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Human Cytotoxic T-Cell Memory: Long-Lived Responses to Vaccinia Virus

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Peripheral T lymphocytes can be classified into two groups: naive and memory T cells. The focus of this study was to examine the duration of T-cell memory in humans. Vaccinia virus replicates in the cytoplasm of infected cells and is not thought to persist or become latent after the acute phase of infection. We identified long-lived vaccinia virus-specific memory cytotoxic T cells in adults who had been immunized against smallpox as children. Initially, we detected vaccinia virus-specific T cells in peripheral blood mononuclear cells while screening for human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses in HIV-1-seropositive subjects. These individuals had not had contact with vaccinia virus since their primary immunization in early childhood. Several vaccinia virus-specific $CD4^+$ T-cell clones were derived from these donors and characterized. Healthy, HIV-1-seronegative donors who had been immunized against smallpox many (35 to 50) years earlier were also screened for vaccinia virus-specific T-cell immunity. We found significant $CD8^+$ and $CD4^+$ cytotoxic T-cell responses to vaccinia virus after in vitro stimulation, indicating that these memory cells are maintained in vivo for many years. The peripheral blood mononuclear cells of young adults with no history of immunization against smallpox did not develop vaccinia virus-specific T-cell responses after in vitro stimulation. Precursor frequency analysis of the vaccinia virus-specific memory $CD4^+$ T cells from a donor immunized with vaccinia virus 35 years earlier revealed a frequency of 1 in 65,920 $CD4^+$ T cells. We concluded that specific vaccinia virus T-cell immunity can persist for up to 50 years after immunization against smallpox in childhood in the presumed absence of exposure to vaccinia virus.

The ability of a T cell to recognize a specific peptide epitope in the context of a major histocompatibility complex molecule on the surface of virus-infected cells is provided by its T-cell receptor (15, 16). The interaction between the T-cell receptor and the antigen-major histocompatibility complex triggers proliferation and clonal expansion of specific T cells (15, 16). The proliferative T-cell response continues during viral infections until the cells expressing the viral epitopes are eliminated or the virus becomes latent within cells and is no longer detectable by T cells. Some of the progeny of the antigen-responsive T cells develop into antigen-specific memory T cells. This subpopulation of T cells is maintained within the host and provides immune surveillance. In the event of reactivation of latent virus and expression of viral antigens, or subsequent natural reexposure to the virus, specific memory T cells become activated and clonally expand with greater magnitude than during the initial response. It is generally accepted that specific immune T-cell memory persists after an encounter with an antigen and may help to protect the immune host against subsequent exposure to that pathogen. The underlying mechanisms which contribute to immunological T-cell memory are poorly understood and have only recently received much attention (11, 14, 17, 20, 22).

Vigorous and long-lasting protective immune responses have long been associated with smallpox vaccination. After a single immunization with vaccinia virus, virus-specific $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes (CTL) are generated, as evidenced by in vitro restimulation of memory T cells (7, 19). Specific immunity is believed to be maintained for many years, and booster immunizations are recommended every 10 years for those at risk for infection. Because of the decreased risk of smallpox infection and side effects of vaccination, vaccinia virus has not been generally used since routine vaccinations were discontinued over 20 years ago. Laboratory workers using vaccinia virus and some members of the military continue to be vaccinated. Vaccinia virus does not cause persistent or latent infections, and therefore repeated endogenous antigenic stimulation is not thought to occur (5). These characteristics of vaccination against smallpox led us to investigate the longevity of vaccinia virus-specific memory responses in humans.

Peripheral blood mononuclear cells (PBMC) of human immunodeficiency virus type 1 (HIV-1)-seropositive donors exhibit vaccinia virus-specific cytotoxic activity against autologous B-LCL cells expressing vaccinia antigen. Cryopreserved PBMC from 22 asymptomatic HIV-1-seropositive donors and 8 HIV-1-seronegative, healthy donors were tested directly in a ^{51}Cr release assay as previously described for HIV-1 envelope antigen-specific cytotoxicity by using autologous B-LCL target cells that were either uninfected or infected with vaccinia virus or the recombinant V/gp160 (4, 18). We detected significant HIV-1 envelope antigen-specific cytotoxicity in the PBMC of 2 (9%) of the HIV-1-seropositive donors tested (data not shown), but significant lysis of target cells expressing vaccinia virus antigens was observed using the PBMC of 6 (27%) of the HIV-1-seropositive donors, as shown in Table 1. K562 cells were used as target cells to measure natural killer cell activity.

Establishment of vaccinia virus-specific $CD4^+$ CTL lines. Several cell lines were obtained that specifically lysed vaccinia virus-infected target cells after stimulation of isolated PBMC with γ-irradiated allogeneic PBMC, anti-CD3 monoclonal antibody 12F6 (0.1 μg/ml), kindly supplied by J. Wong as previ-

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TABLE 1. Vaccinia virus-specific cytotoxic activity in PBMC of asymptomatic HIV-1-seropositive donors[a]

Donor no.	% Specific lysis of target cells			
	Uninfected B-LCL cells	Vaccinia virus-infected B-LCL cells	V/gp160-infected B-LCL cells	Uninfected K562 cells
2	17.2	29.8	23.8	1.8
11	8.9	31.8	29.0	1.7
12	10.1	25.9	18.4	2.3
13	2.8	13.5	15.4	2.1
26	7.0	17.4	13.1	2.5
27	2.3	17.8	15.4	3.3

[a] Unstimulated PBMC were tested directly in a CTL assay. Cytotoxicity was determined in a 6-h ^{51}Cr release assay effector-target cell at an ratio of 50:1. ^{51}Cr release was calculated by the formula 100 × (mean experimental release − mean spontaneous release)/(mean total release − mean spontaneous release). The results of an assay were excluded if the mean level of spontaneous release was >30%.

ously described ([28]), and recombinant human interleukin-2. These cell lines were expanded and subcloned by limiting dilution at 0.3, 1, or 3 cells per well. We isolated vaccinia virus-specific cytotoxic T-cell clones from each of three HIV-1-seropositive donors (Table 2). These cell lines lysed autologous target cells infected with vaccinia virus, and the lysis of K562 cells was low. Phenotypic analysis of these CTL lines revealed that they were all $CD3^+$ $CD4^+$ $CD8^-$ $Leu11^-$.

Demonstration of long-lived vaccinia virus-specific $CD8^+$ CTL responses. Presumably, the HIV-1-seropositive donors from whom the $CD4^+$ clones were generated had not been exposed to vaccinia virus since childhood vaccination, because the donors were over 30 years of age, had not served in the military, and had not been vaccinated since early in childhood. To determine whether vaccinia virus-specific CTL precursors are maintained in the peripheral blood of individuals for long periods of time after immunization in early childhood, two healthy HIV-1 antibody-negative donors, VA15 and VA16, were identified who were known to have been immunized as children with vaccinia virus 35 and 50 years earlier, respectively. These donors stated that they had no subsequent exposure to vaccinia virus or to any other poxvirus. We stimulated these two donors' PBMC with live vaccinia virus in vitro in an attempt to detect vaccinia virus-specific memory $CD8^+$ CTL because we had only isolated $CD4^+$ major histocompatibility complex class II-restricted CTL from the HIV-1-seropositive donors by using anti-CD3 antibody and interleukin-2 stimulations. This method of stimulation with live vaccinia virus had been used earlier to successfully generate vaccinia virus-specific $CD8^+$ major histocompatibility complex class I-restricted CTL in vitro (7).

Donor PBMC were stimulated in vitro with live vaccinia virus, which induced a modest level of vaccinia virus-specific proliferation, and the cultures were restimulated with anti-CD3 antibody on day 7. On day 14, the cultures were restimulated with live vaccinia virus and assayed on day 21 for cytotoxic activity. The bulk cultured cells derived from donors VA15 and VA16 lysed vaccinia virus-infected autologous B-LCL cells at levels of 50.8 and 44.2%, respectively, at an effector-target cell ratio of 50:1 (Table 3). Incubation of effector cells with anti-$CD3^+$ or anti-$CD8^+$ specific monoclonal antibodies in the presence of complement reduced the levels of lysis significantly, indicating that $CD8^+$ CTL were the major effector cells in this virus-stimulated population. There was some decrease in the level of lysis after treatment with either anti-$CD4^+$ antibodies and complement or anti-CD16 antibodies and complement, suggesting a contribution by vaccinia virus-specific $CD4^+$ CTL and NK cells (Table 3).

To confirm that the vaccinia virus-specific CTL activity observed in these cultures was not a result of primary in vitro stimulation, the PBMC of two healthy, young adult donors who had no history of immunization with vaccinia virus were used as controls. The PBMC from these donors, VA21 and VA23, were stimulated in a fashion identical to that used for the PBMC of donors VA15 and VA16, were assayed on day 21 for vaccinia virus-specific CTL activity, and had none (Table 3).

Frequency analysis of vaccinia virus-specific CTL memory cells. As described above, the PBMC isolated from donor VA15, who had been immunized with vaccinia virus more than 35 years ago, exhibited vaccinia virus-specific CTL activity after stimulation in vitro. We wanted to determine the frequency of vaccinia virus-specific memory T cells within the PBMC of this donor. We assumed that the precursor frequency of vaccinia virus-specific CTL would be low, so preliminary experiments were performed to ensure that we could detect a positive response. Sorting of donor PBMC by fluorescence-activated cell sorter analysis yielded a relatively pure population of $CD4^+$ T cells to assay for vaccinia virus-specific memory T cells. Microcultures were initiated under limiting-dilution conditions with 5,000 to 60,000 sorted $CD4^+$ T cells per well in 24 replicate wells. Each microculture received 2×10^5 γ-irradiated autologous PBMC in 200 μl of RPMI 1640 medium supplemented with 20% fetal calf serum, 40 U of interleukin-2 per ml, and vaccinia virus antigen. Vaccinia virus antigen was prepared by infecting a confluent monolayer of CV-1 cells with virus at a multiplicity of infection of 10. After 24 h of incubation, when the cytopathic effect was extensive, the cells were harvested by scraping. After a freeze-thaw cycle, sonication was performed and the cell-virus extract was boiled for 10 min to inactivate any residual infectious virus before use. Generally, a confluent monolayer of vaccinia virus-infected CV-1 cells in a 75-cm^2 tissue culture flask (approximately 12×10^6 cells) would yield 2 ml of cell-virus extract. At 3-day intervals, one-half of the culture medium was removed and replaced with

TABLE 2. Lysis of vaccinia virus-infected target cells by T-cell clones from HIV-1-seropositive donors[a]

Donor no.	Clone no.	% Specific lysis of target cells					
		Uninfected B-LCL cells	Vaccinia virus-infected B-LCL cells	V/gag-infected B-LCL cells	V/pol-infected B-LCL cells	V/gp160-infected B-LCL cells	Uninfected K562 cells
11	214	0	71.8	62.8	64.8	73.5	10.4
2	140	3.1	27.7	29.6	29.3	12.6	9.8
12	109	11.4	32.8	26.5	NT[b]	18.5	3.3

[a] Cytotoxicity was determined in a 5-h ^{51}Cr release assay at an effector-target cell ratio of 4:1. Percent specific ^{51}Cr release was calculated as described in Table 1, footnote *a*.

[b] NT, not tested.

TABLE 3. Characterization of bulk culture cytotoxicity activity

Donor (day) and treatment	% specific ^{51}Cr release from target cells[a]		
	Uninfected B-LCL cells	Vaccinia virus-infected B-LCL cells	Uninfected K562 cells
VA15 (21)			
Complement	−0.2	50.8	23.1
Anti-CD4$^+$ antibodies, complement	−0.3	32.9	14.7
Anti-CD8$^+$ antibodies, complement	−3.3	−5.5	9.8
Anti-CD3$^+$ antibodies, complement	−3.4	−5.1	2.7
Anti-CD16$^+$ antibodies, complement	3.8	37.4	13.0
VA16 (21)			
Complement	3.3	44.2	35.3
Anti-CD4$^+$ antibodies, complement	0.0	36.9	27.3
Anti-CD8$^+$ antibodies, complement	1.7	3.4	19.0
Anti-CD3$^+$ antibodies, complement	−1.3	−0.2	15.7
Anti-CD16$^+$ antibodies, complement	2.3	36.2	25.9
VA21 (21)			
Complement	−1.9	−3.5	0.3
Anti-CD3$^+$ antibodies, complement	−1.1	−4.9	1.1
Anti-CD16$^+$ antibodies, complement	−2.8	−2.7	0.4
VA23 (21)			
Complement	3.2	7.2	−0.1
Anti-CD3$^+$ antibodies, complement	0.6	3.3	−0.7
Anti-CD16$^+$ antibodies, complement	3.6	2.3	−0.2

[a] Effector-target cell ratio, 50:1.

fresh medium without vaccinia virus antigen as described above. On day 10, each well was split and cells were assayed for cytotoxicity on autologous uninfected B-LCL cells or B-LCL cells infected with vaccinia virus. Individual wells were considered positive if the calculated specific lysis of the virus-infected target cells was greater than 3 standard deviations above the mean levels of lysis calculated from negative wells. Precursor frequencies were calculated by using χ^2 analysis as described by Taswell (27), by using a computer program kindly provided by Richard Miller (University of Michigan, Ann Arbor). Analysis of the precursor frequency of CD4$^+$ vaccinia virus-specific memory T cells resulted in a calculated frequency of 1 in 65,920 sorted CD4$^+$ T cells (95% confidence interval, 48,731 to 101,844 cells) or 3 in 10^6 PBMC (Fig. 1). Three experiments were performed on the sorted CD4$^+$ T cells, and the calculated frequencies for each experiment were consistent, differing by less than 2%. This calculated frequency is lower than the reported precursor frequencies for varicella-zoster virus-, HIV-1-, and cytomegalovirus-specific T cells (3, 12, 13), but those viruses cause persistent and/or latent infections, unlike vaccinia virus.

The long-lived T-cell memory responses we observed are striking; however, a recent study suggested that memory T-cell responses to vaccinia virus may be long lasting. In a randomized phase 1 trial reported by Cooney et al., 35 healthy, HIV-1-seronegative, young adult males, 31 of whom had a history of smallpox immunization and 4 of whom were vaccinia virus naive, were immunized with a recombinant vaccinia virus vaccine expressing the gp160 envelope gene of HIV-1 (6). Individuals who had been immunized as young children with vaccinia virus had poor immune responses to the HIV-1 gp160 antigen compared with those who had no previous exposure to vaccinia virus. The results from this trial suggested that long-lasting immunity to vaccinia virus limited replication of the recombinant vaccinia virus used for immunization (6). The observations made during that study and data reported recently on the use of vaccinia virus recombinants expressing herpes simplex virus gene products in mice (8, 24) are consistent with our finding that vaccinia virus-specific memory T-cell responses are long lasting and may persist for life.

The results from the complement depletion assay using bulk-cultured cells demonstrated that CD8$^+$ memory CTL activity was dominant in short-term virus stimulated bulk cultures. We expected that the precursor frequency of the CD8$^+$

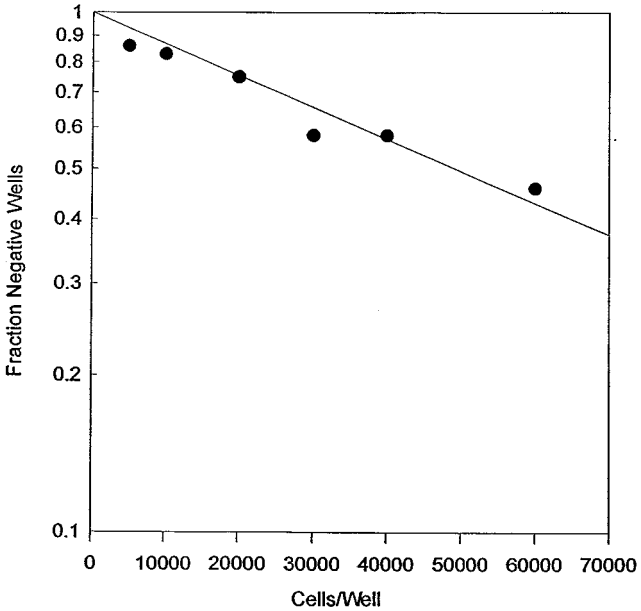

FIG. 1. Frequency of vaccinia virus-specific lytic effectors among sorted CD4$^+$ lymphocytes. Sorted CD4$^+$ T cells were stimulated with vaccinia virus antigen. Lytic activities were measured in a CTL assay on day 10. The frequency of vaccinia virus-specific CD4$^+$ CTL in donor VA15 was calculated as 1 in 65,920 CD4$^+$ T cells.

memory T-cell population would be greater than that observed for the $CD4^+$ T cells; however, we found it difficult to determine the precursor frequency of $CD8^+$ memory T cells. The number of $CD8^+$ cells isolated by fluorescence-activated cell sorter analysis was only one-third of the number of $CD4^+$ T cells, and our culture conditions with live virus may not have been optimal for detection of relatively low numbers of vaccinia virus-specific $CD8^+$ T cells.

The underlying mechanisms which contribute to immunological memory are poorly understood and have only recently received much attention. The major question with respect to persistent immunological T-cell memory is: how is it maintained in vivo? There is controversy about whether the maintenance of memory T cells requires periodic interaction with antigen-presenting cells expressing the relevant peptide (11, 22) or whether T-cell "memory" might be maintained in the absence of specific antigen stimulation (17, 20). Oehen et al. reported that adoptive transfer of immune spleen cells into syngeneic recipient mice required the presence of viral antigen for maintenance of the antiviral protective capacity of the transferred cells (22). Gray and Matzinger reported similar results (11). Other reports have challenged those findings (17, 20). Lau et al. used adoptive-transfer experiments in the lymphocytic choriomeningitis virus mouse model and reported that memory $CD8^+$ CTL persist and retain the memory phenotype indefinitely in the apparent absence of priming antigen, and these CTL apparently protected mice against virus challenge for up to 2 years (17). Mullbacher utilized a similar approach in a mouse model of influenza virus and also concluded that CTL memory is long-lived in the apparent absence of antigen (20). Our results obtained with PBMC of adult humans following immunization with vaccinia virus in early childhood also suggest that persistence of antigen is not required for long-term maintenance of T-cell memory. Although there is no evidence for the persistence of vaccinia virus or antigens in vivo, dendritic cells may sequester antigen for periods of time, making it available for persistent stimulation of the immune system (10).

If antigen persistence is not required, what other mechanisms contribute to long-lived, specific T-cell memory? One mechanism that has been suggested is immunological cross-reactivity between viruses (2, 21, 25). Selin et al. used percursor frequency analyses to study virus cross-reactive T-cell responses in mouse models and postulated that exposure to one virus might provide a boost in immunity to an unrelated virus (25). They could not rule out the possibility of enhanced nonspecific stimulation by lymphokines generated during the immune response to the heterologous virus, but their results tend to support the cross-reactivity hypothesis. In the absence of significant homology among unrelated viruses, they suggested that the observed T-cell cross reactivity may be due to cross-reactive epitopes possessing major amino acid differences but having discrete critical residues in common (25). This hypothesis may be reasonable in light of what is known about the phenotype of memory T cells (1, 9). When T cells acquire a memory phenotype, they upregulate the expression of several surface adhesion molecules in addition to the interleukin-2 receptor and become more sensitive to stimulation by a low-affinity, T-cell-specific peptide (21, 25). This "promiscuous" behavior may allow a memory T cell to become activated through an interaction of its T-cell receptor with an antigen-presenting cell presenting a peptide epitope from a virus unrelated to the virus that induced the original immune response. Shimojo et al. characterized a T-cell line generated against an influenza virus-encoded peptide which specifically recognized a dissimilar rotavirus-derived peptide (26), which supports this hypothesis. There is an increasing number of examples of immunological cross reactivity between proteins of infectious organisms and human proteins (23), and this molecular mimicry at the peptide level may play a role in T-cell cross reactivity in vivo. The interaction of T cells with antigen-presenting cells expressing self peptides which mimic peptide epitopes of infectious agents may also stimulate their propagation. Thus, memory T cells may be promiscuous in their ability to recognize various peptides and may be stimulated in a cross-reactive fashion.

The data presented here are perhaps the first clear evidence that virus-specific T-cell memory can persist for up to 50 years in humans in the presumed absence of antigen. We believe that human subjects with prior exposure to vaccinia virus years earlier provide an excellent model for the study of human T-cell memory. Further elucidation of the underlying mechanisms which contribute to the maintenance of T-cell memory will have an impact on our understanding of the basis of immunological memory and on the design of vaccines.

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REFERENCES

1. **Ahmed, R.** 1992. Immunological memory against viruses. Semin. Immunol. **4:** 104–109.
2. **Beverley, P. C. L.** 1990. Is T-cell memory maintained by crossreactive stimulation? Immunol. Today **11:**203–205.
3. **Borysiewicz, L. K., S. Graham, J. K. Hickling, P. D. Mason, and J. G. P. Sissons.** 1988. Human cytomegalovirus-specific cytotoxic T cells: their precursor frequency and stage specificity. Eur. J. Immunol. **18:**269–275.
4. **Bukowski, J. F., I. Kurane, C.-J. Lai, M. Bray, B. Falgout, and F. A. Ennis.** 1989. Dengue virus-specific cross-reactive $CD8^+$ human cytotoxic T lymphocytes. J. Virol. **63:**5086–5091.
5. **Buller, R. M. L., and G. J. Palumbo.** 1991. Poxvirus pathogenesis. Microbiol. Rev. **55:**80–122.
6. **Cooney, E. L., A. C. Collier, P. D. Greenberg, R. W. Coombs, J. Zarling, D. E. Arditti, M. C. Hoffman, S.-L. Hu, and L. Corey.** 1991. Safety of and immunological response to a recombinant vaccinia virus vaccine expressing HIV envelope glycoprotein. Lancet **337:**567–572.
7. **Demkowicz, W. E., Jr., and F. A. Ennis.** 1993. Vaccinia virus-specific $CD8^+$ cytotoxic lymphocytes in humans. J. Virol. **67:**1538–1544.
8. **Flexner, C., B. R. Murphy, J. F. Rooney, et al.** 1988. Successful vaccination with a polyvalent live vector despite existing immunity to an expressed antigen. Nature (London) **335:**259–262.
9. **Gray, D.** 1993. Immunological memory. Annu. Rev. Immunol. **11:**49–77.
10. **Gray, D., M. Kosco, and B. Stockinger.** 1991. Novel pathways of antigen presentation for the maintenance of memory. Int. Immunol. **3:**141–148.
11. **Gray, D., and P. Matzinger.** 1991. T cell memory is short lived in the absence of antigen. J. Exp. Med. **174:**969–974.
12. **Hickling, J. K., L. K. Borysiewicz, and J. G. P. Sissons.** 1987. Varicella-zoster virus-specific cytotoxic T lymphocytes (Tc): detection and frequency analysis of HLA class I-restricted Tc in human peripheral blood. J. Virol. **61:**3463–3469.
13. **Hoffenbach, A., P. Langlade-Demoyen, G. Dadaglio, E. Vilmer, F. Michel, C. Mayaud, B. Autran, and F. Plata.** 1989. Unusually high frequencies of HIV-specific cytotoxic T lymphocytes in humans. J. Immunol. **142:**452–462.
14. **Hou, S., L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty.** 1994. Virus-specific CD8+ T cell memory is determined by clonal burst size. Nature (London) **369:**652–654.
15. **Jorgensen, J. L., et al.** 1992. Molecular components of T cell recognition. Annu. Rev. Immunol. **10:**835–873.
16. **Kaye, J., et al.** 1991. Structure and specificity of the T cell antigen receptor. Semin. Immunol. **3:**269–281.
17. **Lau, L. L., B. D. Jamieson, T. Somasundaram, and R. Ahmed.** 1994. Cytotoxic T-cell memory without antigen. Nature (London) **369:**648–652.
18. **Littaua, R. A., M. B. A. Oldstone, A. Takeda, C. Debouck, J. T. Wong, C. U. Tuazon, B. Moss, F. Kievits, and F. A. Ennis.** 1991. An HLA-C-restricted CD8+ cytotoxic T-lymphocyte clone recognizes a highly conserved epitope on human immunodeficiency virus type 1 *gag*. J. Virol. **65:**4051–4056.
19. **Littaua, R. A., A. Takeda, J. Cruz, and F. A. Ennis.** 1992. Vaccinia virus-specific human $CD4^+$ cytotoxic T-lymphocyte clones. J. Virol. **66:**2274–2280.
20. **Mullbacher, A.** 1994. The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. J. Exp. Med. **179:**317–321.
21. **Nahill, S. R., and R. M. Welsh.** 1992. Polyclonality of the cytotoxic T lymphocyte response to virus infection. Proc. Soc. Exp. Biol. Med. **200:**453–457.

22. **Oehen, S., H. Waldner, T. M. Kudig, H. Hengartner, and R. M. Zinkernagel.** 1992. Antivirally protective cytotoxic T cell memory to lymphocytic choriomeningitis virus is governed by persisting antigen. J. Exp. Med. **176:**1273–1281.
23. **Oldstone, M. B. A.** 1987. Molecular mimicry and autoimmune disease. Cell **50:**819–820.
24. **Rooney, J. F., C. Wohlenberg, K. J. Cremer, B. Moss, and A. L. Notkins.** 1988. Immunization with a vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D: long-term protection and effect of revaccination. J. Virol. **62:**1530–1534.
25. **Selin, L. K., S. R. Nahill, and R. M. Welsh.** 1994. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. J. Exp. Med. **179:**1933–1943.
26. **Shimojo, N., W. L. Maloy, R. W. Anderson, W. E. Biddison, and J. E. Coligan.** 1989. Specificity of peptide binding by the HLA-A2.1 molecule. J. Immunol. **143:**2939–2947.
27. **Taswell, C.** 1981. Limiting dilution assays for the determination of immunocompetent cell frequencies. J. Immunol. **126:**1614–1619.
28. **Walker, B., C. Flexner, K. Birch-Limberger, L. Fisher, T. Paradis, A. Aldovini, R. Young, B. Moss, and R. Schooley.** 1989. Long-term culture and fine specificity of human cytotoxic T-lymphocyte clones reactive with human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **86:**9514–9518.