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Cytotoxic T cells against herpes simplex virus in Behçet's disease

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SUMMARY

Lymphocytes from 36 patients with Behçet's disease (20 in remission and 16 in active phase) were stimulated *in vitro* with herpes simplex virus and then tested for their ability to generate cytotoxic T cell responses to the virus. Significant cytotoxic responses were found. $CD4^+$ and $CD8^+$ subpopulations from the patients in remission generated specific cytotoxic activity against autologous target cells. These observations suggested that $CD4^+$ and $CD8^+$ cytotoxic T cells may have an important host response in herpes virus infection in Behçet's disease.

Keywords Behçet's disease herpes simplex virus cytotoxic T cells

INTRODUCTION

The aetiology and pathogenesis of Behçet's disease are still poorly understood, and no micro-organism has been consistently isolated from patients with the disease. There is, however, some evidence for the involvement of herpes simplex virus (HSV-1) in the immunopathogenesis of Behçet's disease, Denman *et al.* (1980) found that, unlike healthy controls, phytohaemagglutinin (PHA) transformed lymphocytes of patients were unable to support the growth of HSV-1. Eglin, Lehner & Suback-Sharpe (1982) have shown by *in situ* DNA–RNA hybridization, that at least part of the HSV-1 genome is transcribed in peripheral blood mononuclear cells (PBMC) of patients with Behçet's disease. This was confirmed by Bonass *et al.* (1986) who detected HSV-1 DNA by 'dot-blot' DNA–DNA hybridization in patients with Behçet's disease. The possible relationship of HSV-1 to the pathogenesis of the disease has been recently investigated by Young, Lehner & Barnes (1988). When stimulated with HSV-1, $CD4^+$ cells of patients with Behçet's disease produced low proliferative responses, in contrast to seropositive controls. The impaired cellular response appeared to be specific to HSV-1, since neither cytomegalovirus (CMV) nor *Varicella zoster* showed similar, depressed, cellular responses to HSV-1. Interferon-gamma (IFN-γ) (which is an inducer of cytotoxic T lymphocytes; CTL), was detected in high concentrations in sera and culture supernatant from Behçet's disease (Ohno *et al.*, 1982; Fujii *et al.*, 1983; Bacon *et al.*, 1984; Hamzaoui *et al.*, 1989). The non-acquisition of virus-specific T lymphocytes against HSV-1 in patients with Behçet's disease could be a critical component of the host response to HSV-1. The importance of CTL for the clearance of virus-infected cells in Behçet's disease has not been studied.

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The aim of the present study is to examine HSV-1-specific CTL responses in patients with Behçet's disease. We used HSV-1-infected autologous lymphocytes as targets to evaluate the virus-specific cytotoxicity during active and remission stage.

MATERIALS AND METHODS

Patients

We studied 36 men aged 15–49 years (median 32) of whom 16 had active Behçet's disease and 20 were in the remission phase. Patients with active disease had at least three major symptoms of the proposed criteria of Lehner & Barnes (1979): aphthous ulcer; genital ulcer; ocular symptoms; and skin lesions (the minor criteria are arthritis; gastro-intestinal lesions; and central nervous system involvement). Clinical activity was assessed at the time of blood sampling. Ten patients with active disease were studied before treatment; the other six had been treated with corticosteroids without showing any clinical improvement. Twelve seronegative controls and 10 seropositive controls for HSV-1 were included in our study. All control subjects were in good health. When HSV-1 neutralization antibody titre was 1/8 or higher, controls were designated seropositive; when HSV antibody was $< 1/8$ they were recorded seronegative. All seropositive patients had an HSV-1 antibody titre of $\geqslant 1/16$, and the antibody titre in patients with Behçet's disease ranged from 1/32 to 1/256 (Hamzaoui *et al.*, 1989).

Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized samples using conventional Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) centrifugation and then washed once with phosphate-buffered saline (PBS).

Lymphocyte subpopulations involved in HSV-1-specific cytotoxicity

PBMC were separated into T and non-T cells by rosetting with sheep erythrocytes as described (Hamzaoui *et al.*, 1989). This T cell fraction contained less than 2% SIg^+ and more than 93% Leu 4^+ cells. Cells that bound to the sheep erythrocytes were adjusted to 6×10^7/ml in PBS with 5% fetal calf serum (FCS, GIBCO, Grand Island, NY) and incubated with mouse monoclonal antibody (MoAb) NKH-1^+ (mouse IgG1; Coulter Corporation) at 0·25 $\mu g/10^6$ cells for 60 min at 4°C. The cells were then centrifuged and resuspended at 5×10^6/ml in PBS with 5% FCS and poured into 100-mm plastic Petri dishes (Miles Laboratories). Each dish had been incubated overnight at 4°C with 6 ml of F $(ab)'_2$ goat anti-mouse IgG Fc (20 mg/ml) (Ortho Diagnostics) followed by the addition of 15 ml of PBS with 5% bovine serum albumin (BSA; Sigma), incubated for 1 h at room temperature, then washed three times with PBS and once with PBS with 1% FCS. After 90-min incubation at 4°C, the non-adherent T cells (NKH-1^-/$CD3^+$ cells) were removed by gentle swirling and aspiration. This $CD3^+$ fraction contained $< 1\%$ NKH-1^+ cells and more than 95% $CD3^+$ cells as determined by anti-Leu 4 MoAb (Becton Dickinson). In order to determine the subsets of effector cells involved in HSV-1 cytotoxicity, $CD3^+$ cells were fractionated by the panning procedure as described above. The panning technique permitted consistent separation of mutually exclusive subpopulations of $CD3^+$ cells into $CD4^+$ or $CD8^+$ subsets. The purity of both positively (bound to pans) and negatively (unbound to pans) selected subpopulations was determined by direct immunofluorescence analysis. When OKT8 (anti-CD8) MoAb was used as the first stage incubating antibody, $> 94\%$ of the bound cells and $< 6\%$ of the unbound cells were $CD8^+$. Similar subset purity was obtained when OKT4 (anti-CD4) MoAb was used as the first stage reagent.

Generation of effector cells

Four millilitres of cells at 2×10^6 cells/ml were cultured in tissue culture flasks (Costar, Cambridge, U.K.) in the presence of u.v.-inactivated HSV-1, or control antigens (uninfected tissue culture) in an incubator with 5% CO_2 and 95% air for 6 days, as described by Tsutsumi *et al.* (1986). On the day of testing, the cells were washed and the number of viable cells was determined by trypan blue exclusion. The cells were resuspended in RPMI 1640 medium supplemented with 25 mM HEPES buffer and 10% heat inactivated FCS (complete medium).

Target cells

For the generation of autologous or allogenic targets, cell suspension (10^6/ml) was maintained under conditions similar to those described for effector cells. Subsequently, PHA (Difco) in a final concentration of 0·2% was added 3 days before the cytotoxicity testing. One day before testing, PHA-stimulated lymphocytes were incubated at 37°C for 90 min with live HSV-1 at a multiplicity of infection (MOI) of 40–50 in 0·5 ml of complete medium without FCS. The cells were further incubated for 20 h. The target cells prepared in this manner contained 60–80% of cells bearing HSV-specific surface antigens as determined by indirect membrane immunofluorescence (Anderson *et al.*, 1985; Tsutsumi *et al.*, 1986). This was done using rabbit immunoglobulin (to HSV-1) and fluorescein-conjugated pig anti-rabbit immunoglobulin. Target cells infected with mumps virus were prepared under conditions

Table 1. Virus specificity of HSV-1-sensitized effector cells *in vitro*

Effector lymphocytes*	Autologous target cells†		
	Infected HSV-1	Infected with mumps virus	Uninfected
HSV-1 Ag-stimulated lymphocytes of Behçet's disease‡	37·2 ± 5·6 ($n = 5$)	2·8 ± 0·5 ($n = 5$)	1·7 ± 0·9 ($n = 5$)
HSV-1 Ag-stimulated cells (NKH-1^-) of Behçet's disease	28·6 ± 7·3§ ($n = 5$)	0·0 ± 0·0 ($n = 4$)	0·0 ± 0·0 ($n = 5$)
Control¶	2·1 ± 0·5 ($n = 5$)	0·3 ± 0·1 ($n = 5$)	0·0 ± 0·0 ($n = 5$)

An effector: target cell (E:T) ratio of 50:1 was used. Patients sensitized to HSV-1 were negative for mumps virus.

Results are mean percent lysis ± s.d. Cytotoxicity against autologous HSV-1 infected cells was significantly different ($P < 0·01$) than cytotoxicity against mumps virus or uninfected cells.

* From patients with Behçet's disease.

† Six-day *in vitro*, autologous lymphocytes were infected with HSV-1 or mumps virus (90 min for HSV, 60 min for mumps virus).

‡ Peripheral blood T cells were incubated *in vitro* 6 days with u.v.-inactivated HSV-1 antigen (Ag).

§ HSV-1 Ag-stimulated lymphocytes ($CD3^+$/NKH1^-).

¶ Uninfected effector cells (peripheral blood T cells).

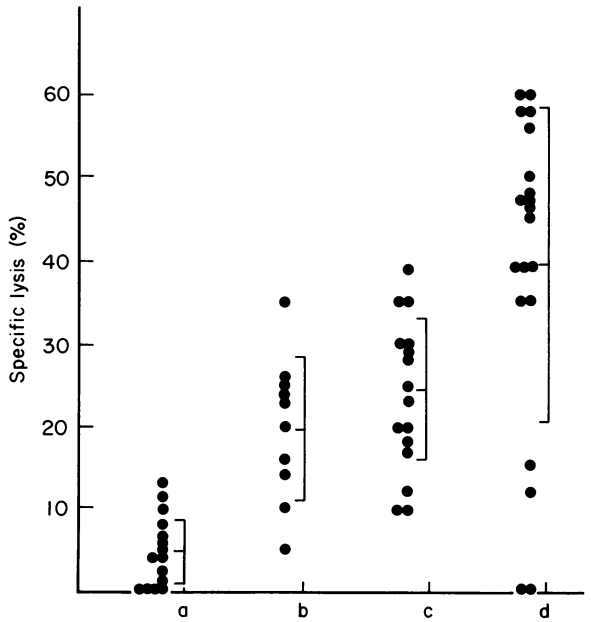

Fig. 1. Cytotoxic activity to autologous HSV-1-infected cells of sensitized unfractionated lymphocytes from seronegative (a), seropositive (a) controls and from patients with Behçet's disease in active (c) and in remission stage (d). An E:T ratio of 50:1 was used. Individual results and mean values ± s.d. are indicated for each group.

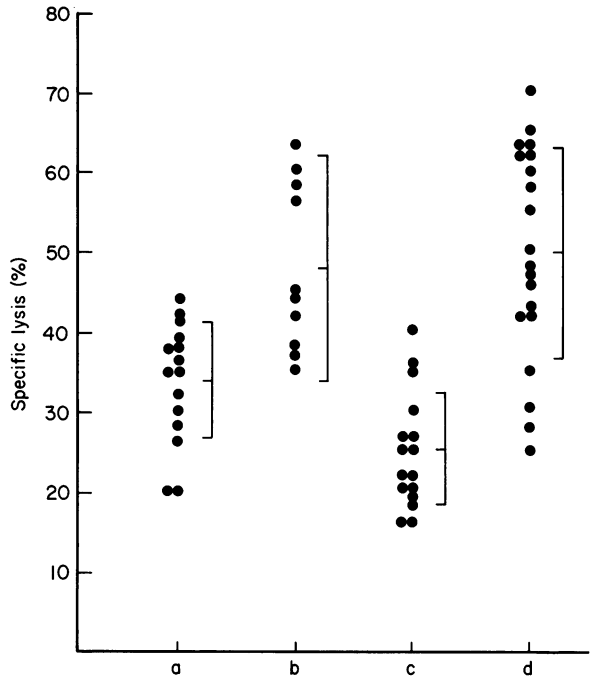

Fig. 2. Natural killer activity of K562 cells of sensitized lymphocytes from unfractionated control seronegative (a), control seropositive (b), patients with Behçet's disease in active (c) and in remission stage (d). An E:T ratio of 50:1 was used. Individual results and mean values ± s.d. are indicated for each group of subjects.

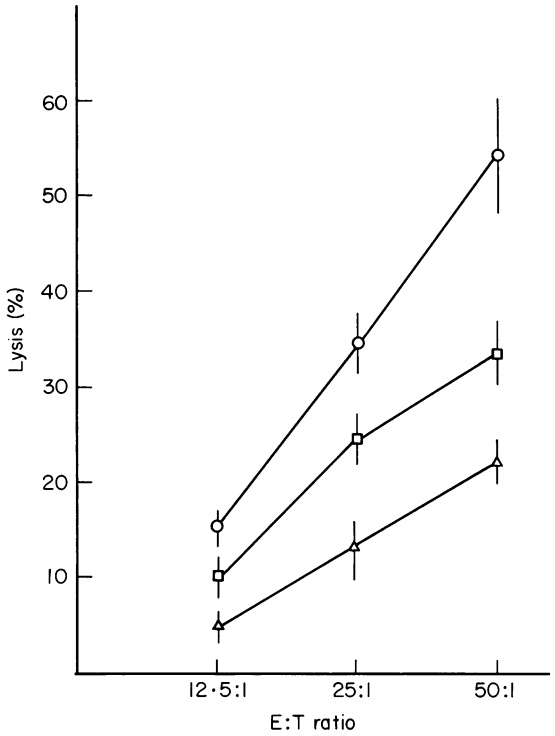

Fig. 3. Analysis of herpes simplex virus-specific cytotoxic effector $CD3^+$ (○) ($CD3^+/NKH\text{-}1^-$) cells, $CD4^+$ (□) ($NKH\text{-}1^-$) and $CD8^+$ (△) ($NKH\text{-}1^-$) subpopulations in 10 patients with Behçet's disease in remission stage. PBMC were sensitized against HSV-1 and assayed 6 days later on autologous HSV-1 infected target cells.

similar to those described for preparation of effector cells in presence of u.v.-inactivated mumps virus. The cells were stimulated with PHA 4 days before the cytotoxicity test was performed. The PHA-stimulated cells were washed and incubated with 12000 haemagglutination units (HAU) of live mumps virus (0·4–0·6 of the stock virus) at 37°C for 1 h. The target cells prepared in this manner constituted 70–80% of cells with virus-specific surface antigen as determined by FITC anti-mumps virus rabbit antibody. Finally, K562 cells served as reference target for natural killing because of their remarkable sensitivity to NK cell cytotoxicity. PHA-stimulated, non-infected mononuclear cells were used as negative controls for virus specificity.

Cytotoxicity assay

Target cells (3×10^6 in 200 μl complete medium) were labelled by incubation with 100 μl (100μ Ci/ml) of ^{51}Cr in the form of sodium chromate (CEA, France) for 60 min at 37°C; the cells were then washed twice in 50 ml of RPMI 1640, and resuspended at 10^5 cells/ml; 0·1 ml of this suspension was then mixed with 5×10^5 effector cells in a final volume of 0·2 in flat-bottomed microtitre plates (Greiner, FRG). Effector-to-target (E:T) ratios of 50:1, 25:1 and 12·5:1 were plated in triplicate. After 4-h incubation at 37°C in a 5% CO_2 humidified incubator, the plates were centrifuged, and 100 μl of the supernatants were collected, and the radioactivity released was counted in a gamma counter (LKB, Bromma, Sweden). A spontaneous release control and a maximum release control were incubated in all assays and consisted of 0·1 ml complete medium or HCl 6 N, respectively, added on, target cells. Mean counts (ct/min) in triplicate tests were determined. Percentage of specific lysis were calculated as:

$$\% \text{ Lysis} = \frac{\text{Experimental} - \text{Spontaneous release}}{\text{Maximal} - \text{Spontaneous release}} \times 100.$$

The spontaneous release usually ranged from 1–18% of the maximum ^{51}Cr released in the HCl controls. The difference between the percentage of lysis by control effector cells and HSV-1-stimulated effector cells was considered as the percentage of specific lysis.

Virus

HSV-1 (HF strain) was grown on Vero cells monolayer culture by infecting at low MOI in modified Eagle's medium (MEM, Gibco). Virus preparations were lightly sonicated. The virus was concentrated by centrifugation at 80000 *g* for 50 min, and cell debris was removed by centrifugation (1000 *g* for 30 min) and the pellet was resuspended in serum-free RPMI 1640 medium in 1/10 of its original volume. These preparations contained 5×10^8 plaque-forming units (PFU) per ml of HSV-1. The control antigen was prepared from uninfected Vero-cells. Inactivated non-infectious virus was prepared by u.v. irradiation for 20 min at a distance of 8 cm. PBMC effector cells were initially cultured with various concentrations of HSV-1 antigen, ranging from 16×10^6 to 10×10^6 PFU. Maximal cytotoxic activity was observed with effector cells cultured with 5×10^6 of PFU of u.v.-inactivated HSV-1.

Mumps virus was prepared by centrifugation of mumps virus-infected chick amniotic fluid at 36000 *g* for 2 h after the

Table 2. Cytotoxic activity against autologous and allogenic HSV-1 infected target cells (effector-to-target cell ratio 50:1)

Effector cells*	Specific lysis of HSV-1-infected target cells (%)	
	Autologous†	Allogenic (A2, A28, B8, B40, CW3)
A2 A9 B5 DR5 DR7	35	13
A9 B5 DR4 DR6	42	10
A9 A28 B12 CW4	28	7
A1 A9 B51 B21	45	15
A1 A30 B17 B27	24	10

* Peripheral blood T cells.

† Cytotoxic activity against autologous target cells was significantly different ($P < 0.01$) from cytotoxic activity against allogenic target cells.

crude host materials had been removed by centrifugation at 4000 *g* for 30 min and by resuspending the pellet into PBS, at the original volume. This antigen had 500–1000 HAU/0·025 ml by conventional haemagglutination testing.

HLA typing

HLA typing was performed by the standard microcytotoxicity assay (Hamza *et al.*, 1988).

Statistical analysis

Results were analysed using Student's *t*-test.

RESULTS

Virus specificity of HSV-1-sensitized effector cells in vitro

The specificity of the effector cells in recognizing viral surface antigen was examined on autologous HSV-1-infected, autologous mumps virus-infected, and uninfected target cells (Table 1). Effector cells from patients with Behçet's disease stimulated with HSV-1 could effectively lyse autologous HSV-1 target cells but could not lyse autologous mumps virus target cells. This suggested that these target cells were susceptible to HSV-1-specific, cell-mediated cytotoxicity. HSV-1-stimulated autologous effector cells could not lyse uninfected cells.

Autologous cytotoxic activity

The data on cytotoxic activity to HSV-1 infected autologous target was presented in Fig. 1. Low activity was detected in seronegative controls (4·60 ± 4·17%) when compared with seropositive controls (19·80 ± 8·47; $P < 0.01$). Significant cytotoxic activity was detected in patients with active Behçet's disease (24·25 ± 8·78% $P < 0.01$) and in patients in remission phase (39·20 ± 18·76%; $P < 0.01$). The difference of cytotoxic activity was not statistically significant between seropositive controls and patients.

NK activity

Seropositive and seronegative controls exhibited extremely variable NK activity. Seronegative controls have NK cytotoxicity similar to that which we recently reported in other healthy controls (Hamzaoui *et al.*, 1988). All seropositive controls exhibited an increased NK activity (Fig. 2). Patients with Behçet's disease also showed similar results to those we have recently reported (Hamzaoui *et al.*, 1988, 1989): cytotoxicity to K562 target cells in active Behçet's disease was in the same range as seronegative controls; patients in remission have an increased NK activity ($P < 0.01$) when compared with those groups.

Identification of the cells responsible for HSV-1-specific cytotoxicity

We analysed the nature of the precursor cells needed for the production of HSV-1-specific killer cells. Virus-specific lysis of autologous lymphocytes mediated by the various T lymphocyte subpopulations is shown in Fig. 3. Unfractionated HSV-stimulated lymphocytes from patients in remission (selected for their high specific cytotoxicity) exhibited virus-specific lysis of HSV-infected autologous cells at 12·5:1, 25:1 and 50:1 E:T ratios (17 ± 4%, 38·9 ± 4·2% and 58 ± 3%). Unfractionated cells as well as $CD3^+$ populations were capable of generating HSV-1 immune cytotoxic cells. As shown in Table 1, depletion of NK cells resulted in moderate reduction (not statistically significant) in virus specific lysis. These results indicated that T cells are sufficient to generate killer cells and that the precursor of HSV-1 immune cytotoxic effectors are T cells. Next we examined the characteristics of the HSV-1-specific effector cells from Behçet's disease patients which were generated *in vitro* (Fig. 3). At an E:T ratios of 12·5:1, 25:1 and 50:1, the mean virus-specific lysis mediated by $CD3^+$ cells was, respectively, 15 ± 2%, 34 ± 3% and 54 ± 6%. This activity was significantly increased when compared with specific cytotoxicity mediated by $CD4^+$ or $CD8^+$ effector T cells ($P < 0.01$). Mean virus-specific lysis mediated by $CD4^+$ T cells at all E:T ratios was 10 ± 2·5%, 24·5 ± 3·6% and 33·5 ± 3%, respectively. $CD4^+$ T cells exhibited a specific cytotoxicity significantly higher ($P < 0.01$) than specific cytotoxicity mediated by $CD8^+$ T cells (5 ± 1·5%, 13 ± 3% and 22 ± 2·5%, respectively, at 12·5:1, 25:1 and 50:1 E:T ratios). These results showed that HSV-specific $CD4^+$ and $CD8^+$ CTL are both present in peripheral blood of patients with Behçet's disease.

Role of allogenic restriction

Cytotoxic activity against autologous, allogenic HSV-1-infected target cells was tested in patients with Behçet's disease. Significant reduction of cytotoxic activity ($P < 0.01$) was observed

when PBMC effector cells were tested against allogenic target cells (Table 2).

DISCUSSION

In this study we have demonstrated that specific cell-mediated cytotoxic activity to HSV-1 is generated after *in vitro* stimulation of PBMC in patients with Behçet's disease. This activity was directed against autologous, HSV-1-infected as well as K562 target cells, but not against mumps virus target cells or uninfected autologous target cells. Part of the HSV-1 genome is present and transcribed in PBMC, probably lymphocytes, of patients with Behçet's disease (Eglin *et al.*, 1982; Bonass *et al.*, 1986). The raised level of hybridization of the HSV DNA probes of patients with Behçet's disease may be due to transcription of cell DNA/HSV/DNA, thus altering the homologous sequence (Peden, Mounts & Hayword, 1982). We have separated control subjects into seronegative (those sera that do not have significant anti-HSV 1 antibodies) and seropositive group (those that show anti-HSV), as only PBMC from seropositive subjects generated high proportions of specific cytotoxic activity against autologous targets. Fifty-six per cent of patients with active disease and 80% of patients in remission generated significant cytotoxic lysis (>20%) of HSV-1 autologous target cells. The reduction of cytotoxic activity against allogenic target cells shows that an allogenic restriction exists; however, in the present work we did not determine any specific HLA-low restriction for the specific cytotoxicity.

The generation of cytotoxic T effector cells after HSV-1 stimulation may be explained by the presence of part of the HSV-1 genome in PBMC of patients with Behçet's disease (Eglin *et al.*, 1982; Bonass *et al.*, 1986; Young *et al.*, 1988). Cytotoxic precursor or memory T cells from Behçet's disease may respond to HSV-1 antigen with the subsequent generation of cytotoxic T lymphocytes directed against HSV-1 infected autologous cells.

An analysis of the HSV-1-specific CTL indicated that cytotoxic activity in this system is mediated by $CD4^+$ and $CD8^+$ cells. Our results were similar to those obtained by Tsutsumi *et al.* (1986) in studying cytotoxic T cells in patients with recurrent labial herpes infection. Tsutsumi *et al.* (1986) noted the presence of $CD4^+$ anti-HSV-1 CTL activity in short-term cultures. Several investigators have shown that cloned populations of human $CD4^+$ $CD8^-$ cells are capable of a virus-specific cytolytic activity restricted by HLA class II molecules (Meuer *et al.*, 1983; Kaplan *et al.*, 1984; Sterkers *et al.*, 1985; Bourgault *et al.*, 1989). Several investigators (Diaz *et al.*, 1989; Schmid, 1988; Biddison *et al.*, 1982; Jacobson, Flerlage & McFarland, 1985) reported that the lysis of HSV-infected targets, other than the Epstein–Barr virus, was mediated by class II-restricted $CD4^+$ T lymphocytes. Yasukawa & Zarling (1984a, 1984b), reported that specific cytotoxic lymphocyte clones to HSV-1 were exclusively restricted by $CD4^+$ cells, but did not rule out the existence of $CD8^+$ T cells directed against the HSV-infected autologous targets. Activation of both $CD4^+$ and $CD8^+$ CTL by stimulation with u.v.-inactivated HSV antigen was also reported by Torpey, Lindsley & Rinaldo (1989). Recently, Yasukawa, Inatsuki & Kobayashi (1989) suggested that viral infection due to cell-free viruses is fought by $CD4^+$ CTL, which have previously been reported to possess both cytotoxicity and helper function.

Our results show that $CD4^+$ CTL precursor cells are present in patients with Behçet's disease; that they appear consistently *in vitro* in bulk cultures after 6 days antigenic stimulation; and that their cytolytic activity is comparable to that of $CD8^+$ CTL in terms of lysis of autologous target cells.

The high frequency of Behçet's disease patients whose cells generate strong *in vitro* HSV-1-specific CTL may reflect the immunogenetic predisposition of these patients to repeated infection with HSV-1. The capacity of HSV-1 to establish a persistent infection could be due to suppression of cellular immune responses in Behçet's disease.

The biological role of HSV-1-specific CTL in Behçet's disease remains unknown. Three questions remain unanswered: (i) does cytotoxic activity against HSV-1, mediated by $CD4^+$ and $CD8^+$ cells, exist *in vivo* in patients with the disease? (ii) why does the cytolytic potential of $CD4^+$ cells appear *in vitro*? and (iii) what is their *in vivo* significance?

These CTL could eliminate HSV-1-infected cells and have an important role in limiting the spread of the virus *in vivo*. It cannot be ignored, however, that they could appear *in vivo* in certain micro-environments resembling our culture conditions, possibly playing a useful or harmful role during HSV-1 infection. Future investigations may demonstrate new links between viral infections and the alteration that remain unexplained in Behçet's disease.

REFERENCES

Anderson, J., Byrne, J.A., Schreiber, R., Patterson, S. & Oldstone, M.B.A. (1985) Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus: clearance of virus and in vitro properties. *J. Virol.* **53,** 552.

Bacon, T.H., Ozabakir, F., Elms, C.A. & Denman, A.M. (1984) Interferon-γ production by peripheral blood mononuclear cells from patients with Behçet's syndrome. *Clin. exp. Immunol.* **58,** 541.

Biddison, W.E., Rao, P.E., Talle, M.A., Goldstein, G. & Shaw, S. (1982) Possible involvement of the OKT4 molecule in T cell recognization of class II HLA antigens: evidence from studies of cytotoxic lymphocytes specific for SB antigens. *J. exp.Med.* **156,** 1065.

Bonass, W.A., Stewart, J.A., Chamberlain, M.A. & Halliburton, I.W. (1986) Molecular studies in Behçet's disease. In *Recent Advances in Behçet's Disease* (ed. by T. Lehner & C. G. Barnes) p. 37. Royal Society of Medicine, London.

Bourgault, I., Gomez, A., Gomrad, E., Picard, F. & Levy, J.P. (1989) A virus specific $CD4^+$ cell mediated cytolytic activity revealed by $CD8^+$ cell elimination regulatory develops in uncloned human antiviral cell lines. *J. Immunol.* **142,** 252.

Denman, A.M., Fialkow, P.J., Pelton, B.K., Salon, A.C., Appleford, D.J. & Gilschrist, C. (1980) Lymphocyte abnormalities in Behçet's syndrome. *Clin. exp. Immunol.* **42,** 175.

Diaz, P.S., Smith, S., Hunter, E. & Arvin, A.M. (1989) T lymphocyte cytotoxicity with natural varicella-zoster virus infection and after immunization with live attenuated varicella vaccine. *J. Immunol.* **142,** 636.

Eglin, R.P., Lehner, T. & Suback-Sharpe, J.H. (1982) Detection of RNA complementary to herpes simplex virus in mononuclear cells from patients with Behçet's syndrome and recurrent oral ulcers. *Lancet,* **ii,** 1356.

Fujii, N., Minagawa, T., Nakane, A., Kato, F. & Ohno, S. (1983) Spontaneous production of γ-interferon in cultures of T lymphocytes obtained from patients with Behçet's disease. *J. Immunol.* **130,** 1683.

Hamza, M., Ayed, K., Hamzaoui, K. & Bardi, R. (1988) Behçet's disease and major histocompatibility complex class II antigens in Tunisians. *Disease Markers,* **6,** 263.

HAMZAOUI, K., AYED, K., HAMZA, M. & TOURAINE, J.L. (1988) Natural killer cells in Behçet's disease. *Clin. exp. Immunol.* **71,** 126.

HAMZAOUI, K., AYED, K., SLIM, A., HAMZA, M. & TOURAINE, J.L. (1989) Natural killer cell activity, interferon-gamma and antibodies to herpes viruses in patients with Behçet's disease. *Clin. exp. Immunol.* **79,** 28.

JACOBSON, S., FLERLAGE, M.L. & MCFARLAND, H.F. (1985) Impaired measles virus-specific cytotoxic T cell responses in multiple sclerosis. *J. exp. Med.* **162,** 839.

KAPLAN, D.R., GRIFFITH, R., BRACIALE, V.L. & BRACIAL, T.J. (1984) Influenza virus specific human cytotoxic T cell clones: heterogeneity to antigen specificity and restriction by class II MHC products. *Cell Immunol.* **88,** 193.

LEHNER, T. & BARNES, C.G. (1979) *Behçet's Syndrome. Clinical and Immunological Features.* Academic Press, London.

MEUER, S.C., HODGDON, J.C., COOPER, D.A., HUSSEY, R.E., FITZGERALD, K.A. & REINHERZ, E.L. (1983) Human cytotoxic T cell clones directed at autologous virus-transformed targets: further evidence for linkage of genetic restriction to T4 and T8 surface glycoproteins. *J. Immunol.* **131,** 186.

OHNO, S., KATO, F., MATSUDA, H., FUJII, N. & MINAGAWA, T. (1982) Detection of gamma interferon in the sera of patients with Behçet's disease. *Infect. Immun.* **36,** 202.

PEDEN, K., MOUNTS, P. & HAYWORD, G. (1982) Homology between-mammalian cell DNA sequences and human herpes virus genomes detected by high complexity probe hybridisation procedure. *Cell,* **31,** 71.

SCHMID, D.S. (1988) The human MHC-restricted cellular responses 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.

STERKERS, G., MICHON, J., HENIN, Y., COMARD, E., HANNOUN, C., LEVY, J.P. (1985) Fine specificity analysis of human influenza specific cloned cell line. *Cell Immunol.* **94,** 394.

TORPEY, D.J., LINDSLEY, M.D. & RINALDO, C.R. JR (1989) HLA-restricted lysis of herpes simplex virus-infected monocytes and macrophages mediated by $CD4^+$ and $CD8^+$ T lymphocytes. *J. Immunol.* **142,** 1325.

TSUTSUMI, H., BERNSTEIN, J.M., TALTY, M.R., COHEN, E., ORSINI, F. & OGRA, P.L. (1986) Immune responses to herpes simplex virus in patients with recurrent herpes labial is: I. development of cell-mediated cytotoxic responses. *Clin. exp. Immunol.* **66,** 507.

YASUKAWA, M. & ZARLING, J.M. (1984a) Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. I. Lysis restricted by HLA class II M.B. and Dr antigens. *J. Immunol.* **133,** 422.

YASUKAWA, M. & ZARLING, J.M. (1984b) Human cytotoxic T cell clones directed against herpes simplex virus infected cells. II. Bifunctional clones with cytotoxic and virus induced proliferative activity exhibit herpes simplex virus type 1 and 2 specific or type common reactivities. *J. Immunol.* **133,** 2736.

YASUKAWA, M., INATSUKI, A. & KOBAYASHI, Y. (1989) Differential in vitro activation of $CD4^+$ $CD8^-$ and $CD8^+$ $CD4^-$ herpes simplex virus-specific human cytotoxic T cells. *J. Immunol.* **143,** 2051.

YOUNG, C., LEHNER, T. & BARNES, C.G. (1988) CD4 and CD8 cell responses to herpes simplex virus in Behçet's disease. *Clin. exp. Immunol.* **73,** 6.