

## Variation in lymphoproliferative responses during recrudescent orofacial herpes simplex virus infections

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### SUMMARY

Sixty-five patients with recrudescent orofacial herpes simplex virus (HSV) infections all had circulating HSV-specific antibody measured by ELISA and cell-mediated immunity (CMI) to HSV by *in vitro* lymphoproliferation. Thirteen control subjects with no history of HSV were negative for both tests. Thirty-three patients, repeatedly investigated during 6 to 38 months, had between 1 and 8 recrudescences each. Lymphoproliferative responses to HSV were low during recrudescence, rose to a peak a few weeks later and then declined to a positive background level. However, ELISA titres and lymphoproliferative responses to concanavalin A were high throughout, and peripheral blood mononuclear cell (PBMC) subset numbers measured by fluorescent flow cytometry remained within normal limits. During HSV lesions, depressed lymphoproliferation to HSV was abrogated by removal of CD8<sup>+</sup> T cells from PBMC either by using a panning technique (nine patients) or by cell sorting (three patients). Reconstitution of the CD8-depleted population suppressed the lymphoproliferative response to HSV. Depletion of CD8<sup>+</sup> T cells did not affect lymphoproliferation to HSV outwith recrudescence (four patients), nor lymphoproliferative responses to another antigen (PPD; five patients) during recrudescence. Thus, reduced lymphoproliferation to HSV during recrudescence may be due to HSV-specific CD8<sup>+</sup> suppressor T lymphocyte function, rather than lack of HSV-responsive lymphocytes. This may result in depression of normal CMI responses to the virus during an asymptomatic recurrence allowing recrudescent lesions to develop.

**Keywords** herpes simplex virus cell-mediated immunity recrudescent infections T lymphocyte subsets suppressor T lymphocytes

### INTRODUCTION

Following primary orofacial herpes simplex virus (HSV) infection virus becomes latent in the nervous system, particularly in the cell bodies of sensory neurones in the trigeminal ganglion. Within the nervous system the virus is in a site of immunological privilege: little synthesis of viral RNA or viral antigen occurs in infected neurones, and no local immune response to it is generated (Stevens *et al.*, 1988). From time to time the virus reactivates, migrates to the epidermis and replicates to produce either an asymptomatic recurrence or recrudescent lesions (Wildy, Field & Nash, 1982). Between 2 and 9% of seropositive patients shed HSV into oral secretions intermittently (Overall, 1984) and 16–45% suffer recrudescences (Nahmias & Josey, 1978). Several well-known triggering factors often precede human recrudescent infections (e.g. u.v. irradiation, local trauma) and in animal models re-activation, recurrence and

recrudescence are provoked by similar stimuli (Wildy, Field & Nash, 1982).

Cell-mediated immunity (CMI) is considered to be of paramount importance in recovery from HSV infection, although antibodies may help prevent the spread of HSV to the nervous system (Wildy & Gell, 1985). Following primary HSV infection, normal patients develop delayed-type hypersensitivity (DTH) to HSV *in vivo* (Anderson & Kilbourne, 1961), *in vitro* proliferation of peripheral blood mononuclear cells (PBMC) to HSV antigen and HSV-antigen driven effector responses (e.g., production of lymphokines by T cells, Rattray *et al.*, 1980). These responses are persistent and indicate the development of immune memory (Iwasaka, Sheridan & Aurelian, 1983). Brisk *in vitro* lymphoproliferative responses to HSV in primary genital HSV infection are associated with reduced severity and duration of symptoms, lesions and viral secretion compared with patients with less marked CMI (Corey, Reeves & Holmes, 1978; Rattray *et al.*, 1980). Acquired immunity to HSV reduces the severity of subsequent infection with HSV-1 or -2 in normal individuals (Corey & Spear, 1986).

Most patients with recrudescent HSV are otherwise healthy, and variations in different manifestations of CMI have been

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measured in relation to recrudescences with conflicting results. Our study was designed to clarify the relation between lymphoproliferative and antibody responses to HSV in subjects during and after recrudescence HSV infection. Any fluctuations found were correlated with the frequency or timing of lesions and any associated triggering factors.

## MATERIALS AND METHODS

### Patients

We studied 65 subjects with recrudescence facial HSV infections but good general health (21 men and 44 women; age range 9–60 years, mean 34) and 13 healthy controls with no history of HSV (five men and eight women; age range 22–45 years, mean 32). Volunteers were either staff members or persons who had been referred to the Dermatology Department at the Royal Infirmary of Edinburgh because of HSV. Blood samples were collected in the early morning into preservative-free heparin for measurement of HSV-specific antibody by ELISA, and neutralizing antibody CMI by *in vitro* lymphoproliferation, full blood count, biochemical profile and circulating immunoglobulin and complement levels. Dipstick urinalysis was performed and during recrudescences virus was typed as described previously (Vestey *et al.*, 1988).

### Preparation of HSV antigen

Antigen was prepared for ELISA or CMI estimation by glycine extraction of Vero cells infected with a clinical isolate of HSV-1 as already outlined (Vestey *et al.*, 1988). Uninfected Vero cells, similarly extracted, acted as control antigen. u.v.-inactivated HSV (u.v.-HSV) was prepared from the same isolate by exposure of infected cell cultures to 300 mJ/cm<sup>2</sup> u.v. B radiation under two Philips TL 20w/12 u.v. lamps (270–350 nm); it was used as an additional test antigen for CMI, with u.v.-irradiated uninfected Vero cells as control antigen, on nearly all occasions in parallel with the glycine extract.

### Lymphoproliferation assay

This method was described previously (Vestey *et al.*, 1988). Stimulation indices (SI) were calculated as:

$$SI = \frac{\text{mean ct/min with HSV antigen}}{\text{mean ct/min with control antigen}}$$

In preliminary experiments dilutions of the HSV glycine-extracted antigen and control antigen were tested at protein concentrations of between 1 µg/ml and 60 µg/ml and u.v.-HSV antigen was tested between 10<sup>3</sup> and 10<sup>7</sup> plaque-forming units (PFU)/ml (pre u.v.-inactivation). Glycine extract at 30 µg/ml and u.v.-HSV at 10<sup>6</sup> PFU/ml produced optimal lymphoproliferation, with similar SIs and ct/min, and were used in subsequent experiments. The T cell mitogen concanavalin A (con A; Sigma UK, Poole, UK) was added to parallel cultures (5 µg/ml) as a general index of CMI. In some experiments tuberculin-purified protein derivative (PPD; Evans Medical, Horsham, UK; 60 units/ml final concentration) was used as an additional test antigen.

### Fluorescent flow cytometry

Peripheral blood mononuclear cells (PBMC) were stained with murine monoclonal antibodies to label the following cell surface antigens: CD3, CD4, CD8 (Dako T3, T4 or T8, respectively;

Dakopatts, High Wycombe, UK), Leu 7 (Becton-Dickinson, Mountain View, CA), CD11 (OKM1; Ortho Diagnostic Systems, High Wycombe, UK), pan-MHC class II antigen (DP, DQ, DR: DA 6.231), DP (B7/21) (gifts from Dr K. Guy) and DR (L243; ATCC). Cells were then washed and restained with sheep anti-mouse IgG F(ab')<sub>2</sub> conjugated to FITC (Sigma). PBMC were also stained with rabbit anti-human IgM (FITC-conjugated F(ab')<sub>2</sub> fraction; Dakopatts). Stained cells were analysed on a Coulter EPICS 'C' flow cytometer as described before (Vestey *et al.*, 1988).

### Measurement of ELISA and neutralizing antibody to HSV

The ELISA method was described previously (Vestey *et al.*, 1988). Neutralizing antibody was measured using a standard, plaque-inhibition assay in Vero cells. The neutralizing antibody titre was estimated as the dilution of serum giving a 50% mean reduction in viral plaques in test wells compared with pooled negative control serum.

### Removal of CD8<sup>+</sup> cells by panning or by cell sorting

The panning technique was that used before (Vestey *et al.*, 1988). Cell sorting (CS) of PBMC stained for CD8 surface antigen was performed using the EPICS on the basis of CD8<sup>+</sup> green fluorescence acquired initially by gating of the PBMC population in a two-parameter histogram measuring forward angle light scatter and 90° light scatter (flow rate 2000 cells/sec, laser output 150 mW at 488 nm). Sorted cells were collected into sterile culture medium, aliquots were re-analysed on the EPICS to measure CD8<sup>+</sup> cell enhancement, and CD8-depleted PBMC were tested in the lymphoproliferation assay. CD8<sup>+</sup> cells were reconstituted in parallel CD8-depleted cultures by adding 3 × 10<sup>5</sup>/ml CD8-enriched cells prepared by panning or CS and tested in the lymphoproliferation assay.

## RESULTS

Subjects varied in the frequency of their recrudescence HSV infections, ranging from less than one, to 20 attacks per year [median 4, interquartile range (IQR) 2–6]. All recrudescences consisted of mild perioral papulovesicular lesions ('cold sores') lasting less than 2 weeks and uncomplicated by systemic symptoms. All subjects remained in good health throughout the study and none of the routine screening investigations revealed results outwith the normal ranges for our hospital. Four patients had genital HSV and four had herpetic whitlow as well as facial lesions. HSV-1 was isolated from 38 subjects with facial lesions, and HSV-2 from the four with genital lesions. Some subjects noted that a number of triggering events frequently preceded the onset of recrudescence HSV: 19 out of 65 thought exposure of local skin sites to sun or u.v. radiation precipitated recrudescences, seven emotional stress or tiredness, six mild upper respiratory infections, three mild local trauma, two febrile illnesses, one frost exposure, and five women often had lesions during the last few days of their menstrual cycle; 22 associated no particular triggering factor with lesions.

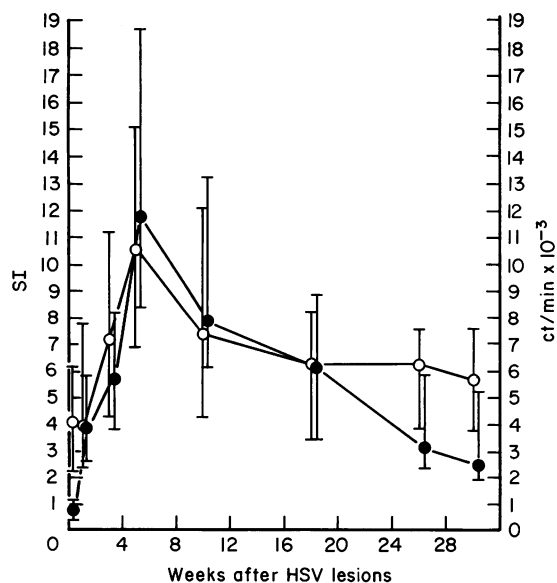
### ELISA and neutralizing antibodies

All 65 patients with a history of HSV had positive ELISA (titre > 1:100) to HSV regardless of the frequency of or time since a lesion (up to 10 years), and in 63 the titre was > 1:1600. In addition to ELISA, neutralizing antibodies were measured and

**Table 1.** Variations in cell-mediated immunity (CMI) to HSV antigens and Con A after recrudescence of perioral HSV

	Follow up (months)	Nil (ct/min $\pm$ s.e.)	Con A (ct/min $\pm$ s.e.)	(Con A Si)	HSV glycine antigen (ct/min $\pm$ s.e.)	HSV glycine control (ct/min $\pm$ s.e.)	HSV glycine SI	u.v.-HSV antigen SI	ELISA
Lesion	0	294 $\pm$ 27	28 853 $\pm$ 2678	98	673 $\pm$ 86	295 $\pm$ 11	2.3	ND	1:6400
	1	490 $\pm$ 35	28 764 $\pm$ 3321	59	7301 $\pm$ 721	422 $\pm$ 53	17.3	ND	1:3200
	4	460 $\pm$ 45	29 831 $\pm$ 1531	65	6642 $\pm$ 622	471 $\pm$ 42	14.1	ND	1:3200
	6	553 $\pm$ 47	27 650 $\pm$ 1480	50	2044 $\pm$ 223	584 $\pm$ 53	3.5	ND	1:6400
Lesion	12	329 $\pm$ 63	32 622 $\pm$ 3458	99	548 $\pm$ 38	155 $\pm$ 8	3.5	3.9	1:3200
	14	785 $\pm$ 50	25 375 $\pm$ 1119	32	8374 $\pm$ 263	837 $\pm$ 36	10.0	11.9	1:3200
	15.5	574 $\pm$ 98	34 747 $\pm$ 1882	60	6164 $\pm$ 856	648 $\pm$ 50	9.5	9.2	ND
Lesion	17	320 $\pm$ 38	22 727 $\pm$ 647	71	769 $\pm$ 70	285 $\pm$ 23	2.7	3.5	1:3200
	17.5	561 $\pm$ 59	21 627 $\pm$ 647	38	3231 $\pm$ 362	600 $\pm$ 72	5.4	6.3	1:3200
	18.5	802 $\pm$ 42	25 839 $\pm$ 1395	32	8204 $\pm$ 388	855 $\pm$ 43	9.6	14.0	1:3200
	19	882 $\pm$ 73	29 822 $\pm$ 1360	34	4185 $\pm$ 220	981 $\pm$ 74	4.3	6.2	ND
	21	789 $\pm$ 74	23 943 $\pm$ 1886	30	3733 $\pm$ 491	773 $\pm$ 28	4.8	4.8	ND
Lesion	23	380 $\pm$ 40	17 160 $\pm$ 701	45	1071 $\pm$ 78	232 $\pm$ 52	4.6	5.3	1:3200
	25	931 $\pm$ 68	35 577 $\pm$ 1223	38	13 892 $\pm$ 374	920 $\pm$ 51	15.1	16.5	ND
	25.5	799 $\pm$ 61	29 960 $\pm$ 719	37	10 872 $\pm$ 965	893 $\pm$ 100	12.2	10.7	ND
Lesion	26	ND	ND	ND	ND	ND	ND	ND	ND
	27	596 $\pm$ 58	39 932 $\pm$ 1843	67	4864 $\pm$ 327	685 $\pm$ 45	7.1	5.2	ND
	28	815 $\pm$ 82	24 305 $\pm$ 831	30	12 361 $\pm$ 442	850 $\pm$ 60	14.5	13.0	ND
	28.5	919 $\pm$ 23	33 293 $\pm$ 3790	36	8425 $\pm$ 914	799 $\pm$ 51	10.5	9.9	ND
Lesion	29	272 $\pm$ 39	14 554 $\pm$ 352	54	1482 $\pm$ 356	315 $\pm$ 45	4.7	4.0	ND
	30	447 $\pm$ 41	11 249 $\pm$ 551	25	5802 $\pm$ 277	455 $\pm$ 27	12.8	12.1	ND
	31	625 $\pm$ 110	15 407 $\pm$ 353	25	5903 $\pm$ 253	759 $\pm$ 84	7.8	5.1	ND

ND not done.

**Fig. 1.** Variation of stimulation index (SI;  $\circ$ ) and ct/min ( $\bullet$ ) in peripheral blood mononuclear cells with glycine-extracted HSV antigen (30  $\mu$ g/ml) with time following recrudescence of HSV (median  $\pm$  interquartile range) in 33 patients.

titres were  $> 1:32$  in all patients with positive ELISA titres, but between 1:8 and 1:32 in association with low ELISA titres ( $< 1:1600$ ) in one patient. The four patients from whom HSV-2 was isolated had positive ELISA, neutralizing antibodies and CMI to HSV-1. Control subjects with no history of HSV were negative for both tests (ELISA  $< 1:100$ ; neutralizing antibodies  $< 1:4$ ).

#### Lymphoproliferation assays

Subjects with histories of HSV had evidence of CMI to HSV by lymphoproliferation (SI  $> 3$ ) using either HSV antigen preparation, but controls with no history of HSV were negative to both antigens (SI  $< 1$ ). All subjects had high lymphoproliferative responses (SI  $> 10$ ) to Con A regardless of HSV status.

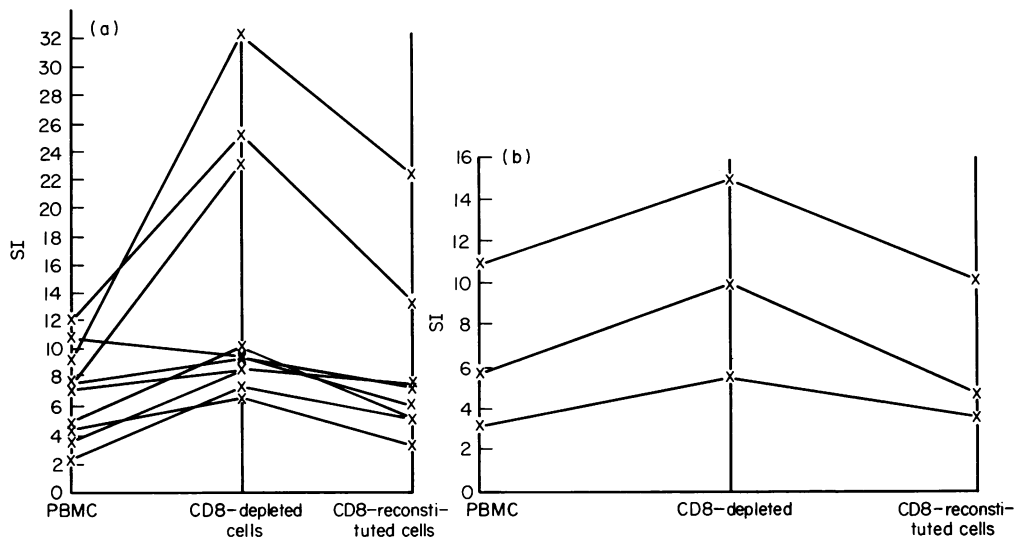
#### Fluctuations in lymphoproliferation to HSV with time after recrudescences

Thirty-three subjects with recrudescence of HSV, who were repeatedly investigated during 6 to 38 months of follow up, had from 1 to 8 recrudescences each (median 2, IQR 1-3). The SI and ct/min fluctuated with time after recrudescence; they were low when lesions were present, rose to a peak 5-8 weeks later, and then slowly declined to a positive background level over subsequent weeks. Lymphoproliferation and ELISA results of a patient followed through 6 recrudescences over 31 months are shown in Table 1; despite marked variation of SI and ct/min with time after lesions, Con A responses were high throughout and ELISA titres were high during the first 23 months of follow up.

**Table 2.** The percentage of peripheral blood mononuclear cells staining with monoclonal antibodies in relation to recrudescent HSV lesions

Monoclonal antibody	T3	T4	T8	OKM1	Leu7	IgM	DA6.231	L243	B7/21
Mean of 21 patients outwith lesions	60 (7)	44 (10)	23 (4)	16 (2)	9 (0.2)	11 (2)	16 (2)	13 (2)	7 (1)
Mean during HSV recrudescence	55 (9)	45 (9)	20 (4)	15 (3)	9 (2)	13 (4)	15 (2)	13 (2)	6 (2)

s.d. in parentheses.

**Fig. 2.** (a) The effect of CD8<sup>+</sup> cell depletion by panning on the lymphoproliferative response (SI) to HSV in 10 patients during recrudescent infections; (b) The effect of CD8<sup>+</sup> cell depletion by cell sorting on the lymphoproliferative response (SI) to HSV in three patients during recrudescent infections.**Table 3.** Lack of enhancement of stimulation index (SI) to PPD by CD8<sup>+</sup> cell depletion during recrudescent HSV

Patient	SI		
	PBMC	CD8-depleted cells	CD8-reconstituted cells
1*	3.2	2.9	ND
2*	7.1	7.6	9.0
3*	5.5	3.6	2.1
4†	3.3	2.6	3.1
5†	3.2	3.7	3.2

\* Depletion by panning.

† Depletion by cell sorting

ND not done.

Pooled results of SI and ct/min after recrudescences for 33 patients followed for a prolonged period are shown in Fig. 1. The difference between the SI and ct/min during a lesion and the peak 5–8 weeks later, and the difference between the peak SI and ct/min and the background level 24 weeks after a lesion were both highly significant ( $P < 0.01$ , Wilcoxon rank sum test), as were paired differences for the rise and fall in SI and ct/min for individual patients after recrudescences (Wilcoxon signed-ranks test,  $P < 0.01$ ). The variation in SI and ct/min after recrudescence occurred regardless of triggering factors associated with lesions, the time since a previous infection, or the frequency of recrudescences. In 13 patients seen less than 6 weeks before a recrudescence (median SI 10, IQR 9–15; median ct/min 6080, IQR 3457–8973) the SI during the subsequent recrudescence was depressed (median SI 4, IQR 3–5; median ct/min 954, IQR 421–1582;  $P < 0.01$ , Wilcoxon rank sum test), confirming that the low SI with recrudescent lesions was not just due to a gradual decline in lymphoproliferative response since the peak level.

#### *Lack of variation in T cell subsets after recrudescences*

PBMC subsets measured by fluorescent flow cytometry during and after recrudescence of HSV in 21 patients were within normal ranges for the local population and did not fluctuate significantly with the infectious cycle (Table 2). Circulating T helper/suppressor cell ratios in individual patients varied slightly on different occasions but there was no correlation between the CD4<sup>+</sup>:CD8<sup>+</sup> cell ratio and the time since HSV lesions (Pearson's correlation coefficient,  $r=0.142$ ;  $P>0.1$ ). The CD4<sup>+</sup>:CD8<sup>+</sup> cell ratio was not depressed nor the number of CD8<sup>+</sup> or MHC class II<sup>+</sup> cells increased during recrudescence lesions (Table 2).

#### *Depletion of CD8<sup>+</sup> cells by panning*

In order to investigate whether depression of SI with recrudescence of HSV was due to T suppressor cell function, as preliminary results had suggested (Vestey *et al.*, 1988), we examined the effect on the SI of depleting CD8<sup>+</sup> T cells from PBMC using a panning technique both during and outwith recrudescence. Panning depleted CD8<sup>+</sup> T cells from PBMC in 12 of 13 patients investigated: the mean CD8<sup>+</sup> cell concentration in PBMC was  $21.7\% \pm 2$  s.e.m.), and in the CD8-depleted population it was  $14.4 \pm 1\%$ . The difference between these concentrations was statistically significant ( $P<0.01$ , two sample Student's *t*-test). PBMC from 10 subjects were depleted of CD8<sup>+</sup> T cells during recrudescence of HSV and in nine the SI was enhanced (Fig. 2a). Reconstitution of CD8-depleted cultures suppressed the SI back nearly to the level of undepleted PBMC in six experiments (mean CD8<sup>+</sup> cell concentration  $41.3 \pm 5.1\%$ ). In a typical experiment depletion of CD8<sup>+</sup> cells by panning enhanced the SI of PBMC to HSV glycine extract from 2, ct/min  $1572 \pm 238$  to SI 8.8, ct/min  $6015 \pm 664$ ; and reconstitution of CD8<sup>+</sup> cells suppressed the SI to 3.4, ct/min  $2312 \pm 180$ . Paired data for enhanced SI and ct/min after panning and suppression after CD8<sup>+</sup> cell reconstitution in each experiment were highly significant ( $P<0.01$ , Wilcoxon signed-ranks test, for both manoeuvres). The suppression in ct/min produced by reconstitution of CD8<sup>+</sup> cells was significant in another experiment (ct/min in CD8-depleted PBMC  $27515 \pm 410$ , CD8 reconstituted PBMC  $20989 \pm 810$ ;  $P<0.01$ , two sample Student's *t*-test) but the SI was still above that of undepleted PBMC (ct/min  $9523 \pm 362$ ; highest line Fig. 2a). Reconstitution was not carried out in two experiments due to low yields of CD8<sup>+</sup> cells. In the 10th patient the SI with PBMC (SI 10.9, ct/min  $11679 \pm 364$ ) was not enhanced by depletion of CD8<sup>+</sup> T cells (Fig. 2a; SI 9.6; ct/min  $11448 \pm 269$ ) but reconstitution of CD8<sup>+</sup> cells did produce a slight but significant reduction in ct/min ( $7439 \pm 112$ ;  $P<0.01$ , two sample Student's *t*-test; SI 6.1) suggesting that suppressor T cells were present but that panning was not efficient enough at depleting CD8<sup>+</sup> cells to produce an enhanced SI. PBMC from two patients depleted of CD8<sup>+</sup> cells 6 and 9 months, respectively, after a recrudescence (SIs for HSV 6.4 and 3.2 before depletion) were neither enhanced by depletion nor suppressed by reconstitution of CD8<sup>+</sup> cells.

#### *Depletion of CD8<sup>+</sup> cells by CS*

Since the panning technique was rather inefficient at removing CD8<sup>+</sup> cells from PBMC, we used CS to increase CD8<sup>+</sup> cell depletion from PBMC in five subjects (mean CD8<sup>+</sup> concentration PBMC  $22.4 \pm 1.8\%$ ; in depleted cells  $3.9 \pm 1.1\%$ ; in CD8-enriched population  $63.3 \pm 5.0\%$ ). CS was undertaken in three

subjects during recrudescence and the SI was enhanced by depletion of CD8<sup>+</sup> cells and suppressed when the CD8<sup>+</sup> cells were reconstituted (Fig. 2b): both enhancement and suppression of ct/min were significant in each experiment ( $P<0.01$ , two sample Student's *t*-test). In two subjects tested 2 and 8 months after recrudescence of HSV, SIs in PBMC (6.4 and 8.5) were not enhanced by depletion of CD8<sup>+</sup> cells using CS (SIs 5.1 and 7.4, respectively) nor suppressed after CD8<sup>+</sup> reconstitution. Thus there was no non-specific enhancement or depression of SI by this technique.

#### *Suppression of CMI during recrudescence is HSV specific*

Parallel experiments with PPD as the test antigen instead of HSV were undertaken simultaneously with panning and CS experiments. Only five of the 13 subjects with CD8<sup>+</sup> cell mediated suppression of SI to HSV during recrudescence (Fig. 2 a,b) had CMI to PPD (SI  $>3$ ), possibly because of reduced use of prophylactic BCG in Scotland in recent years. Neither CD8<sup>+</sup> cell depletion by panning nor by CS enhanced the SI to PPD during recrudescence (Table 3), despite enhancing the SI to HSV.

## DISCUSSION

All subjects with a history of HSV infection had CMI and humoral immunity to HSV and their ELISA and neutralizing antibody titres were high, however long after a lesion they were investigated, however frequent their recrudescences, and whatever triggering factors were associated with infections, as reported in most studies (Lopez & O'Reilly, 1977; Shillitoe, Wilton & Lehner, 1977; Zwerink & Stanton, 1981).

Some triggering factors, e.g. febrile viral illness, menstruation, mental stress, or tiredness may produce general antigen-independent alterations in immune responsiveness leading to temporary depression of systemic CMI to HSV and allowing recrudescence to emerge. Other triggering factors, such as local trauma, u.v. irradiation, local thermal or frost injury are associated with recrudescence of HSV within local dermatomes in humans and also cause reactivation of virus latent in local peripheral nerves and asymptomatic recurrences in animal models (Wildy, Field & Nash, 1982). Such stimuli may produce local antigen-dependent or -independent depression of CMI to HSV, for instance by impaired release of cytokines, e.g. epidermal cell-derived thymocyte-activating factor (Sauder *et al.*, 1983), by increased elaboration of immune mediators, e.g. prostaglandin E<sub>2</sub> (Wildy, Field & Nash, 1982; Sheridan *et al.*, 1987) by epidermal cells, or by damage to epidermal APC function (Howie *et al.*, 1987).

Depressed lymphoproliferation to HSV during and just after lesions has been documented previously (Lopez & O'Reilly, 1977; El Araby *et al.*, 1978; Corey, Reeves & Holmes, 1978; Rattray *et al.*, 1980; Kalimo, Joronen & Havu, 1983). However, Tsutsumi *et al.* (1986) found peak lymphoproliferation to u.v.-HSV during recrudescence in 16 patients who were only followed up for 2–3 weeks after recovery during which a 'modest decline' in the response occurred. Shillitoe, Wilton & Lehner (1977) tested 23 subjects using  $\beta$ -propiolactone-inactivated HSV-1 and -2 as test antigen and found maximal SIs during recrudescence, then a steady decline over 4 weeks to the level in seropositive controls. Depressed SIs during recrudescence were noted when T lymphocytes partially purified by pre-adsorption

on nylon wool columns were used (Shillitoe, Wilton & Lehner, 1978). Nylon wool-purified T cells from patients tested at various times after HSV-1 lesions showed high SIs within 12 weeks of a lesion and significantly lower responses thereafter (Rasmussen & Merigan, 1978).

In this study depressed SIs during recrudescence were shown to be due to CD8<sup>+</sup> T lymphocyte-mediated suppression that was HSV antigen-specific and not accompanied by variations in T cell subset numbers. Panning was relatively inefficient for depleting CD8<sup>+</sup> cells from PBMC but produced enhancement of SIs during recrudescence, as did CS. It is possible that the plastic-adherent cells removed by panning included cells other than CD8<sup>+</sup> T cells, such as suppressor-inducer macrophages, but this was unlikely to happen during CS. Sheridan *et al.* (1982; 1985; 1987) noted little change in lymphoproliferative responses to HSV during recrudescence human genital HSV-1 or -2 infections but found a relative increase in the proportion of circulating CD8<sup>+</sup> and DR<sup>+</sup> lymphocytes, and hence a reduced CD4<sup>+</sup>:CD8<sup>+</sup> cell ratio, from an early stage of infection. The CD8<sup>+</sup> cells produced factors that inhibited enhancement of natural killer (NK) cell activity, leucocyte migration inhibitory factor production by lymphocytes, and lymphoproliferation. Recrudescence HSV-2 infection in guinea pigs is accompanied by induction of T suppressor cells in the spleen and peripheral blood, which elaborate soluble factors capable of inhibiting HSV-2-specific lymphoproliferation (Iwasaka, Sheridan & Aurelian, 1983). u.v.-HSV was reported to suppress phytohaemagglutinin-driven *in vitro* lymphoproliferation in humans especially just before and during recrudescence and in patients prone to frequent rather than rare infections. However, significant suppression only occurred when u.v.-HSV was used at 10PFU/lymphocyte or more, which is 10 times the concentration used in this study (Wainberg *et al.*, 1985). The HSV-specific systemic immunosuppression detected with recrudescence in this study may be associated with impaired or delayed local CMI responses to asymptomatic recurrent HSV infection thus allowing recrudescence lesions to develop. A limited local T suppressor response may be required late in the course of recrudescence HSV to limit normal CMI and help prevent immunologically mediated tissue damage (Rouse & Horohov, 1986).

There is considerable interest at the present time in determining which viral antigens, including both structural and non-structural proteins, may be important in generating specific aspects of the immune response to HSV (Glorioso *et al.*, 1985; Blacklaws, Nash & Darby, 1987; Martin *et al.*, 1988; Heber-Katz *et al.*, 1988). It may be possible to identify specific epitopes capable of generating DTH or cytotoxic T cell responses with a reduced risk of inappropriate suppressor T cell responses. This approach may be useful in the development of a subunit vaccine for HSV.

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