JOURNAL OF VIROLOGY, Nov. 1996, p. 8165–8168 Vol. 70, No. 11
0022-538X/96/$04.00+0

High Frequency of CD8$^+$ Cytotoxic T-Lymphocyte Precursors Specific for Herpes Simplex Viruses in Persons with Genital Herpes

CHRISTINE M. POSAVAD,[1] DAVID M. KOELLE,[2] AND LAWRENCE COREY[1,2]*

Departments of Laboratory Medicine[1] and Medicine,[2] University of Washington, Seattle, Washington 98195

Received 23 May 1996/Accepted 26 July 1996

Herpes simplex virus (HSV)-specific CD8$^+$ cytotoxic T lymphocytes (CTL) have rarely been detected in humans, presumably because of virus-induced mechanisms that downregulate major histocompatibility complex class I expression. We have developed a method that has allowed us to consistently demonstrate HSV-specific CD8$^+$ precursor CTL (pCTL) from HSV type 1- and 2-seropositive persons. Major histocompatibility complex-restricted HSV-specific CD8$^+$ pCTL were found in 10 consecutively tested HSV type 1- and 2-seropositive subjects at frequencies ranging from 1 in 21,000 to 1 in 300 (median, 1 in 6,000) versus a pCTL frequency of 1 in 100,000 in HSV-seronegative donors. These results suggest that CD8$^+$ CTL are important effector cells in resolving HSV lesions.

Herpes simplex virus type 2 (HSV-2) is the major cause of ulcerative lesions of the genital tract (6). The prevalence of genital HSV-2 infections is increasing worldwide, and over 20% of the U.S. population is infected with HSV-2 (16). The infections reactivate in over 90% of patients infected with HSV-2, and severe infections are common in immunocompromised individuals and newborns (6). A large body of work has shown the importance of T-cell immunity in the control and resolution of HSV-2 infections. However, little is known about which T-cell effector responses are relevant in humans.

In contrast to other herpesviruses, in which major histocompatibility complex (MHC)-restricted CD8$^+$ cytotoxic T lymphocytes (CTL) have been readily isolated (1, 2, 3, 9), almost all published reports of HSV-specific CTL have found these cells to be CD4$^+$ and HLA class II restricted (11, 12, 17, 20, 21). Proposed explanations for this have revolved around several virus-induced mechanisms to decrease the expression of peptide-class I complexes on the surface of antigen-presenting cells (7, 10, 14, 22), hence hindering the activation and expansion of HSV-specific CD8$^+$ CTL.

This report describes a recently developed method that demonstrates that MHC-restricted HSV-specific CD8$^+$ CTL are present in high precursor frequency in all patients with HSV-1 and HSV-2. In preliminary experiments, we compared the use of UV-inactivated HSV-2 (HSV-Ag) (12) with the use of paraformaldehyde-fixed phytohemagglutinin (PHA)-blasts previously infected for 18 h with HSV-2 strain 333 (HSV-blasts) as methods of stimulation for the activation of HSV-specific precursor CTL (pCTL) from peripheral blood mononuclear cells (PBMC) in standard limiting-dilution analyses (LDA). In contrast to unstimulated PBMC, which are nonpermissive for HSV infection, PHA-stimulated PBMC support virus replication (4, 8). In parallel cultures established, handled, and measured identically, the frequency of HSV-specific CD8$^+$ pCTL from patient A was 1 in 1,000 from cultures using HSV-blasts as stimulators versus 1 in 17,000 in those using soluble HSV-2 antigen (Fig. 1). HSV-blasts were used as stimulators in all subsequent experiments.

With this method, HSV-specific CD8$^+$ pCTL were detected in all 10 consecutively tested HSV-2-seropositive patients. pCTL frequencies ranged from 1 in 21,000 to 1 in 300, with a median of 1 in 6,000 (Table 1). In contrast, the frequencies of pCTL in five HSV-seronegative patients ranged from 1 in 538,000 to 1 in 47,000, with a median of 1 in 99,000, levels significantly lower than those of HSV-seropositive patients ($P = 0.001$ [Mann-Whitney]). To determine the reproducibility of pCTL frequencies in a single patient, LDA were performed three times with three HSV-seropositive patients, and pCTL frequencies all fell within the same 95% confidence interval (data not shown).

To determine if HSV-specific CD8$^+$ CTL generated from HSV-blast stimulation were MHC restricted, cells within LDA test wells were tested for lytic activity against MHC class I-mismatched, HSV-2-infected lymphoblastoid cell lines (LCL). Large differences were seen between the frequency of CD8$^+$ CTL that lysed HSV-infected autologous LCL and the frequency of CD8$^+$ CTL that lysed HSV-infected allogeneic LCL mismatched at MHC class I (Fig. 2). Similar results were seen in two additional patients (data not shown). Moreover, on a clonal basis, all HSV-specific CD8$^+$ CTL lysed target cells in an MHC class I-restricted manner (see below).

To determine the virus type specificity of HSV-specific pCTL, CD8$^+$ responder cells were selected from patient K, who was seropositive for HSV-1 only; patients G and E, who were seropositive for HSV-2 only; and patient J, who was seropositive for both HSV-1 and HSV-2. Positively selected CD8$^+$ cells were stimulated with PHA-blasts infected with HSV-1 strain E115 or HSV-2 in an LDA. LDA cultures were tested for lytic activity against autologous LCL infected with HSV-1 or HSV-2. No significant differences were seen in HSV-specific pCTL frequencies in patient K, J, or E when HSV-1 blasts or HSV-2 blasts were used to stimulate CTL and HSV-1 LCL or HSV-2 LCL were used as target cells (Fig. 3). In contrast, patient G, who was seropositive for HSV-2 only, had a significantly greater CD8$^+$ pCTL response to HSV-2 blasts than to HSV-1 blasts (Fig. 3). However, HSV-2 stimulation of CD8$^+$ responder cells from patient G had only slightly lower pCTL frequencies directed against HSV-1 LCL versus HSV-2 LCL. Therefore, type-common responses were detected in the four patients studied regardless of their serostatus.

Wells were also tested for lytic activity against the NK-

* Corresponding author. Mailing address: Department of Laboratory Medicine, Box 358260, University of Washington, Seattle, WA 98195. Phone: (206) 621-4177. Fax: (206) 621-4178. Electronic mail address: lcorey@u.washington.edu.

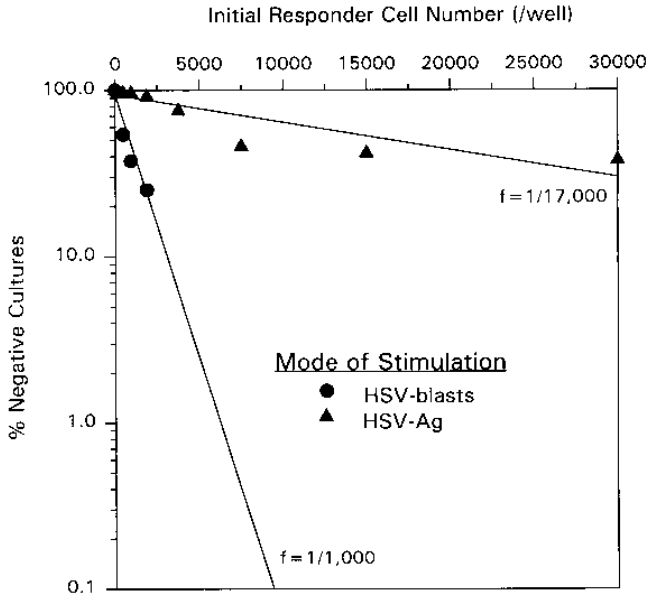

FIG. 1. Frequency of HSV-specific $CD8^+$ pCTL stimulated with HSV-blasts versus HSV-Ag. $CD8^+$ cells were positively selected from PBMC with CD8 microbeads (Miltenyi Biotec Inc., Auburn, Calif.) from patient A and were aliquoted in 100 μl in seven serial twofold dilutions starting at 3×10^4 $CD8^+$ cells per well in 96-well round-bottom plates. Twenty-four replicates were plated for each dilution with 5×10^4 irradiated autologous PBMC (feeders) per well and 1×10^4 HSV-blasts per well or a 1:100 dilution of HSV-Ag (UV-inactivated HSV-2). LDA cultures were fed 5 U of interleukin-2 (Schiaperelli Biosystems, Columbia, Md.) per ml on days 2, 5, and 8. After 10 days, wells were tested for lytic activity against 2×10^3 ^{51}Cr-labeled mock-infected or HSV-infected autologous LCL in a standard ^{51}Cr release assay. A well was scored as positive if the radioactivity in the experimental well was greater than 3 standard deviations above the mean ^{51}Cr release value obtained from 24 control wells (containing no $CD8^+$ responder cells). Wells positive for both HSV-infected and mock-infected LCL were excluded. The percentage of negative cultures was plotted against the initial responder cell number per well and the frequency (f) calculated by the chi-square minimization method (5, 18). This comparison was repeated two additional times for different individuals: with HSV-blasts, the pCTL frequencies were 1 in 14,000 and 1 in 14,000 versus 1 in 80,000 and 1 in 23,000 with HSV-Ag-stimulated cells.

sensitive cell line K562 to determine the level of NK-like killing generated by stimulating $CD8^+$ cells with HSV-blasts. Although a low frequency of HSV-blast-stimulated cells lysed K562 cells, the frequencies of $CD8^+$ cells that lysed K562 cells were not statistically significantly different between HSV-seropositive patients (median, 1 in 26,000; range, 1 in 77,000 to 1 in 4,000; $n = 6$) and HSV-seronegative patients (median, 1 in 37,000; range, 1 in 250,000 to 1 in 28,000; $n = 5$) ($P = 0.16$ [t test]). The low frequency of HSV-blast-stimulated $CD8^+$ cells capable of lysing K562 cells further confirms that the effector cells in the LDA wells were classic MHC-restricted $CD8^+$ CTL.

To ensure that $CD8^+$ T cells were responsible for the CTL activity measured after 10 days of culture with HSV-blasts, the cell phenotypes of six LDA test wells were determined by fluorescence-activated cell sorter analysis. The cells in all six wells were >95% $CD3^+$ $CD8^+$ and <5% $CD4^+$ (Table 2). Moreover, all six wells specifically lysed HSV-infected autologous LCL and not HSV-infected allogeneic LCL mismatched at class I MHC, indicating that $CD8^+$ CTL were expanded during culture and were the effectors responsible for specifically lysing infected target cells.

To verify the presence of HSV-specific $CD8^+$ CTL in PBMC of persons with genital herpes, eight $CD8^+$ HSV-specific CTL clones were isolated from two different patients. All eight clones killed autologous HSV-2-infected LCL but not mock-infected autologous LCL, allogeneic HSV-LCL, or K562 cells (Table 3). Clones from patient A were prevented from lysing HSV-2 infected LCL w6/32, a monoclonal antibody that inhibits MHC class I-restricted killing, demonstrating that these CTL were MHC class I restricted. Of the eight $CD8^+$ CTL clones, four were type common in that they recognized HSV-1- and HSV-2-infected LCL while four were type specific, lysing only HSV-2-infected LCL (Table 3).

Discussion. Our study is the first to consistently demonstrate the presence of HSV-specific $CD8^+$ T cells in high precursor frequencies in persons with mucocutaneous HSV-2 infections. All 10 consecutively tested persons with recurrent genital herpes had HSV-2-specific $CD8^+$ pCTL at frequencies of 1 in 21,000 to 1 in 300 (median, 1 in 6,000). The frequencies of pCTL determined by identical methods in HSV-seronegative donors were 20 to 100 times lower. HSV-specific $CD8^+$ CTL were MHC class I restricted and displayed low levels of NK-like activity as detected in both bulk and cloning assays.

The pCTL frequencies we observed are similar to those seen with infections with other herpesviruses, such as cytomegalovirus (2), Epstein-Barr virus (3), and varicella-zoster virus (1, 9). The high frequency of $CD8^+$ CTL in HSV-2-seropositive persons in our current study, that was not seen in previous studies, is likely due to the selection of $CD8^+$ cells from PBMC prior to culture initiation and the utilization of paraformaldehyde-fixed, HSV-infected PHA-blasts as stimulator cells.

One interesting feature of our study is that the pCTL frequencies we observed were relatively similar in persons with HSV-2 antibodies only versus persons seropositive for HSV-1 and HSV-2. Both type-common and type-specific CTL clones were isolated. Type-common CTL responses were detected in patients whether they were seropositive for HSV-1 only, HSV-2 only, or both HSV-1 and HSV-2. While we studied only

TABLE 1. Frequencies of HSV-specific pCTL in PBMC from HSV-seropositive and -seronegative patients[a]

Patient	HSV serostatus[b]	HSV-specific $CD8^+$ pCTL	
		Frequency[c]	95% confidence interval
A	2	1/1,000	1/800–1/1,400
B	2	1/14,000	1/11,000–1/19,000
C	1 + 2	1/8,000	1/6,000–1/11,000
D	2	1/3,000	1/2,000–1/4,000
E	2	1/7,000	1/5,000–1/10,000
F	2	1/300	1/270–1/450
G	2	1/5,000	1/3,000–1/7,000
H	1 + 2	1/1,000	1/800–1/1,200
I	1 + 2	1/14,000	1/11,000–1/21,000
J	1 + 2	1/21,000	1/14,000–1/30,000
L	Neg	1/538,000	1/200,000–1/750,000
M	Neg	1/99,000	1/64,000–1/150,000
N	Neg	1/90,000	1/67,000–1/137,000
O	Neg	1/47,000	1/40,000–1/57,000
P	Neg	1/170,000	1/120,000–1/300,000

[a] LDA was performed on positively selected $CD8^+$ cells from PBMC of HSV-2-seropositive and -seronegative donors by using HSV-blasts as stimulators. Wells were tested for lytic activity against ^{51}Cr-labeled targets: HSV-infected and mock-infected autologous LCL. Wells with positive lytic activity for mock-infected autologous LCL were excluded. The frequency was calculated by the chi-square minimization method.

[b] Patients were seropositive for HSV-1 only, HSV-2 only, or both HSV-1 and HSV-2 or were seronegative for both HSV-1 and HSV-2 (Neg).

[c] The media frequencies for patients A to J and L to P were 1 in 6,000 and 1 in 99,000, respectively.

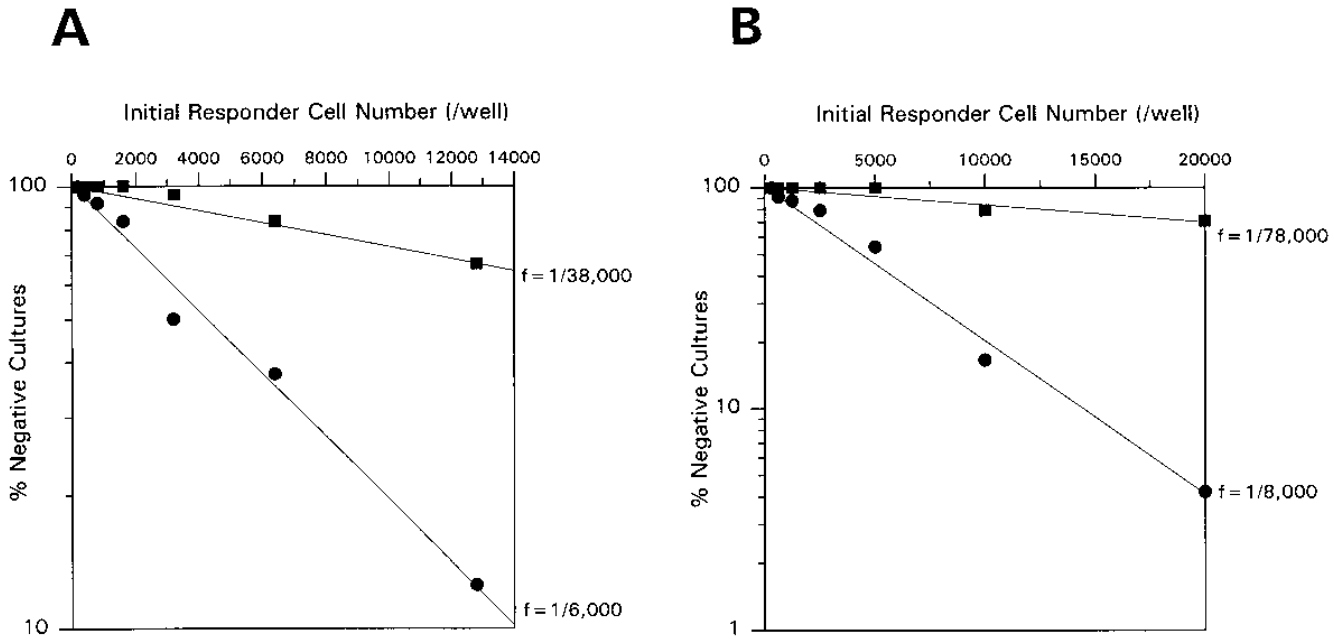

FIG. 2. MHC restriction of HSV-specific $CD8^+$ CTL from PBMC. $CD8^+$ cells were positively selected from PBMC from patients E (A) and C (B), set up at various concentrations with HSV-blasts, and incubated for 10 days. Wells were tested for lytic activity against the following ^{51}Cr-labeled targets: HSV-infected autologous LCL (●) and HSV-2-infected allogeneic LCL (■). The percentage of negative cultures was plotted against the number of initial responder cells per well, and the frequency (f) was calculated by the chi-square minimization method.

one person who was seropositive for HSV-1 only, the pCTL frequency in this person was 1 in 4,000, similar to that of the persons seropositive for HSV-2.

The detection of high frequencies of HSV-specific MHC-restricted $CD8^+$ pCTL is interesting in light of the recently identified mechanism by which ICP47 downregulates the expression of MHC class I molecules on HSV-infected fibroblasts (7, 9, 22). There are several explanations for these observations. A previous study has shown that the downregulation of class I expression by HSV is quantitatively much lower in LCL and monocyte-macrophages than in keratinocytes and fibroblasts (14). These cells, even if infected with HSV, are likely to be capable of helping to elicit a $CD8^+$ CTL response. This cell-specific downregulation could allow the virus to achieve productive infection in epithelial cells and hence facilitate its transmission. However, antigen presentation by cells less susceptible to the effects of ICP47 could promote a $CD8^+$ T-cell response to resolve the infection. In addition, the downregulation of peptide-class I complexes on the surfaces of HSV-infected cells may be overcome by the host. Gamma interferon, levels of which are markedly elevated during reactivation of

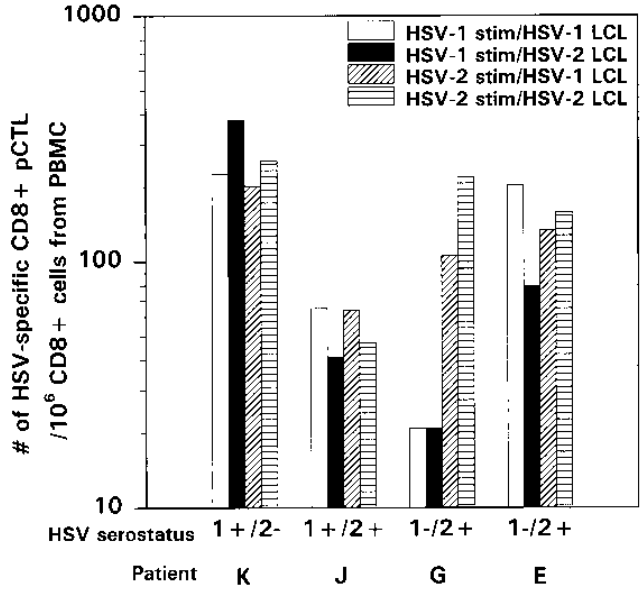

FIG. 3. Type specificity of HSV-specific $CD8^+$ pCTL responses. LDA were performed on positively selected $CD8^+$ cells from patient K (seropositive for HSV-1 only), patient J (HSV-1 and HSV-2 seropositive), and patients G and E (seropositive for HSV-2 only) that were stimulated (stim) with PHA-blasts infected with HSV-1 or HSV-2. Wells were tested for lytic activity against ^{51}Cr-labeled autologous LCL infected with HSV-1 or HSV-2. pCTL frequencies were determined as described in the legend to Fig. 1.

TABLE 2. Analysis of LDA wells after 10 days of incubation of $CD8^+$ T cells and HSV-blasts: cell phenotype and MHC restriction of CTL activity[a]

Well no.	Cell phenotype		% Specific Cr release	
	% CD4	% CD8	Auto HSV-2	Allo HSV-2
1	3	84	37	6
2	2	92	44	11
3	1	95	43	3
4	5	93	35	7
5	2	94	40	15
6	3	94	55	7

[a] Six wells were chosen from the highest dilution of 13,600 input $CD8^+$ cells from patient G after 10 days of incubation of stimulators and responders in an LDA. The phenotype of cells in each well was determined by fluorescence-activated cell sorter analysis. Cells remaining were tested in a ^{51}Cr release assay for killing against autologous (Auto) and allogeneic (Allo) LCL infected with HSV-2.

TABLE 3. Characteristics of $CD8^+$ HSV-specific CTL clones isolated from PBMC[a]

Clone	Cell phenotype		% Specific Cr release					
	% CD4	% CD8	Auto mock	Auto HSV-1	Auto HSV-2	+w6/32	Allo HSV-2	K562
A1	1	99	2	2	34	5	3	2
A7	2	98	1	68	77	6	4	11
A9	4	98	2	57	55	0	2	8
C3	1	99	3	32	29	ND[b]	−1	ND
C4	2	95	−4	−2	49	ND	−3	ND
C17	1	98	1	−4	45	ND	1	ND
C22	0	99	−2	47	51	ND	2	ND
C27	1	97	−2	5	42	ND	2	ND

[a] PBMC from patients A and C were stimulated with HSV-blasts for 10 days. $CD8^+$ T cells were positively selected and cloned at 1 cell per well with PHA, allogeneic feeder cells, and interleukin-2. Clones which lysed HSV-infected LCL were expanded with PHA, allogeneic feeder cells, and interleukin-2, and the cell phenotype was determined by flow cytometry. Clones were tested in a ^{51}Cr release assay by using HSV-1-infected LCL (Auto HSV-1); HSV-2-infected LCL (Auto HSV-2); HSV-2-infected LCL in the presence of w6/32, a monoclonal antibody that inhibits MHC class I-restricted lysis (+w6/32); HSV-2-infected allogeneic LCL (Allo HSV-2); mock-infected autologous LCL (Auto mock); and K562 cells as target cells. Effector-target cell ratio, 15:1.

[b] ND, not done.

HSV-2 (13), appears to overcome the inhibitory effects of ICP47 by reducing MHC class I downregulation by HSV infection (15). Thus, while the virus has apparently devised methods to reduce host $CD8^+$ CTL responses, the host has also evolved mechanisms to counteract the immune response evasion tactics of HSV-2 to permit the activation and expansion of HSV-specific $CD8^+$ CTL.

Alternatively, ICP47 may affect the antigen specificity of the $CD8^+$ CTL response. Virion proteins brought into the cell by the virus or viral proteins made early in infection, such as immediate-early proteins, may serve as major target antigens that can be presented to $CD8^+$ CTL prior to MHC class I downregulation by ICP47. It is of interest that five of the seven HSV-specific $CD8^+$ CTL clones reported by Tigges et al. (19) were directed at virion proteins.

In summary, the high frequencies of HSV-specific $CD8^+$ pCTL we have measured in patients with recurrent genital herpes suggest an important role for these cells in resolving HSV infections.

This work was supported by National Institutes of Health grants AI-20381 (L. Corey) and AI-34616 (D. M. Koelle) and the Medical Research Council of Canada and National Health Research Development Program of Canada (postdoctoral fellowship [C. M. Posavad]).

We thank Mary Shaugnessy and Gail Barnum for specimen collection and Matthew Johnson, Aimee Ekstrom, and Excel Guerrero for excellent technical assistance.

REFERENCES

1. **Arvin, A. M., M. Sharp, S. Smith, C. M. Koropchak, P. S. Diaz, P. Kinchington, W. Ruyechan, and J. Hay.** 1991. Equivalent recognition of a varicella-zoster virus immediate early protein (IE62) and glycoprotein I by cytotoxic T-lymphocytes of either $CD4^+$ or $CD8^+$ phenotype. J. Immunol. **146:**257–264.
2. **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.
3. **Bourgault, I., A. Gomez, E. Gomard, and J. P. Levy.** 1991. Limiting-dilution analysis of the HLA restriction of anti-Epstein-Barr virus-specific cytolytic T lymphocytes. Clin. Exp. Immunol. **84:**501–507.
4. **Braun, R. W., H. K. Teute, H. Kirchner, and K. Munk.** 1984. Replication of herpes simplex virus in human T lymphocytes: characterization of the viral target cell. J. Immunol. **132:**914–919.
5. **Clouse, K. A., P. W. Adams, and C. G. Orosz.** 1989. Enumeration of viral antigen-reactive helper T lymphocytes in human peripheral blood by limiting dilution for analysis of viral antigen-reactive T-cell pools in virus-positive and virus-negative individuals. J. Clin. Microbiol. **27:**2316–2323.
6. **Corey, L., and P. G. Spear.** 1986. Infections with herpes simplex viruses. N. Engl. J. Med. **314:**386–391.
7. **Fruh, K., K. Ahn, H. Djaballah, P. Semp'e, P. M. van Endert, R. Tamp'e, P. A. Peterson, and Y. Yang.** 1995. A viral inhibitor of peptide transporters for antigen presentation. Nature (London) **375:**415–418.
8. **Hammer, S. M., W. P. Carney, V. R. Iacoviello, B. R. Lowe, and M. S. Hirsch.** 1982. Herpes simplex virus infection of human T-cell subpopulations. Infect. Immun. **38:**795–797.
9. **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.
10. **Hill, A., P. Jugovic, I. York, G. Russ, J. Bennink, J. Yewdell, H. Ploegh, and D. Johnson.** 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature (London) **375:**411–415.
11. **Koelle, D. M., H. Abbo, A. Peck, K. Ziegweid, and L. Corey.** 1994. Direct recovery of herpes simplex virus (HSV)-specific T lymphocyte clones from recurrent genital HSV-2 lesions. J. Infect. Dis. **169:**956–961.
12. **Koelle, D. M., L. Corey, R. L. Burke, R. J. Eisenberg, G. H. Cohen, R. Pichyangkura, and S. J. Triezenberg.** 1994. Antigenic specificities of human $CD4^+$ T-cell clones recovered from recurrent genital herpes simplex virus type 2 lesions. J. Virol. **68:**2803–2810.
13. **Koelle, D. M., J. G. Goddard, W. van Hoorhis, L. Barrett, M. L. Remington, D. B. Corry, R. M. Locksley, and L. Corey.** 1994. Enrichment of interferon-gamma, IL-2, IL-4, and IL-10. mRNA in skin biopsy tissue from a recurrent HSV-2 skin lesion, abstr. 52-A, p. 305. *In* Abstracts of the 32nd Annual IDSA Meeting. Infectious Diseases Society of America, Orlando, Fla.
14. **Koelle, D. M., M. A. Tigges, R. L. Burke, F. W. Symington, S. R. Riddell, H. Abbo, and L. Corey.** 1993. Herpes simplex virus infection of human fibroblasts and keratinocytes inhibits recognition of cloned $CD8^+$ cytotoxic T lymphocytes. J. Clin. Invest. **91:**961–968.
15. **Mikloska, Z., A. M. Kesson, M. E. T. Penfold, and A. L. Cunningham.** 1996. Herpes simplex virus protein targets for CD4 and CD8 lymphocyte cytotoxicity in cultured epidermal keratinocytes treated with interferon-γ. J. Infect. Dis. **173:**7–17.
16. **Nahmias, A. J., F. K. Lee, and S. Beckman-Nahmias.** 1990. Sero-epidemiological and sociological patterns of herpes simplex virus infection in the world. Scand. J. Infect. Dis. Suppl. **69:**19–36.
17. **Schmid, D. S.** 1988. The human MHC-restricted cellular response to herpes simplex virus type 1 is mediated by $CD4^+$ $CD8^-$ T cells and is restricted to the DR region of the MHC complex. J. Immunol. **140:**3610–3616.
18. **Taswell, C.** 1981. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. J. Immunol. **126:**1614–1619.
19. **Tigges, M. A., D. Koelle, K. Hartog, R. E. Sekulovich, L. Corey, and R. L. Burke.** 1992. Human $CD8^+$ herpes simplex virus-specific cytotoxic T-lymphocyte clones recognize diverse virion protein antigens. J. Virol. **66:**1622–1634.
20. **Yasukawa, M., A. Inatsuki, and Y. Kobayashi.** 1989. Differential in vitro activation of $CD4^+$ $CD8^-$ and $CD8^+$ $CD4^-$ herpes simplex virus-specific human cytotoxic T cells. J. Immunol. **143:**2051–2057.
21. **Yasukawa, M., and J. M. Zarling.** 1984. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. I. Lysis restricted by HLA class II MB and DR antigens. J. Immunol. **133:**422–427.
22. **York, I. A., C. Roop, D. W. Andrews, S. R. Riddell, F. L. Graham, and D. C. Johnson.** 1994. A cytosolic herpes simplex virus protein inhibits antigen presentation to $CD8^+$ T lymphocytes. Cell **77:**525–535.