Selective Expansion of Cross-reactive CD8\(^+\) Memory T Cells by Viral Variants

By John B.A.G. Haanen, Monika C. Wolkers, Ada M. Kruisbeek, and Ton N.M. Schumacher

From the Department of Immunology, The Netherlands Cancer Institute, Amsterdam 1066 CX, The Netherlands

Summary

The role of memory T cells during the immune response against random antigenic variants has not been resolved. Here, we show by simultaneous staining with two tetrameric major histocompatibility complex (MHC)-peptide molecules, that the polyclonal CD8\(^+\) T cell response against a series of natural variants of the influenza A nucleoprotein epitope is completely dominated by infrequent cross-reactive T cells that expand from an original memory population. Based on both biochemical and functional criteria, these cross-reactive cytotoxic T cells productively recognize both the parental and the mutant epitope in vitro and in vivo. These results provide direct evidence that the repertoire of antigen-specific T cells used during an infection critically depends on prior antigen encounters, and indicate that polyclonal memory T cell populations can provide protection against a range of antigenic variants.

Key words: influenza virus • major histocompatibility complex tetramers • peptides • C57BL mice • in vivo

The small size of the contact surface between the TCR and MHC-bound peptide suggests that the peptide specificity in TCR-MHC interactions is limited (1). Indeed, a large number of studies have demonstrated that the sequence requirements for ligand recognition by T cell clones in vitro are quite minimal (2–5). However, in certain situations, variations in CTL epitopes may lead to a total or partial loss of functional recognition by cytotoxic T cells, due to qualitatively different TCR signaling upon interaction with these altered peptide ligand-MHC complexes (for a review, see reference 6). Indeed, altered peptide ligands have been shown to antagonize antigen-specific T cell responses both in vitro and in vivo (7–14). These experiments have firmly established that selected mutations in T cell epitopes can abolish productive T cell recognition. However, it is unresolved whether such abortive T cell responses are common upon in vivo encounter of antigen variants. In an MHC outbred population, T cell epitope mutations encountered during transient infections are likely to be random, and we therefore set out to examine how a polyclonal T cell population would react to such random antigenic variants in an in vivo model.

After infection with influenza A viruses, large numbers of influenza A-specific cytotoxic T cells can be recovered from pulmonary tissue, lymphoid organs, and peripheral blood in mice and humans (15–17). In C57BL/10 mice, the immunodominant CTL epitope of influenza A viruses is located in the viral nucleoprotein (NP), amino acids 366–374. Within the TCR-exposed side chains of the COOH-terminal region of this peptide (positions 6, 7, and 8), significant variation exists among naturally occurring influenza A strains. Early work from Townsend and Skehel (18) showed that certain influenza A virus NP-specific T cell lines can recognize viral variants in in vitro assays, but the extent and in vivo relevance of such cross-reactivity have remained elusive. To assess the consequences of exposure to naturally occurring variants on T cell reactivity in an in vivo setting, we have analyzed the effects of polyclonal T cell memory, formed during a primary influenza A virus infection, on the subsequent response against a series of influenza A virus variants. To directly visualize T cells displaying antigen receptors that are monospecific for a certain viral variant or that cross-react between different variants, we used differentially labeled oligomeric peptide-MHC class I complexes (19). Contrary to conventional functional assays, such as \(^{51}Cr\) release, this strategy allows for a direct assessment of the potential for cross-recognition of individual cells in mixed T cell populations. The results thus obtained show that cross-reactive memory T cells generated during a primary infection dominate the T cell response during a secondary infection with a variant virus even when such cross-reactive cells are rare.
in the original memory T cell pool. The implications of these findings for peripheral T cell repertoire selection and viral variation are discussed.

Materials and Methods

Animals. C57BL/10 mice at 5–6 wk of age were obtained from the animal department of the Netherlands Cancer Institute. Mice were handled at all times in accordance with institutional guidelines.

MHC Tetramers and Peptides. Peptides were produced using standard solid-phase FMOC chemistry. Soluble fluorochrome (PE or APC)-labeled MHC tetramers were produced as described previously (17, 19) and stored frozen in Tris-buffered saline/16% glycerol/0.5% BSA.

Viruses and Cells. Influenza virus strains A/NT/60/68 and A/HKx31 were provided by Dr. R. Gonsalves, National Institute for Medical Research, London, U.K. Influenza virus B/Leu/40 was obtained from the American Type Culture Collection. Mice were killed at indicated time points after infection, and organs were removed for further analysis. Inflamed lung tissue and spleens were minced in single chamber mesh filters. The single cell suspensions obtained were treated with NH4Cl to get rid of contaminating erythrocytes, before staining for flow cytometry purposes.

The influenza virus (A/NT/60/68) N-P-derided H-2D- restricted CTL epitope, ASNENMDAM, was introduced into EL4 tumor cells by retroviral insertion as a COOH-terminal fusion with the enhanced green fluorescent protein (eGFP) gene product (Wolkers, M.C., manuscript in preparation).

Flow Cytometry. In all instances, mononuclear cells were stained with directly labeled mAbs or MHC tetramers. Analysis was performed on a FACSAir™ (Becton Dickinson) using CELLQuest™ software (Becton Dickinson). Before staining, propidium iodide (PI) was added to gate for PI-negative (living) lymphocytes.

Cytotoxicity Assay. Mononuclear cells were stained with anti-CD8–APC (PharMingen) or FITC-labeled isotype control antibody (PharMingen). Analyses were performed on a FACSAir™ (Becton Dickinson) using CELLQuest™ software. Isotype control antibodies resulted only in background staining (data not shown).

In some experiments, cells were incubated with peptide (0.5 μM) for 5–6.5 h at 37°C in the presence of recombinant human (rh)IL-2 (50 U/ml) and Brefeldin A (0.1 μM/ml). After incubation, cells were surface stained with anti-CD8–APC (PharMingen), incubated in Cytofix/Cytoperm solution (PharMingen) for 20 min on ice, washed, and stained for intracellular cytokine with anti-IFN-γ–FITC (PharMingen) or FITC-labeled isotype control antibody (PharMingen). Analyses were performed on a FACSAir™ (Becton Dickinson) using CELLQuest™ software. Isotype control antibodies resulted only in background staining (data not shown).

Results

Selective Expansion of C cross-reactive Influenza A Virus-specific T Cells. To assess the effect of random antigen variation on the dynamics of T cell responses in vivo, we infected mice with pairs of influenza A viruses. These viruses expressed either the same NP366-374 epitope or epitope variants. The specificity of the resulting T cell repertoire was assessed by two-parameter MHC tetramer staining, and association of MHC tetramers to NK receptors was ruled out through analysis of CDb8-expressing cells only. When mice are infected once or twice with either influenza virus strain A/NT/60/68 or A/PR/8/34, which differ in the sequence of the immunodominant NP CTL epitope at positions 7 and 8 (ASNENMDAM vs. ASNE METM), the vast majority of the resulting NP-specific T cells selectively recognize the epitope of the strain encountered and not that of the opposite strain (Fig. 1 A, panels 1 and 3). This dominant role of the peptide side chains at positions 7 and 8 in ligand recognition by the majority of T cells is in accord with the predominant contribution of p8 and especially p7 to the TCR-exposed surface of this peptide–MHC complex (22).

This virus strain specificity of the N-P-reactive CTLs contrasts sharply with the apparent lack of strain specificity during secondary responses against a variant strain. When mice that had recovered from a previous infection with influenza virus strain A/NT/60/68 are challenged with viral strain A/PR/8/34, most if not all N P8-reactive T cells are fully cross-reactive between the two N P variants (Fig. 1 A, panel 4). This phenomenon is reciprocal: in mice that have previously experienced infection with strain A/HKx31 (a reassortant strain with the NP gene from A/PR/8/34), the N-P-reactive T cell population that emerges upon infection with A/NT/60/68 is fully cross-reactive between the two viral strains (Fig. 1 A, panel 2).

Although somewhat variable between individual mice, the extent of binding of the two MHC tetramers appears independent of the order in which the epitopes were encountered over a large series of experiments. To compare the affinity for primary and secondary antigen in a more direct manner, competition studies were performed. These experiments demonstrate that the binding of fluorochrome-labeled MHC tetramers can be inhibited by similar concentrations of ASNE METM - and ASNE METM - containing monomers, indicating equal affinity of the cross-reactive TCRs for either antigen (Fig. 1 B). In addition, these data rule out the possibility of dual TCR expression (23, 24) by the cross-reactive T cells, since both MHC monomers compete for binding of ASNE METM -containing MHC tetramers.

Cross-reactivity Correlates with Cross-recognition. The above results indicate that the subsequent encounter of variants of a T cell epitope results in the expansion of a cross-reactive T cell population, as established by biochemical assays. In fact, this expansion inhibits the expansion of the largely strain-specific population observed during a regular primary response. However, T cell recognition of ligands with similar affinities can have drastically different functional outcomes, due to differences in off rates of TCR–ligand interactions.
reactive T cell population, we examined the kinetics and composition of this T cell pool. The cross-reactive cytotoxic T cell population appears 2–3 d earlier at the site of infection (i.e., pulmonary tissue; data not shown) than the antigen-specific T cells during infection of naive mice (15–17), suggesting that these cells originate from a pre-existing memory T cell population. Several studies have shown a narrowing of the antigen-specific polyclonal TCR repertoire during recall infection, due to the preferential outgrowth of a subpopulation of memory cells (30–33). In naive mice that are infected with influenza virus A/PR/8/34, the repertoire of NP-specific T cells involves a variety of BV elements (Fig. 3 A). The slight preferential usage of the BV8.3 element reported previously for C57BL animals infected with influenza virus A/PR8/34 was not observed in these experiments (34). In contrast, in A/NT/60/68-primed mice that are infected with influenza virus A/PR/8/34, the repertoire of A/PR/8/34-specific T cells is highly restricted (Fig. 3 A). This narrow T cell repertoire is likely to reflect the affinity maturation observed previously (33) compounded by the low number of cross-reactive T cells within the original memory population. In certain animals, the oligoclonal nature of the cross-reactive T cell population appears to be directly visible from double-tetramer analyses of the influenza-reactive CD8+ T cell population. In these mice,
the expanded cross-reactive T cell population appears as two to three separate populations (an example is shown as Fig. 3 B), which may reflect slightly distinct affinities for the primary and secondary antigen.

Specificity of Cross-reactive T Cells. Several reports have indicated that the requirements for activating memory T cells differ from those for activating naive T cells, making them more susceptible to low-affinity TCR triggering or cytokine-mediated stimulation (35–39). To address the possible contribution of aspecific or more broadly reactive T cells in the formation of the cross-reactive T cell population, influenza A/NT/60/68-primed mice were infected with the antigenically unrelated influenza B virus (B/Lee/40). Both at day 4 (data not shown) and day 8 after infection (Fig. 4, panel 1), no expansion of the influenza A/NT/60/68-specific memory T cell population is observed. This is in agreement with results by others showing that bystander activation during an acute viral infection is minimal (20, 40, 41). Furthermore, the influenza A virus-specific T cells in mice that underwent sequential infections with A/NT/60/68 and A/PR/8/34 do not bind peptide-MHC tetramers that contain an adenovirus E1A-derived CTL epitope (Fig. 4, panel 2). Thus, the cross-reactive T cell population that expands upon infection of primed mice does require a significant structural homology between two antigens that are encountered sequentially, and is specific for these two peptide-MHC complexes. Finally, selective outgrowth of cross-reactive T cells does not take place to an appreciable extent when the two related antigens are introduced simultaneously (Fig. 4, panel 3). This suggests that the selective advantage of cross-reactive cells relies on quantitative or qualitative traits of the T memory population, such as homing properties or the requirements for costimulatory signals.

Structural Requirements for Cross-reactivity. To provide a first estimate of the extent of structural homology between two antigens that is required for the selective expansion of cross-reactive T cells, we searched the National Center for Biotechnology Information (NCBI) GenBank database for other natural variants of the H-2D<sup>b</sup>-restricted influenza NP epitope. Thus far, 10 variants of this epitope have been identified, 7 of which contain mutations that affect TCR-
exposed side chains (positions 6, 7, and 8) (reference 22, and Table I). Because influenza A virus is not a common mouse pathogen, this set of mutants should be random with respect to T cell recognition. Synthetic peptides corresponding to these mutants were generated, and mice that had previously been exposed to influenza A/NT/60/68 or A/HKx31 were challenged by subcutaneous immunization. To circumvent the need of helper T cells for the induction of an effective CTL response, mice were treated with anti-CD40 mAb (FGK.45) after vaccination (21, 42–44). These experiments establish that in most if not all cases where influenza A virus–primed mice are confronted with antigens that contain a single mutation within the T cell epitope, an influenza-specific T cell population emerges that is fully cross-reactive between the primary and secondary antigen (Table I). This phenomenon is not only observed for conservative substitutions, but also for more drastic amino acid changes (e.g., Ala8→Asn). For variants that contain multiple alterations in TCR-exposed residues, cross-reactivity is observed for some sequences (e.g., ASNENMDA→ASNENMET), but not for others (e.g., ASNENMDAM→ASNENVEAM). Both the type of mutation and the contribution of the mutated residues to the TCR-exposed surface of the peptide are likely to be determining factors in this regard. For one of the mutant epitopes (ASNENVTAM), the functional behavior of the cross-reactive T cells was also tested. In line with the biochemical data, intracellular IFN-γ staining of spleen cells that were stimulated with the primary antigen

Figure 3. Cross-reactive cells are oligoclonal T memory cell expansions. (A) Cross-reactive CD8+ T cells display a restricted BV repertoire. Naive (top) or A/NT/60/68-primed mice (bottom) were infected with 10 and 200 HAU of influenza A/PR/8/34, respectively. At 7 d after infection, lung-infiltrating A/PR/8/34-specific CD8+ T cells were analyzed for expression of a series of BV elements by using a panel of fluorochrome-labeled anti-BV antibodies (PharMingen). Values represent the percentage of A/PR/8/34-specific CD8+ T cells that stain with a particular anti-BV antibody. (B) Mice were sequentially infected with influenza virus A/HKx31 and A/NT/60/68. 7 d after the second infection, mononuclear cells recovered from pulmonary tissue were stained with anti-CD8 and APC-labeled MHC tetramers with the influenza A/NT/60/68 epitope and PE-labeled MHC tetramers with the influenza A/PR/8/34 epitope, and analyzed by FACS® analysis. The dot plot reveals three distinct populations of selectively expanded cross-reactive CD8+ T cells.
Memory T Cell Responses against Viral Variants

ASNENMDAM, or the peptide variant used for vaccination revealed an identical percentage of responding cells (data not shown).

In Vivo Function of Cross-reactive T Cells

Cross-protection or the lack thereof by an epitope-specific T cell population cannot readily be tested through the use of mutant or recombinant viral strains. Even in settings where B cell immunity is absent, such as in μ-MT mice, it is difficult to exclude a possible contribution of T cell responses against subdominant epitopes that are conserved between the primary and the recall strain. To circumvent these difficulties, we developed a model system in which only the T cell epitope under study is shared between the primary and secondary antigen encounter. The NP366-374 CTL epitope of influenza virus strain A/NT/60/68 was introduced into the EL4 tumor cell line as a COOH-terminal fusion with the eGFP gene product. Although the parental cell line grows progressively when injected subcutaneously in C57BL/10 mice, the NP epitope–expressing tumor is rejected over a 2–3-wk time period. Furthermore, tumor rejection is paralleled by the appearance of an NP366-374–specific T cell population and is markedly enhanced by simultaneous infection with influenza virus A/NT/60/68 (Wolters, M. C., manuscript in preparation). Thus, the introduced NP epitope appears to function as a bona fide (neo)tumor antigen in this setting, as recognition of this antigen is correlated with tumor rejection.

To examine the in vivo effects of cross-reactive T cell populations, mice that had previously been exposed to influenza virus A/NT/60/68 (carrying the homologous NP epitope), A/HKx31 (reassortant of A/PR/8/34; carrying a variant NP epitope), or an unrelated influenza B virus were challenged with EL4-NP366-374 tumor cells. After tumor cell injection, blood samples were taken from individual mice and the frequency of virus strain–specific and cross-reactive CD8+ T cells was measured. In mice that had been infected with the unrelated influenza B virus (Fig. 5, panel 1), tumor growth is comparable to that in uninfected mice (data not shown). As expected, mice that were previously exposed to influenza virus A/NT/60/68, which shares the CTL epitope with the EL4-NP366-374 tumor, showed a strong reduction in tumor growth (Fig. 5, panel 1). This protection is accompanied by a massive and rapid increase in the number of A/NT/60/68-monospecific T cells, which were apparently reactivated from the memory T cell pool by the NP epitope–expressing tumor (Fig. 5, panel 2). Importantly, intermediate tumor growth was seen in mice that had previously been infected with variant virus A/HKx31 (Fig. 5, panel 1). In addition, the reduced tumor outgrowth in these mice is accompanied by the expansion of a large population of CD8+ T cells, which cross-react between the NP366-374 expressing EL4 tumor and the viral strain used for priming (Fig. 5, panel 3). These results illustrate that the presence of a structurally related T cell antigen promotes the expansion of infrequent cross-reactive T cells even in the absence of any further noticeable homology between primary and secondary challenge, and this expansion is accompanied by a significant reduction in tumor outgrowth.
The ability of cytotoxic T cell populations to cross-react between different viral strains was first appreciated by Townsend and Skehel in 1984 (18). They showed that repeated in vitro restimulation protocols of splenic lymphocytes derived from influenza A virus–primed mice could lead to the selection of NP-specific T cell lines that cross-reacted between different influenza A viral strains. At that time, it was unknown whether these T cell lines cross-reacted at the level of the (variable) immunodominant NP<sub>366-374</sub> CTL epitope, or whether shared subdominant epitopes were recognized. However, with the benefit of hindsight, these experiments can be said to have revealed for the first time the capacity of cytotoxic T cells to productively recognize viral variants.

In spite of the early recognition of T cell cross-reactivity in in vitro assays, the biological in vivo significance of this cross-reactivity for the immune system to cope with viral variants has since remained unclear. Our findings now document that prior antigen exposure dramatically affects the repertoire of T cells used in a subsequent response to antigenic variants. The propensity for cross-reactivity between two T cell antigens appears roughly proportional to the sequence similarity between the epitopes tested, and seems to be a common event for antigens that are closely related. These cells that expand in vivo are phenotypically indistinguishable from conventional effector T cell populations (CD44<sup>high</sup>, CD62L<sup>low</sup>; data not shown) and are functional ex vivo and in vivo. This process appears to be due to the selective expansion of cross-reactive T cells present in the memory T cell pool, and is not dependent on shared B or T helper epitopes between primary and recall antigen.

In recent years, several groups have studied the impact of epitope variants on antigen-specific T cell responses (for reviews, see references 6, 45, and 46). These variant epitopes...
may be hypothesized that for such restricted antigen-specific T cell repertoire (33). Indeed, the antigen-specific T cell expansions observed during chronic HIV infection are oligoclonal (47). It is currently not known why such restriction occurs. One possibility is that the T cell repertoire is narrowed during the initial infection as a result of antigenic stimulation. Alternatively, the impaired ability to react to emerging antigens in those cases and the mutations studied to date do not point towards obvious differences (not shown). The selective narrowing of the T cell repertoire could be a more general phenomenon.

We conclude that a polyclonal T cell repertoire responds to the encounter of random variants by the selective expansion of cross-reactive memory T cells. C57BL/10 mice (six mice per group) were infected with either A/NT/60/68 (10 HAU), A/HX31 (100 HAU), or B/Leo/40 (125 HAU). 3 mo after the infection, all mice were injected subcutaneously with 3 × 10⁶ live EL4-NP366-374 tumor cells 7 d after tumor challenge, tumor size was measured and the mean diameter per mouse is plotted (panel 1). At the same time point, peripheral blood samples were taken and surface stained with anti-CD8 mAb (PharMingen) and differentially labeled MHC tetramers containing the ASNENMDAM and ASNENMETM epitopes. The percentages of A/NT/60/68 monospecific CD8+ T cells (panel 2) and cross-reactive CD8+ T cells (panel 3) are plotted for individual mice.

Figure 5. In vivo function of cross-reactive T cells. C57BL/10 mice (six mice per group) were infected with either A/NT/60/68 (10 HAU), A/HX31 (100 HAU), or B/Leo/40 (125 HAU). 3 mo after the infection, all mice were injected subcutaneously with 3 × 10⁶ live EL4-NP366-374 tumor cells 7 d after tumor challenge, tumor size was measured and the mean diameter per mouse is plotted (panel 1). At the same time point, peripheral blood samples were taken and surface stained with anti-CD8 mAb (PharMingen) and differentially labeled MHC tetramers containing the ASNENMDAM and ASNENMETM epitopes. The percentages of A/NT/60/68 monospecific CD8+ T cells (panel 2) and cross-reactive CD8+ T cells (panel 3) are plotted for individual mice.

were generally isolated as immune escape variants, or were identified in in vitro assays by their aberrant recognition by T cell clones. These selected antigen variants function as either partial agonists or antagonists of antigen-specific T cell responses not only in vitro but also in vivo. We sought to examine whether this type of T cell antagonism or partial agonism is a common phenomenon when a polyclonal T cell repertoire is confronted with antigenic variation. To this purpose, we studied the development of antigen-specific T cell repertoire during random natural variants of influenza A viruses. We conclude that during such encounters, the T cell repertoire generally reacts with the outgrowth of a T cell population for which the variant epitope is a full agonist. The TCRs encoded by these T cells bind with equal affinity to MHC molecules complexed with wild-type and variant epitopes. Furthermore, the functional capacity of this T cell population towards target cells expressing the original or the variant antigen is indistinguishable both in vitro and in vivo.

How do these data fit in with previous observations that mutations in T cell epitopes can lead to CTL escape by the virus? During chronic HIV and HBV infections (10, 11), and also in a murine lymphocytic choriomeningitis virus (LCMV) model (12), the mechanism that we have identified apparently does not operate efficiently. In theory, this could be due to the type of amino acid changes in the T cell epitopes involved. However, examination of the type of mutations in the T cell epitopes in those cases and the mutations studied here does not point towards obvious differences (not shown). Alternatively, the impaired ability to react to emerging antigenic variants in those settings may be due to alterations at the T cell level. Specifically, repetitive antigen-specific T cell stimulation results in a narrowing of the reactive T cell repertoire (33). Indeed, the antigen-specific T cell expansions observed during chronic HIV infection are oligoclonal (47). It may be hypothesized that for such restricted antigen-specific T cell populations, a single antigenic variant could antagonize a substantial part of the antigen-specific T cell response, and it will be a challenge to test this notion in a direct manner.

We conclude that a polyclonal T cell repertoire responds to the encounter of random variants by the selective expansion of cross-reactive memory T cells. C57BL/10 mice (six mice per group) were infected with either A/NT/60/68 (10 HAU), A/HX31 (100 HAU), or B/Leo/40 (125 HAU). 3 mo after the infection, all mice were injected subcutaneously with 3 × 10⁶ live EL4-NP366-374 tumor cells 7 d after tumor challenge, tumor size was measured and the mean diameter per mouse is plotted (panel 1). At the same time point, peripheral blood samples were taken and surface stained with anti-CD8 mAb (PharMingen) and differentially labeled MHC tetramers containing the ASNENMDAM and ASNENMETM epitopes. The percentages of A/NT/60/68 monospecific CD8+ T cells (panel 2) and cross-reactive CD8+ T cells (panel 3) are plotted for individual mice.
The authors would like to thank Mireille Toebes and Marjo van Puijenbroek for preparation of MHC tetramers and technical assistance, Helmut Kessels for peptide synthesis, Drs. G. R. Immelzaan and A. Osterhaus (Erasmus University, Rotterdam, The Netherlands) for growing and titrating the various influenza strains, and members of the Kruisbeek and Schumacher labs for discussions.

Address correspondence to T on N.M., Schumacher, The Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam 1066 CX, The Netherlands. Phone: 31-20-512-2072; Fax: 31-20-512-2057; E-mail: tschum@nki.nl

Submitted: 9 July 1999 Revised: 19 August 1999 Accepted: 23 August 1999

References

1. Garboczi, D.N., P. Ghosh, U. Utz, Q.R. Fan, W.E. Biddisson, and D.C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. N. nature. 384:134–141.
2. Lorenz, R.G., A.N. Tyler, and P.M. Allen. 1989. Reconstruction of the immunogenetic peptide R Nase(43–56) by identification and transfer of the critical residues into an unrelated peptide backbone. J. Exp. Med. 170:203–215.
3. Wucherpfennig, K.W., and J.L. Strominger. 1995. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell. 80:695–705.
4. Evavold, B.D., L.J. Sloan, K.J. Wilson, J.B. Rothbard, and P.M. Allen. 1995. Specific T cell recognition of minimally homologous peptides evidence for multiple endogenous ligands. Immunity. 2:655–663.
5. Kersh, G.J., and P.M. Allen. 1996. Structural basis for T cell recognition of altered peptide ligands a single T cell receptor can productively recognize a large continuum of related ligands. J. Exp. Med. 184:1259–1268.
6. Sloan-Lancaster, J., and P.M. Allen. 1996. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. Annu. Rev. Immunol. 14:1–27.
7. De Magistris, M.T., J. Alexander, M.C. Coggeshall, A. Altman, F.C. Gaeta, H.M. Grey, and A. Sette. 1992. Antigen analog-major histocompatibility complexes act as antagonists of the T cell receptor. Cell. 68:625–634.
8. Sloan-Lancaster, J., B.D. Evavold, and P.M. Allen. 1993. Induction of T-cell anergy by altered T-cell receptor ligand on live antigen-presenting cells. Nature. 363:156–159.
9. Evavold, B.D., J. Sloan-Lancaster, B.L. Hsu, and P.M. Allen. 1993. Separation of T helper 1 clone cytolysis from proliferation and lymphokine production using analog peptides. J. Immunol. 150:3131–3140.
10. Kleneman, P., S. Rowlan-Jones, S. M. Adam, J. Edwards, S. Dænke, D. Laloo, B. Koppe, W. R. Rosenberg, D. Boyd, A. Edwards, et al. 1994. Cytotoxic T-cell activity antagonized by naturally occurring HIV-1 Gag variants. Nature. 369:403–407.
11. Bertoletti, A., A. Sette, F.V. Chisari, A. Penna, M. Leverro, M. De Carli, F. Fiaccadori, and C. Ferrari. 1994. Natural variants of cytopotoxic T cells are T-cell receptor antagonists for antiviral cytotxic T cells. Nature. 369:407–410.
12. Kleneman, P., and R.M. Zinkernagel. 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. Nature. 394:482–485.
13. Basu, D., C.B. Williams, and P.M. Allen. 1998. In vivo antagonism of a T cell response by an endogenously expressed ligand. Proc. Natl. Acad. Sci. USA. 95:14332–14336.
14. Plebanski, M., E.A. Lee, C.M. Hannon, K.L. Flanagan, S.C. Gilbert, M.B. Gravenor, and A.V. Hill. 1999. Altered peptide ligands narrow the repertoire of cellular immune responses by interfering with T-cell priming. N. nat. Med. 5:565–571.
15. Flynn, J.K., G.T. Belz, J.D. Altmann, R. Ahmed, D.L. Woolard, and P.C. Doherty. 1998. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. Immunity. 8:683–691.
16. Dunbar, P.R., G.S. Ogg, J. Chen, N. Rust, P. van der Bruggen, and V. Cerundolo. 1998. Direct isolation, phenotyping and cloning of low-frequency antigen-specific cytotoxic T lymphocytes from peripheral blood. Curr. Biol. 8:413–416.
17. Haanen, J.B.A.G., M. Toebes, G.A. Cordaro, M. Wolkers, A.M. Kruisbeek, and T.N.M. Schumacher. 1999. Systemic T cell expansion during localized viral infection. Eur. J. Immunol. 29:1168–1174.
18. Townsend, A.R., and J.J. Skehel. 1984. The influenza A virus nucleoprotein gene controls the induction of both subtype-specific and cross-reactive cytotoxic T cells. J. Exp. Med. 160:552–563.
19. Altmann, J.D., P.A.H. Moss, P.J.R. Goulder, D.H. Barouch, M.G. M Cheyer-Williams, J.L. Bell, A.J. M Chiel, and M.M. Davis. 1996. Phenotypic analysis of antigen-specific T lymphocytes. Science. 274:94–96.
20. Murali, K.K., J.D. Altmann, M. Suresh, D.J. Sourdive, A.J. Zajac, J.D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8 T cells a reevaluation of bystander activation during viral infection. Immunity. 8:177–187.
21. Diehl, L., A.T. den Boer, S.P. Schoenberger, E.I.H. van der Voort, T.N.M. Schumacher, C.J.M. Melief, R. Offringa, and R. Toes. 1999. Peptide-induced peripheral CTL tolerance can be overcome by in vivo CD40 activation. Nat. Med. 5:774–779.
22. Young, A.C., W. Zhang, J.C. Sacchettini, and S.G. Nathans. 1984. The influenza A virus enzyme structural Sin impairs cytototoxic T lymphocyte responses to viruses bearing variant epitopes. Nature. 394:482–485.
25. Matsui, K., J.J. Boniface, P. Steffner, P.A. Reay, and M.M. Davis. 1994. Kinetics of T-cell receptor binding to peptide-I-Ek complexes: correlation of the dissociation rate with T-cell responsiveness. Proc Natl Acad Sci U.S.A. 91:12862-12866.

26. Alam, S.M., P.J. Travers, J.L. Wung, W. Nasholds, S. Redpath, S.C. Jameson, and N.R. Gascoigne. 1996. T-cell-receptor affinity and thymocyte positive selection. Nature 381:616-620.

27. Lyons, D.S., S.A. Lieberman, Y. Chien, L.J. Berg, and M.M. Davis. 1996. A TCR binds to antigenic complexes with lower affinities and faster dissociation rates than to agonists. Immunity 5:53-61.

28. Kersh, G.J., E.N. Kersh, D.H. Fremont, and P.M. Allen. 1996. TCR affinity and thymocyte positive selection. Proc Natl Acad Sci U.S.A. 91:12862-12866.

29. Savage, P.A., J.J. Boniface, and M.M. Davis. 1999. A kinetic basis for T cell receptor repertoire selection during an immune response. Immunity 10:485-492.

30. McHeyzer-Williams, M.G., and M.M. Davis. 1995. Antigen-specific development of primary and memory T cells in vivo. Science 268:106-111.

31. Bachmann, M.F., D.E. Speiser, and P.S. Ohashi. 1997. Functional maturation of an antiviral cytotoxic T-cell response. J Virol. 71:5764-5768.

32. Busch, D.H., I. Pilip, and E.G. Pamer. 1998. Evolution of a complex T-cell receptor repertoire during primary and recall bacterial infection. J Exp Med. 188:61-70.

33. Busch, D.H., and E.G. Pamer. 1999. T-cell affinity maturation by selective expansion during infection. J Exp Med. 189:701-710.

34. Deckhut, A.M., W. Allan, A. McMickle, M. Eichelberger, M.A. Blackman, P.C. Doherty, and D.L. Woodland. 1993. Prominent usage of V beta 8.3 T cells in the H-2Db-restricted response to an influenza A virus nucleoprotein epitope. J Immunol. 151:2658-2666.

35. Tough, D.F., and J. Sprent. 1994. Turnover of naive- and memory-phenotype T cells. J Exp Med. 179:1127-1135.

36. Sprent, J., and D.F. Tough. 1994. Lymphocyte life-span and memory. Science. 265:1395-1400.

37. Tough, D.F., P. Borrow, and J. Sprent. 1996. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science. 272:1947-1950.

38. Pihlgren, M., P.M. Dubois, M. Tomkowiak, T. Sjogren, and J. Maravel. 1996. Resting memory CD8+ T cells are hyperreactive to antigenic challenge in vitro. J Exp Med. 184:2141-2151.

39. Ehl, S., J. Hombach, P. Aichele, H. Hengartner, and R.M. Zinkernagel. 1997. Bystander activation of cytotoxic T cells studies on the mechanism and evaluation of in vivo significance in a transgenic mouse model. J Exp Med. 185:1241-1251.

40. Zanzonico, C.C., and R.M. Welsh. 1997. Minimal bystander activation of CD8+ T cells during the virus-induced polyclonal T cell response. J Exp Med. 185:672-639.

41. Butz, E.A., and M.J. Bevan. 1998. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity. 8:167-175.

42. Bennett, S.R., F.R. Carbono, F. Karamalis, R.A. Flavell, J.F. Miller, and W.R. Heath. 1998. Help for cytotoxic T-cell responses is mediated by CD40 signaling. Nature. 393:478-480.

43. Ridge, J.P., R.F. Di, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature. 393:474-478.

44. Schoenberger, S.P., R.E. Toes, E. van der Vooit, R. Ooffringa, and C.J. Mielie. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 393:480-483.

45. Oldstone, M.B. 1997. How viruses escape from cytotoxic T lymphocytes molecular parameters and players. Virol. 234:179-185.

46. Borrow, P., and G.M. Shaw. 1998. Cytotoxic T-lymphocyte escape viral variants: how important are they in viral evasion of immune clearance in vivo? Immunol. Rev. 164:37-51.

47. Wilson, J.D., G.S. Ogg, R.L. Allen, P.J. Goulder, A. Kelleher, A.K. Sewell, C.A. O’Callaghan, S.L. Rowland-Jones, M.F. Callan, and A.J. McMichael. 1998. Oligoclonal expansions of CD8+ T cells in chronic HIV infection are antigen specific. J Exp Med. 188:785-790.

48. Croft, M., L.M. Bradley, and S.L. Swain. 1994. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. J Immunol. 152:2675-2685.

49. Iezzi, G., K. Karjalainen, and A. Lanzavecchia. 1998. The duration of antigenic stimulation determines the fate of naive and effector T cells. Immunity. 8:89-95.

50. Dutton, R.W., L.M. Bradley, and S.L. Swain. 1998. T cell memory. Annu Rev Immunol. 16:201-223.

51. Selin, L.K., S.M. Varga, I.C. Wong, and R.M. Welsh. 1998. Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. J Exp Med. 188:1705-1715.