Immune responses to herpes simplex virus in patients with recurrent herpes labialis: I development of cell-mediated cytotoxic responses

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SUMMARY

Groups of subjects during acute (0–3 days) and convalescent (2–3 weeks) phase of recurrent herpes labialis (RHL), and other subjects seropositive or seronegative for herpes simplex virus type 1 (HSV-1) antibody without any history of RHL, were tested for the appearance of cell-mediated cytotoxic responses by stimulating peripheral blood leukocytes (PBL) *in vitro* with ultraviolet-inactivated HSV-1 antigen, using the release of radiolabelled chromium (^{51}Cr) from HSV-1-infected autologous, or allogeneic lymphocytes and K562 erythroleukemia cell line as nonspecific targets. Development of HSV specific cytotoxic response using autologous targets was essentially limited to subjects with RHL and in HSV antibody seropositive control subjects. Peak activity was observed during the acute phase of the disease, compared to the activity in the convalescent phase in seropositive subjects with RHL, and was preceded by high lymphoproliferative response to HSV. Higher cytotoxic responses against K562 cells were also observed in RHL subjects compared to the controls. Depletion of Leu-2$^+$, Leu-3$^+$ or Leu-11 effector lymphocytes from HSV-1-stimulated PBL cultures by treatment with complement and appropriate monoclonal antibodies resulted in significant reduction of cytotoxicity to HSV-1-infected autologous cells. However, cytotoxicity to K562 cells was reduced only after depletion of Leu-11$^+$ cells. Low levels of allogeneic restriction were observed for cytotoxicity to HSV-1-infected targets. These observations suggest selective activation of virus specific Leu-2$^+$ and Leu-3$^+$ T cell subsets as well as natural killer cell mediated cytotoxic mechanisms during the active phase of recurrences of herpes simplex virus infection.

Keywords Herpes simplex virus type 1   recurrent herpes labialis   cytotoxic T lymphocyte   natural killer cell

INTRODUCTION

Herpse simplex virus (HSV) is characteristically associated with latency after acute primary infection, and despite the development of virus specific immune responses (Buddingh *et al.*, 1953; Nahmias & Roizman, 1973), the infected host may suffer many episodes of recurrence (Rawls *et al.*, 1970; Rawls & Campione-Piccardo, 1981). A role for the immune response in controlling recurrence

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of latent HSV infection has been suggested by the high incidence of reactivation of HSV infection in patients undergoing immunosuppressive therapy for transplantation (Pien *et al.*, 1973; Rand *et al.*, 1976) and during the use of cytotoxic drugs in cancer patients (Muller, Herrmann & Winkelmann, 1972). Observation in several situations *in vivo* and *in vitro* indicate a primary role for T lymphocytes in HSV immunity, particularly in animal models (Pfizenmaier *et al.*, 1977; Nagafuchi *et al.*, 1979; Sethi, Omata & Schneweis, 1983). For human HSV infection, several testing procedures have been developed to analyse HSV specific cell-mediated immunity. These include lymphocyte transforma- tion (LTF) (Starr *et al.*, 1975; Stilltoe, Wilton & Lehner, 1977; Lopez & O'Reilly, 1977; O'Reilly *et al.*, 1983), lymphokine production especially leukocyte migration inhibition factor (Shilltoe *et al.*, 1977; O'Reilly *et al.*, 1983) and interferon (O'Reilly *et al.*, 1983; Cunningham & Merigan, 1983; 1984), and specific cytotoxic T lymphocyte (CTL) responses (Stethi, Stroehmann & Brandis, 1980; Yasukawa & Zarling, 1984a,b). Previous studies have suggested alterations in parameters *in-vitro* such as LTF (Shilltoe *et al.*, 1977; Lopez & O'Reilly, 1977; O'Reilly *et al.*, 1983) and lymphokine production (Shilltoe *et al.*, 1977; O'Reilly *et al.*, 1983; Cunningham & Merigan, 1983) in patients with recurrent HSV infection. The aim of the present study was to examine cell-mediated immunological events, especially HSV-1 specific CTL responses in patients with recurrent herpes labialis (RHL).

MATERIALS AND METHODS

Study population. The patient population consisted of 18 patients with RHL. The subjects ranged in age from 15 to 50 years, and included seven males and 11 females. All were in good health. Heparinized specimens of peripheral blood were available for immunological investigations in 16 subjects during the acute (0–3 days) phase of disease and in all 18 subjects during the convalescent (2–3 weeks) phase. The diagnosis was made on the basis of the patient's history, the appearance of the typical lesions, and positive virological cultures using Hep 2 and RK13 cell lines. Only patients with virologically documented RHL with recovery of the virus during acute phase of the disease were included in the study. The control population for these studies consisted of 12 HSV antibody seronegative subjects and 10 HSV antibody seropositive subjects without any history of recurrent HSV infection. These subjects were matched for age and sex to the test population described above. Subjects with HSV-1 neutralization antibody titre of 1 : 10 (the lowest detactable titre) or higher were designated seropositive and subjects in whom no HSV antibody was detected (< 1 : 10) were considered to be seronegative. All seropositive patients had an HSV-1 antibody titre of 1 : 40 or greater, and no difference was found between the antibody titre in acute phase and convalescent phase samples (data not shown).

Preparation of effector and target cells: in vitro generation of effector cells. Peripheral blood mononuclear cells were separated from blood specimen processed for preparation of both effector and target cells as described below. Ficoll-Hypaque-separated peripheral blood lymphocytes were suspended in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 25 mM HEPES buffer and 10% heat-inactivated normal human serum to a concentration of 2×10^6 cells/ml. Four millilitres of the cell suspension were cultured in tissue culture flasks (no. 3025, Costar, Cambridge, MA) in the presence of u.v.-inactivated HSV-1 or control antigens (uninfected tissue culture) in an incubator with an atmosphere of 5% CO_2 for 6 days. On the day of the cytotoxicity testing, the cells were washed twice and the number of viable cells was determined by trypan blue exclusion. They were resuspended in RPMI-1640 medium supplemented with 25 mM HEPES buffer and 10% heat- inactivated fetal calf serum (FCS). This medium will be referred to as 'assay medium'.

Target cells. For the generation of autologous or allogeneic targets, aliquots of 1×10^6/ml of Ficoll-Hypaque separated peripheral blood mononuclear cells were suspended in assay medium. The cell suspension was initially maintained under conditions similar to those described for preparation of effector cells. Subsequently, phytohaemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, MI) in a final concentration of 0·2% was added 3 days before the cytotoxicity testing. One day before cytotoxicity 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 serum free RPMI-1640

medium. The cells were incubated for an additional 16–18 h and labelled with 100 μCi of NA$_2$ ^{51}CrO$_4$ (New England Nuclear, Boston, MA) for 1 h at 37°C. The cells were washed extensively and reconstituted in assay medium and employed as target cells. Regardless of the immune status of the subjects the target cells prepared in this manner contained 60 to 82% of cells bearing HSV virus-specific surface antigens as determined by indirect membrane immunofluorescence (Anderson *et al.*, 1985), using rabbit immunoglobulin to HSV-1 and fluorescein-conjugated swine immunoglobulin to rabbit immunoglobulin (Accurate Chemicals, Westbury, NY). PHA stimulated but uninfected mononuclear cells were employed as controls for virus specificity.

K562 suspension cell cultures were employed as heterologous targets for nonspecific and NK cytotoxicity. The cells were grown continuously in RPMI-1640 medium supplemented with 25 mM HEPES buffer and 5% FCS, and control (uninfected PHA stimulated) lymphocytes were also labelled with Na$_2$ ^{51}CrO$_4$ in the same manner.

Cytotoxicity assay. The assay was performed by a micromethod as described previously (Shaw, Nelson & Shearer, 1978). One hundred microlitres of the target cell suspension (5 × 10^3/well) and an equal volume of suspension of effector cells were placed in triplicate wells in round-bottomed microculture plates (Costar, Cambridge, MA). The plates were centrifuged at 250 g for 3 min and incubated for 5 h at 37°C 5% CO$_2$. Total releasable radioactivity was determined by treating target cells with Triton X-100 (Sigma, St Louis, MO). After incubation, supernatants were removed and transferred to tubes for counting in a gamma counter. Spontaneous release of radioactivity was determined from target cells incubated with medium alone. The percent (%) lysis as a reflection of cytotoxicity was calculated as follows:

$$\frac{\text{ct/min experimental release} - \text{ct/min spontaneous release}}{\text{ct/min maximal release} - \text{ct/min spontaneous release}} \times 100$$

The difference between the % lysis by control effector cells and HSV-1-stimulated effector cells was regarded as the % specific lysis. In general, spontaneous release from the target cells was less than 25% of the maximal release.

Determination of LTF response. The proliferative response of cultured peripheral blood lymphocytes was measured by the uptake of [^{3}H]thymidine (New England Nuclear, Boston, MA). On day 6 of culture, the cells were suspended and 100 μl aliquots were transferred in triplicate to microculture plates. Then 0·2 μCi of [^{3}H]thymidine per 20 μ was added, and the cultures were incubated for 6 h in 5% CO$_2$ at 37°C, followed by cell harvesting and assessment of radioactivity in a scintillation counter. The counts per minute (ct/min) of replicate culture stimulated by antigen minus the ct/min of the unstimulated (uninfected tissue culture) controls, the Δ ct/min, reflect proliferative responses *in vitro*.

Other specificity controls: cold target inhibition studies. ^{51}Cr-labelled HSV-infected or uninfected autologous, or K562 target cells (5 × 10^3), were added to corresponding unlabelled (cold) target cells in microtitre wells. A constant number of autologous effector cells were added to each well. Unlabelled cells were also added to wells containing ^{51}Cr-labelled target cells without effector cells. The specific chromium release and cytotoxicity determinations were subsequently carried out as outlined earlier.

Cytotoxic cell subset determinationa. In order to determine the subsets of effector cells involved in cytotoxicity, several depletion experiments were carried out before cytotoxicity assays *in vitro*. Aliquots of Ficoll-Hypaque separated peripheral blood lymphocytes were cultured *in vitro* for 6 days as described above. The cells were extensively washed, and the centrifuged cell pellets were incubated with 15 μl of Leu-2b (suppressor-cytotoxic), Leu-3a (helper-inducer), or Leu-11b (natural killer) specific monoclonal antibodies (Becton Dickinson, Mountain View, CA) for 1 h on ice. The preparations were subsequently incubated with 50% solution of rabbit complement (Pel-Freeze, Rogers, AR) for 1 h at 37°C. The cells were washed twice with medium and viable cells were resuspended in the culture medium after trypan blue exclusion test for cytotoxicity testing (Biddison, Sharrow & Shearer, 1981).

Virus. HSV-1 (HF strain) was grown on Vero cell monolayer cultures by infecting at a low MOI in Eagle's medium (MEM) (Gibco, Grand Island, NY). Virus preparations were lightly sonicated. Concentrated virus was prepared by centrifugation (80,000 g for 50 min) after the crude cell debris

had been removed by centrifugation (1,000 g for 30 min) and by suspending the pellet into serum free RPMI-1640 medium in 1/10 of the original volume. These preparations contained 5×10^8 plaque forming unit (pfu/ml) of HSV-1. The control antigen was prepared from uninfected Vero cells. Inactivated non-infectious virus was prepared by subjecting the virus antigens to ultraviolet (u.v.) irradiation for 20 min with a 30 W CE30 T8 u.v. lamp at a distance of 8–10 cm.

Antibody assay. The plaque reduction neutralization test described by Rawls *et al.* (1970) was used with minor modifications. Beginning at 1 : 10, serial 2-fold dilutions of heat-inactivated serum or plasma was mixed with an equal volume of 2×10^3 pfu/ml of HSV-1 and incubated at room temperature for 1 h. Mixtures, 0·1 ml, were incubated onto each of three wells in a Linbro tissue culture plate (Flow Laboratories, McLean, VA) containing Vero cell monolayers. After 1 h absorption at room temperature, the monolayers were overlaid with MEM containing 2% FCS and 1% methylcellulose. After three days of incubation at 37°C, the plaques in the test and control plates were counted. The dilution which reduced the infectivity of virus by 80% of the control values was considered as the neutralizing antibody titre.

HLA typing. Human leukocyte antigen (HLA)-A, -B and -DR loci typing of limited numbers of these donors had been performed by the standard microcytotoxicity assay (Terasaki & Park, 1976).

Statistics. Differences between study groups in terms of cytotoxic activity and lymphoproliferative response were analysed using Student's *t*-test. Correlation coefficient between autologous cytotoxic activity and lymphoproliferative response was calculated by the methods of least squares.

RESULTS

Cytotoxic responses

Dose responses and virus specificity. To determine the optimal conditions of cytotoxicity to HSV infected autologous targets, PBL effector cells were initially cultured with varying concentrations of

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

Donors*	Antigens *in vitro*	% Lysis of virus-infected and uninfected autologous cells†		
		HSV-1	Mumps‡	Uninfected
1	HSV-1	32·7	12·4	8·7
	Mumps‡	15·4	75·1	16·5
	Control	10·9	−3·5	−1·1
2	HSV-1	12·9	5·4	5·7
	Mumps	9·2	22·8	1·4
	Control	4·5	−2·8	−1·2
3	HSV-1	24·8	7·5	8·7
	Control	4·7	−1·0	−0·8
4	HSV-1	30·3	5·3	1·3
	Control	3·1	−4·6	−2·7
5	HSV-1	20·3	3·0	4·6
	Control	−1·5	−3·2	1·7

* All donors were subjects with RHL.

† An effector cell : target cell (E : T) ratio of 40 : 1 was used.

‡ Mumps virus-sensitized effector cells and mumps virus-infected target cells were prepared as described previously (Tsutsumi *et al.*, 1980).

HSV-1 antigen, ranging from 10^6 to 9×10^6 pfu. Maximal cytotoxic activity was observed with effector cells cultured with 3×10^6 pfu of u.v.-inactivated HSV-1 virus (data not shown), so for subsequent experiments we used lymphocytes cultured for 6 days with 3×10^6 pfu of u.v.-irradiated HSV-1.

The specificity of the effector cells in recognizing viral surface antigen on autologous target cell was examined by using autologous lymphocytes infected with another unrelated non-DNA virus, the mumps virus.

Effector cells of mumps seropositive subjects cultured with u.v.-irradiated mumps virus could effectively lyse autologous mumps virus infected targets (Table 1), suggesting that these target cells were susceptible to virus-specific cell-mediated cytotoxicity (Tsutsumi *et al.*, 1980). However, HSV-1-stimulated autologous effector cells could not lyse mumps virus-infected or uninfected cells. Significantly, however, such effectors exhibited high cytotoxic activity to HSV-1-infected targets (Table 1).
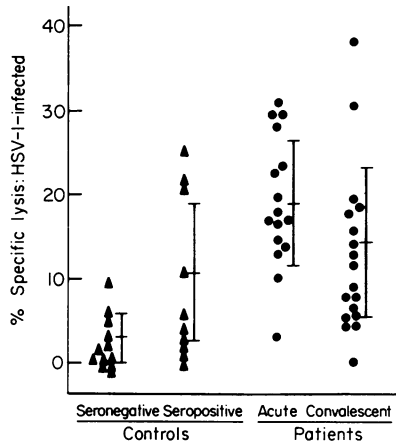

Fig. 1. Cytotoxic activity to autologous HSV-1-infected cells of sensitized lymphocytes from controls (▲) and patients with RHL (●). An E:T ratio of 40:1 was used. Mean values ± s.d. were indicated for each group of subjects.

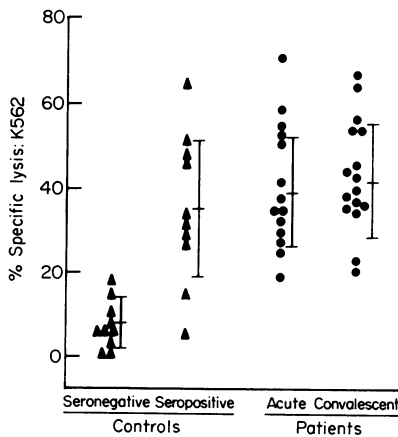**Fig. 2.** Cytotoxic activity to K562 cells of sensitized lymphocytes from controls (▲) and patients with RHL (●). An E:T ratio of 40:1 was used. Mean values ± standard deviation were indicated for each group of subjects.

Autologous cytotoxic activity. The data on cytotoxic activity to HSV-1-infected autologous targets is presented in Fig. 1. Low or no activity was detected in seronegative controls. On the other hand, significant cytotoxicity was observed in patients during the acute phase of the disease ($17.8 \pm 7.9\%$) and the activity declined somewhat during the convalescence phase ($14.2 \pm 9.8\%$). This difference did not appear to be statistically significant ($P > 0.05$). It is, however, significant to note that the cytotoxic activity observed in seropositive controls without RHL ($10.4 \pm 8.8\%$) was significantly ($P < 0.05$) lower than that of patients during acute phase of the recurrences (Fig. 1).

Cytotoxic activity to K562 targets. Although demonstrable cytotoxicity to K562 cells was observed in a few seronegative controls, high activity was observed in all RHL patients both during acute ($39.3 \pm 13.4\%$) and convalescent phases ($43.2 \pm 13.7\%$) (Fig. 2). Seropositive controls without RHL exhibited extremely variable responses ($35.9 \pm 16.6\%$). Although there were no apparent differences in the cytotoxic activity observed in HSV seropositive subjects with or without RHL, the cytotoxicity against K562 targets in HSV seropositive subjects was significantly higher than in seronegative controls ($6.8 \pm 6.3\%$, $P < 0.01$) (Fig. 2).

Competitive inhibition of cytotoxicity. In order to examine the possible heterogeneity of effector cells responsible for such cytotoxic response we did several cold target inhibition experiments. The lysis of autologous ^{51}Cr labelled HSV-1-infected cells was inhibited by unlabelled HSV-1-infected cells in a dose dependent manner, but only partially inhibited by unlabelled K562 cells. Similarly, the lysis of K562 cells was only minimally inhibited by unlabelled HSV-1-infected cells. On the other hand, uninfected autologous targets cells did not inhibit cytotoxic activity of either ^{51}Cr labelled HSV-1-infected cells or of K562 cells.

Cellular subsets of cytotoxicity. An attempt was made to determine whether cytotoxicity of autologous HSV-1-infected or K562 target cells was mediated by two or more different populations of effector cells generated after stimulation *in vitro* by HSV-1. A series of depletion experiments were performed in which PBL effector cells were treated with cell subset specific monoclonal antibody and complement as shown in four representative experiments in Table 2.

In experiments 1 and 2, depletion of Leu-2$^+$, Leu-3$^+$, or Leu-11$^+$ cells resulted in reduction of cytotoxicity to HSV-1-infected cells to 44.0 and 54.3, 28.9 and 49.7, 47.7 and 30.5% respectively, in comparison to the activity of effector cells treated with complement alone (control). In experiment 3, depletion of Leu-2$^+$ or Leu-11$^+$ cells, and in experiment 4, depletion of Leu-3$^+$ or Leu-11$^+$ cells resulted in similar significant reduction of cytotoxicity to HSV-1-infected cells. On the other hand, cytotoxicity to K562 cells was reduced only after depletion of Leu-11$^+$ cells. The extent of the reduction ranged from 13.5–15.0 (experiments 1, 3 and 4) to 37.0% (experiment 2) of activity observed with effector cells treated with complement alone (control). It should be emphasized that no Leu-11$^+$ cells were detectable after treatment with specific antibody and complement. Taken together, these results would indicate that cultured cytotoxic PBL effector cells have at least four

Table 2. Effect of Leu-2$^+$, Leu-3$^+$ or Leu-11$^+$ cell depression from HSV-1-sensitized cultures on lysis of autologous HSV-1-infected cells and K562 cells

Treatment	Exp. 1†		Exp. 2		Exp. 3		Exp. 4	
	HSV-1	K562	HSV-1	K562	HSV-1	K562	HSV-1	K562
C alone	21.8	34.0	15.1	12.7	16.8	63.9	15.6	36.8
Leu-2+C	12.2	35.6	6.9	16.7	20.1	63.8	8.7	36.3
Leu-3+C	15.5	35.7	7.6	14.6	9.6	66.8	15.7	37.2
Leu-11+C	11.4	29.4	10.5	8.0	10.2	54.3	10.4	31.7

* An E:T ratio of 40:1 was used.

† All experiments were done with lymphocytes of RHL patients. No significant lysis of uninfected autologous cells was observed (data not shown).

Table 3. Effect of Leu-2$^+$, Leu-3$^+$, Leu-11$^+$ cells depletion from HSV-1 sensitized cultures on lysis of autologous and allogenic HSV-1-infected and K562 cells

Antigen *in vitro*	Treatment	% Lysis* of		
		Autologous	Allogeneic†	K562
HSV-1	C alone	15·6	5·6	36·8
	Leu-2+C	8·7	6·9	36·3
	Leu-3+C	15·7	5·6	37·2
	Leu-11+C	10·4	3·2	31·7
Control‡	C alone	4·4	0·3	3·7

   * An E:T ratio of 40:1 was used.

   † There was no HLA-A, -B, and -DR shared between autologous and allogeneic cells.

   ‡ Control effector cells had been treated with C alone.

   No significant lysis of uninfected autologous and allogeneic cells was observed (data not shown).
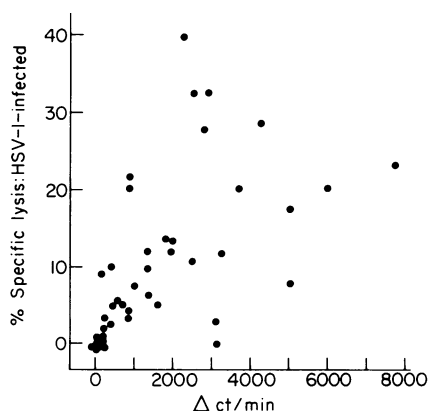

Fig. 3. Relationship between cytotoxic activity against HSV-1-infected autologous targets and LTF response to HSV-1 of sensitized lymphocytes.

subpopulations, Leu-2$^+$ T cells and Leu-3$^+$ T cells that react with HSV-1 infected cells, and Leu-11$^+$ natural killer (NK) cells that kill both HSV-1-infected and K562 cells, and fourth cell population that reacts with only K562 cells.

Role of allogeneic restrictions. The effect of depletion of various effector cell subsets on cytotoxicity against allogeneic HSV-1-infected cells mismatched for HLA-A, -B, and -DR antigens is presented in Table 3. Depletion of Leu-2$^+$ cells resulted in significant reduction of cytotoxic activity against autologous targets (15·6 vs 8·7, Table 3), but no reduction of cytotoxicity was observed against allogenic (5·6 vs. 6·9) and K562 (36·8 vs. 26·3) target cells. We could not determine any specific HLA locus restriction for the cytotoxicity when the effector population was tested on two allogeneic targets. It should be pointed out, however, that a reasonable assessment of the HLA restriction may require assays employing several targets that shared only one HLA antigen, and using a panel of targets that isolate all the effector cell HLA specificities. Unfortunately, these studies could not be considered in these subjects due to lack of large quantities of heparinized blood.

Relationship of cytotoxicity to lymphoproliferation in vitro. Patients in acute phase of RHL consistently exhibited higher ($P < 0.001$) HSV specific lymphoproliferative response when compared to responses observed in seronegative control subjects. The activity declined modestly during the convalescent phase of the disease. Detectable proliferative activity to HSV was observed in some seropositive subjects without clinical evidence of RHL. Careful examination of the proliferative and autologous cytotoxic response indicate a strong correlation between the height of HSV proliferative response to the magnitude of HSV specific cytotoxicity in autologous target as shown in Fig. 3 ($r = 0.533$, $P < 0.005$). These observations are quite similar to the data on seropositive subjects for cytotoxicity against autologous targets.

DISCUSSION

In this study we have demonstrated that cell-mediated cytotoxic activity is generated after stimulation *in vitro* of peripheral blood lymphocytes in patients with recurrent HSV-1 infection. This activity was directed against both autologous HSV-1-infected as well as K562 target cells but not against targets infected with an unrelated (mumps) virus or uninfected autologous targets.

Studies involving cold target competition and lymphocyte subpopulation depletion suggest that the effector cell fractions responsible for these cytotoxic activities are heterogenous. These data suggest that viral specific cytotoxicity may be mediated by Leu-2^{+} and Leu-3^{+} cytotoxic lymphocytes. Studies carried out with influenza virus (Biddison *et al.*, 1981), Epstein-Barr virus (Zarling *et al.*, 1981) and measles virus (Lucas *et al.*, 1982) have suggested that OKT4^{−} (analogue to Leu-3), and OKT8^{+} (analogue to Leu-2) cells mediate specific cytotoxicity. Recently Yasukawa *et al.* (1984a,b) clearly demonstrated that HSV specific cytotoxic lymphocyte clones were exclusively restricted to OKT4^{+}, OKT8^{−} T cells, although they did not rule out the existence of OKT8^{+} T cells directed against the HSV-infected autologous targets. In the present studies, we could detect both Leu-2^{+} and Leu-3^{+} HSV immune cytotoxic cells in cultured lymphocytes from patients with RHL. However, the proportion of the cytotoxic activity to autologous HSV-1-infected cells was not always uniform.

The generation of at least four different populations of effector cells after HSV-1 stimulation may be explained as follows. Cytotoxic precursor or memory T cells from subjects with RHL may respond to HSV-1 antigen with the subsequent generation of Leu-2^{+} CTL and Leu-3^{+} CTL directed against HSV-1 infected autologous cells. In addition stimulation with HSV-1 may augment Leu-11^{+} NK cell activity and may further stimulate the generation of NK activity associated with Leu-11^{−} cell type.

We could detect higher LTF responses to HSV-1 in acute phase samples than in convalescent phase samples of RHL subjects or seropositive subjects without RHL. Wilton *et al.* (1972) and more recently Shilltoe *et al.* (1977) also observed higher LTF activity in acute phase of RHL. In view of the strong correlation observed between LTF and CTL (Fig. 3), the present data strongly support the possibility of generation *in vitro* of the HSV immune CTL in patients with RHL. Similar mechanisms may result in generation *in vivo* of virus specific CTL following antigenic re-exposure during recurrence of infection under natural conditions.

The present studies provide for the first time evidence for cytotoxicity to HSV-1-infected autologous cells in subjects with recurrent HSV-1 infection. Higher cytotoxic activity was observed during acute phase of RHL. As mentioned earlier, such cytotoxicity appears to be mediated also by Leu-11^{+} lymphoid cells. It is, however, apparent that significant cytotoxic activity against HSV-1 is generated in acute phase (0–3 days) of the disease in RHL subjects. The development of such cytotoxic response may have an important role in limiting the spread of virus during reactivation of HSV and in elimination of viral infected cells. Interestingly, some seropositive subjects without RHL also exhibited high cytotoxic response to HSV-1 infected autologous targets. The mechanism underlying this observation may be related to the appreciable degree of viral shedding in previously infected subjects and subsequent endogenous antigenic exposure of the immunologic repertoire even in the absence of clinical disease (Lopez & O'Reilly, 1977; Hill, 1981).

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