. Bevan. 1988. Induction of cytotoxic T lymphocytes by primary in vitro stimulation with peptides. J. Exp. Med. 167:3767.

17. Peace, D.J., W. Chen, H. Nelson, M.A. Cheever. 1991. T cell recognition of transforming proteins encoded by mutated ras proto-oncogenes. J. Immunol. 146:2059.

18. Matsuura, M., D.H. Fremont, P.A. Peterson, and I.A. Wilson. 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science (Wash. DC). 257:927.

19. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H.-G. Rammenner. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature (Lond.). 351:290.

20. Nathenson, S.G., H. Uchara, B.M. Ewenstein, T.J. Kindt, and J.E. Coligan. 1981. Primary structural analysis of the transplantation antigens of the murine H-2 major histocompatibility complex. Annu. Rev Biochem. 50:1025.

21. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic lymphocytes can be defined with short synthetic peptides. Cell. 44:959.

22. Attaya, M., S. Jameson, C.K. Martinez, E. Hermel, C. Aldrich, J. Forman, K.F. Lindahl, M.J. Bevan, and J.J. Monaco. 1992. Ham-2 corrects the class I antigen-processing defect in RMA-S cells. Nature (Lond.). 355:647.

23. Monaco, J.J. 1992. A molecular model of MHC class I-restricted antigen processing. Immunol. Today. 13:173.

24. Townsend, A. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. J. Exp. Med. 167:3767.

25. Peace, D.J., W. Chen, H. Nelson, M.A. Cheever. 1991. T cell recognition of transforming proteins encoded by mutated ras proto-oncogenes. J. Immunol. 146:2059.

26. Matsuura, M., D.H. Fremont, P.A. Peterson, and I.A. Wilson. 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science (Wash. DC). 257:927.

27. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H.-G. Rammenner. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature (Lond.). 351:290.

28. Nathenson, S.G., H. Uchara, B.M. Ewenstein, T.J. Kindt, and J.E. Coligan. 1981. Primary structural analysis of the transplantation antigens of the murine H-2 major histocompatibility complex. Annu. Rev Biochem. 50:1025.

29. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic lymphocytes can be defined with short synthetic peptides. Cell. 44:959.

30. Attaya, M., S. Jameson, C.K. Martinez, E. Hermel, C. Aldrich, J. Forman, K.F. Lindahl, M.J. Bevan, and J.J. Monaco. 1992. Ham-2 corrects the class I antigen-processing defect in RMA-S cells. Nature (Lond.). 355:647.

31. Monaco, J.J. 1992. A molecular model of MHC class I-restricted antigen processing. Immunol. Today. 13:173.

32. Townsend, A. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature (Lond.). 351:290.

33. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H.-G. Rammenner. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature (Lond.). 351:290.

34. Nathenson, S.G., H. Uchara, B.M. Ewenstein, T.J. Kindt, and J.E. Coligan. 1981. Primary structural analysis of the transplantation antigens of the murine H-2 major histocompatibility complex. Annu. Rev Biochem. 50:1025.

35. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic lymphocytes can be defined with short synthetic peptides. Cell. 44:959.

36. Attaya, M., S. Jameson, C.K. Martinez, E. Hermel, C. Aldrich, J. Forman, K.F. Lindahl, M.J. Bevan, and J.J. Monaco. 1992. Ham-2 corrects the class I antigen-processing defect in RMA-S cells. Nature (Lond.). 355:647.
and H.-G. Rammensee. 1991. Exact prediction of a natural T cell epitope. Eur. J. Immunol. 21:2891.
26. Fremont, D.H., M. Matsumura, E.A. Stura, P.A. Peterson, and I.A. Wilson. 1992. Crystal structure of two viral peptides in complex with murine class I H-2K\textsuperscript{b}. Science (Wash. DC). 257:919.
27. Zhang, W., A.C.M. Young, M. Imarai, S.G. Nathenson, and J.C. Sacchettini. 1992. Crystal structure of the major histocompatibility complex class I H-2K\textsuperscript{b} molecule containing a single viral peptide: implications for peptide binding and T-cell receptor recognition. Proc. Natl. Acad. Sci. USA. 89:8403.
28. Zhou, X., L. Berg, U. Motal, and M. Jondal. 1992. In vivo primary induction of virus-specific CTL by immunization with 9-mer synthetic peptides. J. Immunol. Methods. 153:193.
29. Ishioka, G., S. Colon, C. Miles, H. Grey, and R. Chesnut. 1989. Induction of class I MHC-restricted, peptide-specific cytolytic T lymphocytes by peptide priming in vivo. J. Immunol. 143:1094.
30. Raychaudhuri, S., M. Tonks, F. Carbone, T. Ryskamp, W.J. Morrow, and N. Hanna. 1992. Induction of antigen-specific class I-restricted T cells by soluble proteins in vivo. Proc. Natl. Acad. Sci. USA. 89:8308.
31. Schumacher, T.N., M.L. De Bruijn, L.N. Vernie, W.M. Kast, C.J. Melief, J.J. Neefjes, and H.L. Ploegh. 1991. Peptide selection by MHC class I molecules. Nature (Lond.). 350:703.
32. Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature (Lond.). 348:252.
33. Parham, P., C. Clayberger, S.L. Zorn, D.S. Ludwig, G.K. Schoolnik, and A.M. Krensky. 1987. Inhibition of alloreactive cytotoxic T lymphocytes by peptides from alpha 2 domain of HLA-A2. Nature (Lond.). 325:625.
34. Widmann, C., P. Romero, J.L. Marvanski, G. Coradin, and D. Valmori. 1992. T helper epitopes enhance the cytotoxic response of mice immunized with MHC class I-restricted malaria peptides. J. Immunol. Methods. 155:95.
35. Yanuck, N., D.P. Carbone, C.D. Pendleton, T. Tsukui, S.F. Winter, J.D. Minna, and J.A. Berzofsky. 1993. A mutant p53 tumor suppressor protein is a target for peptide-induced CD8\textsuperscript{+} cytotoxic T-cells. Cancer Res. 53:3257.
36. Skipper, J., and H.J. Stauss. 1993. Identification of two cytotoxic T lymphocyte-recognized epitopes in the ras protein. J. Exp. Med. 177:1493.
37. Fenton, R.G., D. Taub, L.W. Kwak, M.R. Smith, and D.L. Longo. 1993. Cytotoxic T-cell response and in vivo protection against tumor cells harboring activated ras proto-oncogenes. J. Natl. Cancer Inst. 85:1294.