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HLA-Restricted T Lymphocyte-Mediated Cytotoxicity Against Herpes Simplex Virus-Infected Cells in Humans

MASAKI YASUKAWA,* TOMOO SHIROGUCHI, AND YUZURU KOBAYASHI

First Department of Internal Medicine, Ehime University School of Medicine, Ehime 791-02, Japan

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Cytotoxic T lymphocytes (CTL) against herpes simplex virus (HSV) were induced in vitro from human peripheral blood lymphocytes by stimulation with HSV antigen. CTL generated by HSV type 1 (HSV-1) antigen stimulation killed not only HSV-1-infected target cells but also HSV type 2 (HSV-2)-infected target cells, though at a lower level. This evidence suggests that CTL against HSV recognize the HSV type-specific and type-common determinants on HSV-infected target cells. These CTL were generated from high responders against HSV-1 antigen as measured by antigen-specific T lymphocyte proliferation in vitro, but not to such an efficient degree from low responders. The cytotoxic activities of CTL against the allogeneic HSV-infected target cells were high when at least one of the HLA-A or -B antigens was shared. However, the HLA-A and -B nonidentical target cells were not killed effectively. The data presented here suggest the possibility of HLA restriction of HSV-specific CTL in humans.

Many of the major histocompatibility complex-restricted phenomena in the immune response described in the murine system also hold true for humans. Antigen-specific T lymphocyte proliferation was only observed when T lymphocytes and macrophages shared the HLA-D/DR antigen (1), and mixed lymphocyte reaction-specific suppressor T lymphocytes also showed HLA-D/DR restriction (7). The restriction phenomena of cytotoxic T lymphocytes (CTL) have been shown against hapten-modified target cells (4), H-Y antigen (9), and virus-infected target cells. Among them, HLA restriction of virus-specific CTL has been demonstrated in both the influenza (13, 27) and Epstein-Barr (EB) viruses (8, 21). However, because of the difficulty of antigen-specific CTL induction in humans, the exact mechanism(s) of CTL recognition has not been clarified in other virus-immune experiments.

In the murine system, in vitro induction of herpes simplex virus (HSV)-specific CTL has been successful (19). H-2 restriction and discrimination between target cells infected with HSV type 1 (HSV-1) or type 2 (HSV-2) were observed (18). These HSV-specific CTL may play an important role in protection against infection and recovery from disease.

In this series of experiments, we attempted to induce CTL against HSV in humans and to clarify the mechanism(s) of their antigen recognition of target cells.

MATERIALS AND METHODS

Virus. The KOS strain of HSV-1, provided by S. Sonoda, Ehime Prefectural Institute of Public Health, Ehime, Japan, was mainly used. The HF strain of HSV-1 and the Savage strain of HSV-2 were kindly donated by R. Mori, Department of Virology, Kyushu University, Fukuoka, Japan.

HSV antigen. HSV was cultivated in Vero cells. Virus-infected cells were rapidly frozen and thawed. After centrifugation at 600 × *g* for 10 min, the supernatant was inactivated by UV irradiation and prepared as the HSV antigen. HSV antigen was stored at −76°C. The titer by plaque-forming unit assay before UV irradiation was 10^7 per ml. No plaques were detected after inactivation.

Preparation of PBL and T lymphocytes. Peripheral blood lymphocytes (PBL) were isolated from heparinized peripheral blood obtained from healthy adults by the Ficoll-Conray gradient method (3). T lymphocytes were separated by the neuraminidase-treated sheep erythrocytes rosetting (E rosette) method (32).

Assay of in vitro T lymphocyte proliferation against HSV antigen. PBL were suspended at 5×10^5 cells per ml in RPMI 1640 medium (MBA, Walkersville, Md.) supplemented wtih 2 mM L-glutamine, 10% heat-inactivated pooled human male AB sera, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. PBL were cultured at 10^5 cells per 0.2 ml of culture medium in a round-bottomed 96-well microtiter plate (Linbro, Hamden, Conn.) with various dilutions of

HSV antigens. The cell cultures were incubated at 37°C in a 5% CO_2 incubator for 7 days, the optimum incubation period as determined in preliminary kinetics studies (data not shown).

For the final 16 h of the incubation period, 1 μCi of [^{3}H]thymidine (Amersham International Ltd., Amersham, England) was added to each well, and the cultured cells were harvested on glass filter paper with a semiautomatic multiple harvester (Flow Laboratories, Inc., Rockville, Md.). Incorporation of [^{3}H]thymidine into PBL was counted with a liquid scintillation counter.

In vitro induction of cytotoxic effector cells. PBL at a concentration of 1×10^7 to 1.5×10^7 cells per 10 ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal calf serum (FCS), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10^6 PFU of UV-irradiated HSV-1 were cultured in upright plastic flasks (Falcon 3013). After 7 days, cultured cells were harvested and prepared as the first stimulated effector cells. Other cultured cells were cultured for 7 days, and then half of the medium was removed and exchanged for fresh medium and 10^6 fresh PFU of UV-irradiated HSV-1 was added. After 3 days, these cultured cells were harvested and prepared as the second stimulated effector cells.

Detection of HSV cell surface antigen. Anti-HSV serum was raised by immunizing a rabbit with the KOS strain of HSV-1 and the Savage strain of HSV-2. HSV-infected cells were incubated with antiserum for 15 min on ice and washed with phosphate-buffered saline at pH 7.2 three times and then suspended with the ^{125}I-labeled goat anti-rabbit IgG serum for 15 min on ice and washed with phosphate-buffered saline twice. ^{125}I labeling of goat IgG was performed as described by Sonoda and Schlamowitz (28). Cell surface expression of HSV antigen was detected by measuring the radioactivity.

Preparation of target cells. Freshly separated PBL, phytohemagglutinin (PHA)-stimulated lymphocytes (PHA-blasts), and EB virus-transformed lymphoblastoid cell lines (LCL) were prepared as the target cells. HSV antigen expression was detected on PHA-blasts and LCL, but not efficiently on PBL (see Fig. 2). Therefore, PHA-blasts and LCL were selected as the target cells. We added 2×10^6 target cells in serum-free RPMI 1640 medium to 10^7 PFU of native HSV. After 1 h of incubation at 37°C, the target cells were washed once and incubated in RPMI 1640 supplemented with 5% FCS. LCL or PHA-blasts incubated with HSV for more than 12 h were prepared as the HSV-infected target cells because cell surface HSV antigen expression was distinct after 10 h of incubation (see Fig. 2). Target cells were incubated with 100 μCi of $Na_2{}^{51}CrO_4$ (Daiichi Radioisotope Laboratory, Tokyo, Japan) at 37°C for 1 h and washed three times with cold RPMI 1640 medium.

Cell-mediated cytotoxicity assay. Target cells (10^4) were cocultured with various amounts of effector cells in 0.2 ml of RPMI 1640 supplemented with 10% heat-inactivated FCS in round-bottomed microtiter plates. Culture plates were centrifuged at $250 \times g$ for 3 min and incubated for 5 h at 37°C in a 5% CO_2 incubator. After 5 h of incubation, the supernatant was collected by the Titertek supernatant collection system (Flow Laboratories), and the ^{51}Cr release level was measured in a gamma counter. Maximum release was obtained by adding 2.5% Triton X-100 (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The percentage of cytotoxicity was calculated by the formula:

$$\% \text{ cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Experiments were performed as triplicate assays, and standard deviations were almost always less than 5%. Preliminary experiments were performed more than twice, and quite similar results were obtained.

Cold target inhibition test. Various numbers of unlabeled "cold" target cells were added to 10^4 ^{51}Cr-labeled target cells. These target cells were incubated with 25×10^4 cytotoxic effector cells in 0.2 ml of RPMI 1640 supplemented with 10% heat-inactivated FCS at 37°C for 5 h. After incubation, the ^{51}Cr release levels were measured.

HLA-A and HLA-B typing. HLA-A and -B antigens were kindly typed by T. Sasazuki, Department of Human Genetics, Tokyo Medical and Dental University, Tokyo, Japan, using the National Institutes of Health standard microcytotoxicity method (30).

RESULTS

In vitro T lymphocyte proliferation against HSV-1 antigen. The immune responses to HSV-1 antigen by about 20 healthy individuals, with or without a history of herpes labialis, were examined. From the preliminary examinations, it was clear that in vitro immune response to HSV antigen measured by the incorporation of [^{3}H]thymidine into PBL reflected macrophage-dependent T lymphocyte proliferation, and a maximum response was obtained on the 7th day of culture (data not shown). Maximum responses of random healthy individuals ranged from 3,000 to 80,000 cpm (Fig. 1). No response was detected when the culture supernatant of HSV-uninfected Vero cells was used as antigen.

Cell surface expression of HSV antigen. Cell surface expressions of HSV-1 and HSV-2 antigen are shown in Fig. 2. After 10 h of incubation, HSV-1 antigen was distinctly detected on PHA-blasts and LCL, but not efficiently on PBL. HSV-2 antigen expression was similar to that of HSV-1.

Induction of cytotoxic effector cells against autologous HSV-1-infected target cells. The cytotoxic activities of various effector cells induced from one of the high responders to HSV-1 antigen against autologous HSV-1-infected target cells are shown in Fig. 3. Unstimulated effector cells had little effect on the target cells. The first stimulated effector cells showed cytotoxicity at a low level (10 to 15%; effector-to-target cell [E:T] ratio, 25:1); however, the second stimulated effector cells exhibited distinct

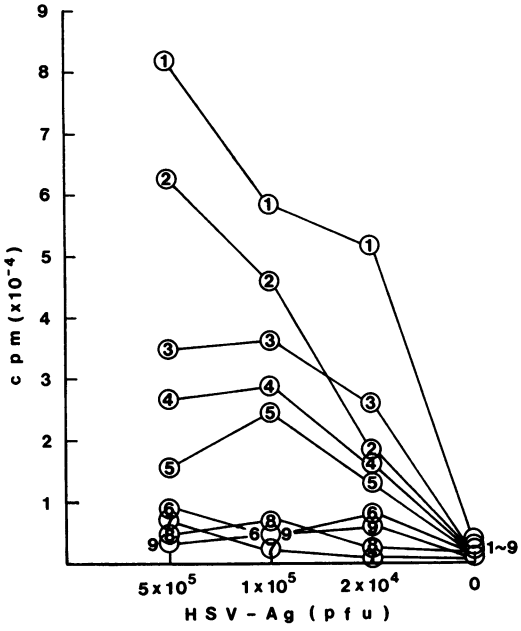

FIG. 1. Immune responses of PBL from healthy individuals against HSV-1 antigen (HSV-Ag). PBL were cultured at 10^5 cells per 0.2 ml of RPMI 1640 with full supplement in a round-bottomed microtiter plate with various doses of HSV-1 antigen for 7 days. Cell proliferation was measured by incorporation of [^{3}H]thymidine into PBL.

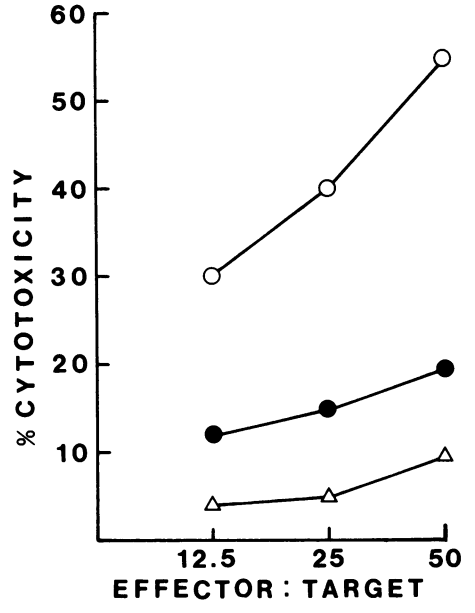

FIG. 3. Cytotoxic activity of first stimulated effector cells (●), second stimulated effector cells (○), and control effector cells (△) against autologous HSV-1-infected target cells at three E:T ratios. Control effector cells were prepared as PBL cultured without HSV antigen for 7 days.

cytotoxicity ranging from 20 to 40% (E:T ratio, 25:1).

Nature of cytotoxic effector cells. Natural killing (NK) activities against K562 cells of various effector cells are shown in Fig. 4A. Unstimulated, fresh effector cells revealed high NK activity; however, the NK activities of the first and second stimulated effector cells apparently decreased. Figure 4B shows the cytotoxic activities of a T lymphocyte-rich fraction and a non-T lymphocyte fraction separated from the second stimulated effector cells. The non-T lymphocyte fraction showed little cytotoxicity, whereas efficient cytotoxicity was observed in the T lymphocyte fraction. Therefore, it was concluded that the cytotoxicity against HSV-infected target cells reflected the activity of CTL.

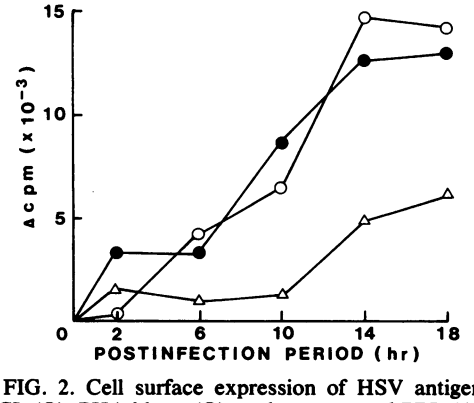

FIG. 2. Cell surface expression of HSV antigen. LCL (○), PHA-blasts (●), and unseparated PBL (△), 2×10^7 of each, were incubated with 10^8 PFU of HSV-1. After 1 h of incubation at 37°C, HSV-1-infected cells were washed once and incubated in RPMI 1640 supplemented with 5% FCS. After various incubation periods, 10^6 cells were harvested, and cell surface expression of HSV antigen was measured as described in the text. Δcpm, cpm bound to HSV-infected cells − cpm bound to noninfected cells.

Virus specificity of CTL. Cytotoxic activities of CTL induced by the KOS strain of HSV-1 antigen were examined by using various autologous target cells infected with the KOS strain of HSV-1, the HF strain of HSV-1, the Savage strain of HSV-2, or without HSV. Only slight cytotoxicity against HSV noninfected target cells was detected, and full cross-reactivity was observed between the two HSV-1 strains. Little cytotoxicity against HSV-2-infected target cells was detected. The same results were obtained in the experiments in which LCL or PHA-blasts were used as the target cells (Fig. 5A and 5B).

Induction of HSV-1-specific CTL from a random population. In vitro induction of HSV-1-specific CTL was attempted from random healthy individuals. Strong cytotoxicity was de-

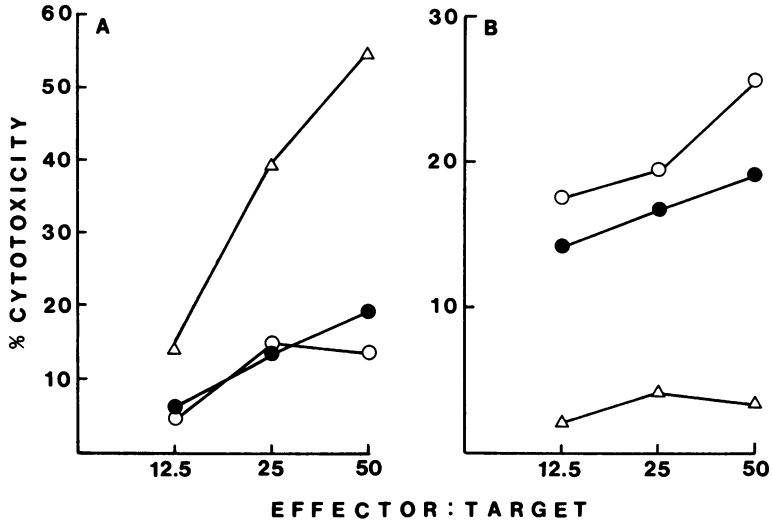

FIG. 4. (A) NK activity of first stimulated effector cells (●), second stimulated effector cells (○), and freshly separated PBL (△) at three E:T ratios. NK activity was measured as the cytotoxicity against 10^4 K562 cells. (B) Cytotoxic activity of various cell populations separated from second stimulated effector cells against autologous HSV-infected target cells at three E:T ratios. Unseparated cells (○), T cells (●), and non-T cells (△) separated by the E rosette method were prepared as cytotoxic effector cells.

tected from the high responders against HSV-1 antigen, but not to such a great extent from the low responders. The high responders had high titers of neutralizing antibody to HSV-1 and almost none of the low responders had demonstrable antibody (Fig. 6).

Cold target inhibition test. The cold target inhibition test was performed with various ^{51}Cr-unlabeled cold target cells to examine the antigen determinants recognized by CTL. As shown in Fig. 7, effective inhibition was detected when autologous HSV-1-infected target cells were added. However, when autologous HSV noninfected cells or HLA-A and -B nonidentical HSV-

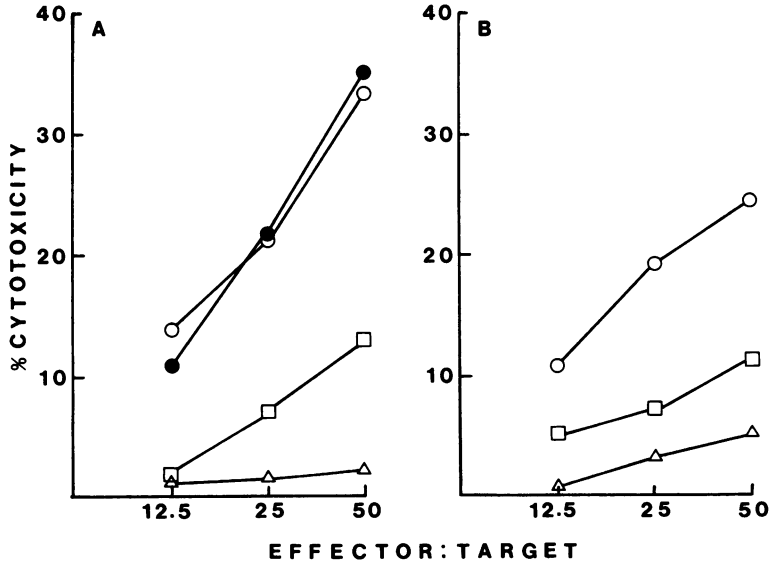

FIG. 5. Cytotoxic activity of second stimulated CTL induced by the KOS strain of HSV-1 antigen against HSV-1- and HSV-2-infected target cells at three E:T ratios. (A) Target cells were prepared as LCL. LCL were infected with the KOS strain of HSV-1 (○), the HF strain of HSV-1 (●), the Savage strain of HSV-2 (□), and without HSV (△). (B) Target cells were prepared as PHA-blasts. PHA-blasts were infected with the KOS strain of HSV-1 (○), the Savage strain of HSV-2 (□), and without HSV (△).

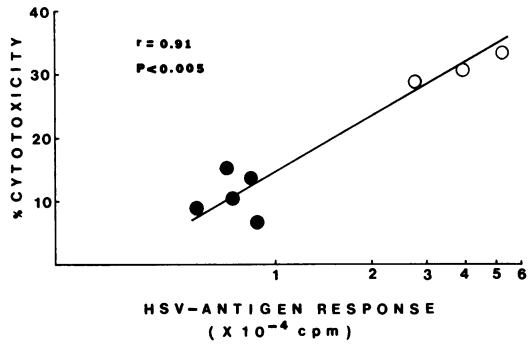

FIG. 6. Correlation between T lymphocyte blastogenesis to HSV-1 antigen and cytotoxicity against HSV-1-infected autologous LCL ($r = 0.91$; $P < 0.005$). T lymphocyte blastogenesis to HSV-1 antigen was measured by the incorporation of [^{3}H]thymidine as described in the text, and maximum response to various diluted HSV-1 antigen was shown. Cytotoxic effector cells were second stimulated CTL induced from HSV-1 seropositive (○) (titer of neutralizing antibody >16) and HSV-1 seronegative (●) (titer of neutralizing antibody <4). E:T ratio, 25:1.

infected target cells were added, only slight inhibition was detected. These data suggest the possibility that HSV-1-specific CTL recognized not only HSV-1 antigen but also HLA-A or -B antigen on the target cells.

HLA-A and HLA-B restriction of CTL against HSV-1. The cytotoxic activities against allogeneic HSV-1-infected target cells were investigated to clarify the mechanism(s) of antigen recognition of CTL. The cytotoxic activities of CTL induced from four high responders to HSV-1 antigen against autologous and allogeneic HSV-1-infected target cells are shown in Fig. 8. These CTL effectively killed the target cells which shared at least one of the HLA-A or -B antigens, except donor MY against target no. 5 and donor TT against target no. 27 (Fig. 8). However, high killing activity was not detected in pairs which shared no HLA-A and -B antigens, except donor MY against target no. 6 and donor AI against target no. 22 (Fig. 8). Table 1 shows the summarized data of 31 combinations presented in Fig. 8. These data suggest the possibility that the HSV-1-specific CTL may recognize at least one of the HLA-A or -B antigens with the target antigen of the target cells.

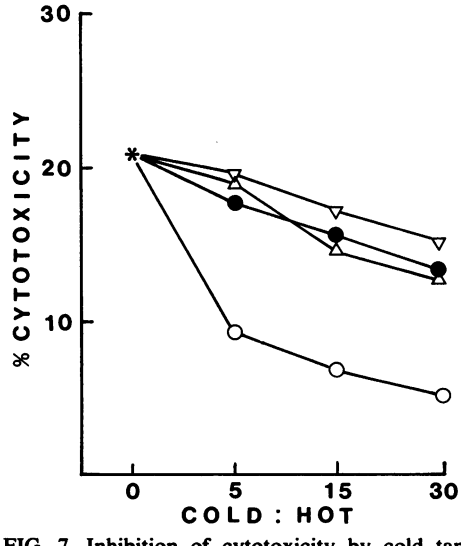

FIG. 7. Inhibition of cytotoxicity by cold target cells. 25×10^4 of second stimulated CTL were incubated with 10^4 ^{51}Cr-labeled hot target cells. Cold target cells used were autologous HSV-1-infected cells (○), autologous HSV noninfected cells (●), and HLA-A and -B nonidentical HSV-1-infected cells (△, ▽). The donor of CTL was MY (A26,Aw31, Bw62,B40); cold target cell (△) is target cell no. 7 (A2,Aw24, Bw35,−); and cold target cell (▽) is target cell no. 8 (A2,A11, Bw35,Bw44), described in the legend to Fig. 8. *, Cytotoxic activity without cold target cells.

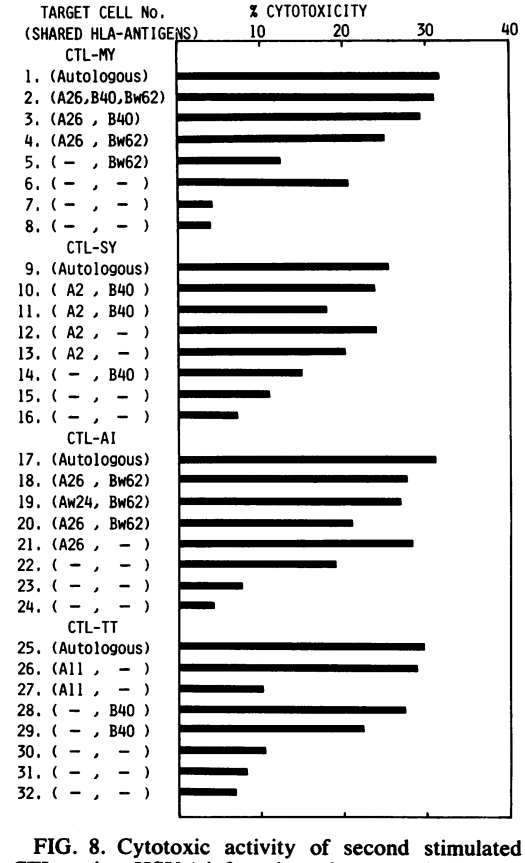

FIG. 8. Cytotoxic activity of second stimulated CTL against HSV-1-infected autologous and allogeneic target cells. CTL were induced from four high responders against HSV-1 antigen. E:T ratio, 25:1. MY, SY, AI, and TT refer to the donors.

TABLE 1. HLA-A and -B restriction of CTL against HSV-infected LCL

No. of shared HLA-A and -B antigens	No. of combinations	Restriction[a]
Autologous	4	100
2	7	81.9 ± 9.6
1	9	73.6 ± 22.9
0	11	31.7 ± 16.3

[a] Mean values ± standard error of (% cytotoxicity against allogeneic HSV-infected LCL/% cytotoxicity against autologous HSV-infected LCL) × 100.

DISCUSSION

It is well known that protection and recovery from HSV infection are T lymphocyte dependent (14, 20). Pfizenmaier et al. first succeeded in the induction of HSV-specific murine CTL by transferring the immune lymphocytes to in vitro culture (19). They also showed that HSV-specific murine CTL were H-2 restricted (18). These findings indicated that the phenomenon reported by Doherty et al. (5) also applied to HSV-specific CTL.

The cytotoxic effector cells induced in this series of studies exhibited cytotoxicity against HSV-infected LCL but not against HSV noninfected LCL. The cytotoxic activity was detected in an E rosette-positive fraction. These cytotoxic effector cells showed cytotoxicity in anti-HSV antibody-free medium, so they were antibody independent. These cells showed low NK activity against K562 cells. Because of the data mentioned above, it was concluded that the cytotoxic activities detected in this experimental system were mediated mainly by CTL against HSV-infected cells.

CTL induced by stimulation with HSV-1 antigen also killed HSV-2-infected target cells, although at a lower level. This cytotoxicity may reflect the CTL recognition of HSV type-common determinants on the HSV-infected target cells. In the murine system, some investigators have reported the induction of CTL against HSV. However, there are contrary results about the cytotoxicity against heterologous HSV-infected cells. Pfizenmaier et al. (18) reported that the cytotoxicity was limited to homologous HSV-infected cells, whereas Sethi and Wolff (26) showed high cross-reacting cytotoxicity between HSV-1 and HSV-2. These contrary results may be caused by the different experimental approaches, and additional studies have been needed to clarify the type specificity of CTL against HSV. Eberle et al. (6) showed HSV-specific CTL recognition of type-specific and type-common surface antigens in the murine system, and the data presented in this communication is in agreement with their observation. It is interesting that HSV-specific CTL distinguish HSV-1 from HSV-2, which closely cross-react on the conventional serological level. The antigenic specificities between HSV-1 and HSV-2 are determined principally by glycoproteins, which may be the most important components recognized by the immune system. Recently, type specificities of major HSV glycoproteins have been confirmed with monoclonal antibodies (17). The presence of type-specific and type-common recognition suggests that the target antigens of HSV-specific CTL are glycoproteins. This supposition is supported by the experiments of Lawman et al. (10), who showed that glycoprotein expression is essential for HSV-specific CTL by using HSV mutants with diminished expression of glycoprotein and chemical inhibition of glycoprotein synthesis.

We attempted the induction of CTL against HSV from healthy individuals. As a result, CTL against HSV were induced from high responders to HSV antigen; however, low responders exhibited little cytotoxicity. In addition, almost all of the high responders were seropositive, and low responders had no detectable antibody to HSV. It is well known that although most adults have been infected with HSV and carry the virus as a latent infection in ganglion cells, reactivations are triggered only in some individuals under stressful conditions (16). Recurrent reactivations of latent HSV infection, whether clinical manifestations are present or not, may stimulate the immune system and lead to the various immune responses. Therefore, the high responders are considered to possess memory cells that induce HSV-specific CTL as a result of recurrent reactivations. However, latent infection with HSV seems to persist in the low responders. High T-lymphocyte-proliferative responses to HSV antigen probably reflect the recurrent reactivation of latent HSV infection; however, the mechanisms of latency and reactivation are not yet known. The correlation between T lymphocyte blastogenesis against HSV antigen and T-lymphocyte-mediated cytotoxicity or neutralizing antibody level suggests that T-lymphocyte-proliferative response against HSV antigen may reflect the helper T cell response to HSV-specific CTL or anti-HSV antibody-producing cells.

As shown in Table 1, CTL induced by our experimental system showed effective cytotoxicity against allogeneic HSV-infected target cells which shared at least one of the HLA-A or -B antigens. These data suggest the HLA restriction of HSV-specific CTL similar to influenza virus- (11, 27) or EB virus-specific (8, 21) CTL. However, some HLA-A and HLA-B nonidentical pairs also exhibited evident killing. One interpretation of this phenomenon is that the cell surface molecules recognized by CTL are not HLA-A and -B molecules themselves but other

products coded by genes closely linked and in linkage disequilibrium with HLA. However, the evidence that influenza- (12) or EB virus-specific (31) CTL cytotoxicities were blocked by monoclonal antibodies to HLA-A and -B antigens suggests that the cytotoxicity between HLA-A and -B nonidentical pairs may be mediated by other mechanisms. Tanaka et al. (29) cultured the cytotoxic effector cells against EB virus-infected cells continuously with T cell growth factor and established clones of cytotoxic effector cells. It was shown that cytotoxic effector cells induced by their system included several subpopulations, i.e., HLA-restricted CTL, HLA-nonrestricted CTL, and NK-like cells. The cytotoxic activity against HSV of the effectors induced in this study was revealed mainly in the E rosette-positive fraction; however, they showed NK activity, although at a low level. In addition, in cold target inhibition tests, slight inhibition was detected by autologous HSV-noninfected target cells or HLA-A and -B nonidentical HSV-infected target cells. These data suggest the possibility of the presence of some cell populations which are distinguished from HLA-restricted HSV-specific CTL. It may be reasonable to postulate that the cytotoxicity against HLA-A and -B nonidentical target cells was induced by some cytotoxic effector cells other than HLA-restricted HSV-specific CTL. Sethi et al. (25) observed more obvious HLA restriction phenomena in the human HSV system in long-term cultures with T cell growth factor.

It has been suggested that resistance to HSV infection is controlled by genes within the major histocompatibility complex. Although the study by Russell and Schlaut (22) demonstrated the association between HLA-A1 and HSV infection, there has been no clear evidence of immune response or immune suppression genes influencing HSV infection as in other immune response systems (23, 24). The present series of experiments suggests the presence of genetic control in the immune response to HSV infection. It has been shown that not all HLA-A and -B antigens were equally effective in recognizing virus-specific CTL. Influenza virus-specific CTL in particular do not kill target cells sharing only HLA-A2 (11). The HLA-A2-associated failure of recognition has also been shown in EB virus-specific CTL (15). In this study, CTL from donor SY showed effective killing against the target cells sharing only HLA-A2. However, the study by Biddison et al. (2) demonstrated that HLA-A2 antigen determinants recognized by CTL are distinguished from the serologically defined HLA antigen. Further investigation is required before this phenomenon can be clarified.

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LITERATURE CITED

1. **Bergholtz, B. O., and E. Thorsby.** 1977. Macrophage-dependent response of immune human T lymphocytes to PPD *in vitro*. Influence of HLA-D histocompatibility. Scand. J. Immunol. **6**:779–786.
2. **Biddison, W. E., M. S. Krangel, J. L. Strominger, F. E. Ward, G. M. Shearer, and S. Shaw.** 1980. Virus-immune cytotoxic T cells recognize structural differences between serologically indistinguishable HLA-A2 molecules. Hum. Immunol. **3**:225–232.
3. **Böyum, A.** 1968. Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. **21**(Suppl. 97):77–89.
4. **Dickmeiss, E., B. Soeberg, and A. Svejgaard.** 1977. Human cell-mediated cytotoxicity against modified target cells is restricted by HLA. Nature (London) **270**:526–528.
5. **Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel.** 1976. Specificity of virus-immune effector T cell for H-2K and H-2D compatible interactions: implication for H-antigen diversity. Transplant. Rev. **29**:89–123.
6. **Eberle, R., R. G. Russell, and B. T. Rouse.** 1981. Cell-mediated immunity to herpes simplex virus: recognition of type-specific and type-common surface antigens by cytotoxic T cell populations. Infect. Immun. **34**:795–803.
7. **Engleman, E. G., A. J. McMichael, M. E. Batey, and H. O. McDevitt.** 1978. A suppressor T cell of the mixed lymphocyte reaction in man specific for the stimulating alloantigen. Evidence that identity at HLA-D between suppressor and responder is required for suppression. J. Exp. Med. **147**:137–146.
8. **Fukukawa, T., T. Hirano, N. Sakaguchi, T. Teranishi, I. Tsuyuguchi, N. Nagao, N. Naito, K. Yoshimura, Y. Okubo, H. Tohda, and A. Oikawa.** 1981. *In vitro* induction of HLA-restricted cytotoxic T lymphocytes against autologous Epstein-Barr virus transformed B lymphoblastoid cell line. J. Immunol. **126**:1697–1701.
9. **Goulmy, E., A. Termijtelen, B. A. Bradley, and J. J. van Rood.** 1977. Y-antigen killing by T cells of women is restricted by HLA. Nature (London) **266**:544–545.
10. **Lawman, M. J. P., R. J. Courtney, R. Eberle, P. A. Schaffer, M. K. O'Hara, and B. T. Rouse.** 1980. Cell-mediated immunity to herpes simplex virus: specificity of cytotoxic T cells. Infect. Immun. **30**:451–461.
11. **McMichael, A.** 1978. HLA restriction of human cytotoxic T lymphocytes specific for influenza virus. Poor recognition of virus associated with HLA A2. J. Exp. Med. **148**:1458–1467.
12. **McMichael, A. J., P. Parham, F. M. Brodsky, and J. R. Pilch.** 1980. Influenza virus-specific cytotoxic T lymphocytes recognize HLA molecules. Blocking by monoclonal anti-HLA antibodies. J. Exp. Med. **152**:195s–203s.
13. **McMichael, A. J., A. Ting, H. J. Zweerink, and B. A. Askonas.** 1977. HLA restriction of cell-mediated lysis of influenza virus-infected human cells. Nature (London) **270**:524–526.
14. **Mori, R., T. Tasaki, G. Kimura, and K. Takeya.** 1967. Depression of acquired resistance against herpes simplex virus infection in neonatally thymectomized mice. Arch. Gesamte. Virusforsch. **21**:459–462.
15. **Moss, D. J., L. E. Wallace, A. B. Rickinson, and M. A. Epstein.** 1981. Cytotoxic T cell recognition of Epstein-Barr virus-infected B cells. I. Specificity and HLA restric-

tion of effector cells reactivated *in vitro*. Eur. J. Immunol. 11:686–693.
16. **Nahmias, A. J., and B. Roizman.** 1973. Infection with herpes simplex viruses 1 and 2 (third of three parts). N. Engl. J. Med. 289:781–789.
17. **Pereira, L., D. V. Dondero, D. Gallo, V. Devlin, and J. D. Woodie.** 1982. Serological analysis of herpes simplex virus types 1 and 2 with monoclonal antibodies. Infect. Immun. 35:363–367.
18. **Pfizenmaier, K., H. Jung, A. Starzinski-Powitz, M. Röllinghoff, and H. Wagner.** 1977. The role of T cells in anti-herpes simplex virus immunity. I. Induction of antigen-specific cytotoxic T lymphocytes. J. Immunol. 119:939–944.
19. **Pfizenmaier, K., A. Starzinski-Powitz, M. Röllinghoff, D. Falke, and H. Wagner.** 1977. T-cell-mediated cytotoxicity against herpes simplex virus-infected target cells. Nature (London) 265:630–632.
20. **Rager-Zisman, B., and A. C. Allison.** 1976. Mechanism of immunologic resistance to herpes simplex virus 1 (HSV-1) infection. J. Immunol. 116:35–40.
21. **Rickinson, A. B., L. E. Wallace, and M. A. Epstein.** 1980. HLA-restricted T-cell recognition of Epstein-Barr virus-infected B cells. Nature (London) 283:865–867.
22. **Russell, A. S., and J. Schlaut.** 1977. Association of HLA-A1 antigen and susceptibility to recurrent cold sores. Arch. Dermatol. 113:1721–1722.
23. **Sasazuki, T., H. Kaneoka, Y. Nishimura, R. Kaneoka, M. Hayama, and H. Ohkuni.** 1980. An HLA-linked immune suppression gene in man. J. Exp. Med. 152:297s–313s.
24. **Sasazuki, T., Y. Kohno, I. Iwamoto, M. Tanimura, and S. Naito.** 1978. Association between an HLA haplotype and low responsiveness to tetanus toxoid in man. Nature (London) 272:359–361.
25. **Sethi, K. K., I. Stroehmann, and H. Brandis.** 1980. Human T-cell cultures from virus-sensitized donors can mediate virus-specific and HLA-restricted cell lysis. Nature (London) 286:718–720.
26. **Sethi, K. K., and M. H. Wolff.** 1980. The nature of host-cell herpes-simplex virus interaction(s) that renders cells susceptible to virus-specific cytotoxic T cells. Immunobiology 157:365–378.
27. **Shaw, S., and W. E. Biddison.** 1979. HLA-linked genetic control of the specificity of human cytotoxic T-cell responses to influenza virus. J. Exp. Med. 149:565–575.
28. **Sonoda, S., and M. Schlamowitz.** 1970. Studies of ^{125}I trace labeling of immunoglobulin G by chloramine-T. Immunochemistry 7:885–898.
29. **Tanaka, Y., K. Sugamura, and Y. Hinuma.** 1982. Heterogeneity of allogeneic restriction of human cytotoxic T cell clones specific for Epstein Barr virus. J. Immunol. 128:1241–1245.
30. **Terasaki, P. I., and M. S. Park.** 1976. Microdroplet lymphocyte cytotoxicity test. NIAID Manual of Tissue Typing Techniques. U.S. Department of Health, Education and Welfare, Washington, D.C.
31. **Wallace, L. E., D. J. Moss, A. B. Rickinson, A. J. McMichael, and M. A. Epstein.** 1981. Cytotoxic T cell recognition of Epstein-Barr virus-infected B cells. II. Blocking studies with monoclonal antibodies to HLA determinants. Eur. J. Immunol. 11:694–699.
32. **Weiner, M. S., C. Bianco, and V. Nussenzweig.** 1973. Enhanced binding of neuraminidase-treated sheep erythrocytes to human T lymphocytes. Blood 42:939–946.