Systemic immunity in herpetic keratitis

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SUMMARY The importance of specific immunity, especially cell-mediated, in resistance to herpes simplex disease is well documented, and animal experiments show that immune responses to previous herpetic infection can give considerable protection against reinfection in epithelial keratitis. Since susceptibility to stromal herpetic keratitis is probably determined in part by the extent of virus proliferation in the epithelium, immune responses to herpes simplex virus were studied in patients with epithelial or stromal keratitis, a group of patients with keratitis who were also severely atopic, and controls. The parameters measured were lymphocyte transformation, production of macrophage migration inhibition factor, and levels of serum antibody and immunoglobulin. No group differences were found except for a slight decrease in the whole blood culture assessment of lymphocyte transformation in stromal keratitis patients compared with seropositive controls and patients with epithelial disease. It is considered that in a small group of patients a deficiency in the specific cell-mediated immune response plays a role in determining the spread of virus into the corneal stroma.

Herpes simplex virus (HSV) can affect the cornea in a number of ways, commonly producing epithelial disease in the form of a dendritic ulcer which heals in 7-10 days, but which can lead to prolonged inflammatory disease in the corneal stroma, with eventual development of scarring in a significant proportion of the patients. Ulcerative herpes simplex keratitis (HSK) occasionally occurs as a primary disease, initially in the absence of immunity. Most patients have had some previous contact with the herpes simplex virus, and recurrent disease of the cornea occurs in the presence of systemic immune responses.

The precise way in which immune processes operate to protect the host against HSV infections is far from clear. Several lines of evidence suggest that cellular immunity plays an important role in combating HSV infections, especially in relation to cell to cell spread. Firstly, HSV infections are more severe in animals whose cell-mediated immunity is suppressed.¹ Secondly, protection against HSV infection can be transferred to immunosuppressed animals by immune T lymphocytes but not by antibody.² Thirdly, patients who have been treated with topical or systemic corticosteroids or suffer from recognised immunodeficiency syndromes are subject to severe corneal or cutaneous herpetic

disease.³ Fourthly, HSV can persist both in vitro and in vivo despite the presence of high concentrations of neutralising antibody.⁴ Fifthly, it has been shown that recovery from ulcerative herpetic keratitis in laboratory animals correlates with the development of a cellular immune response.⁵

We here report the results of an investigation into both humoral and cell-mediated immunity (CMI) in patients with epithelial and stromal keratitis, who are compared with a group of controls, together with our findings in highly atopic and immunosuppressed patients, groups who seem to be at risk of developing particularly severe keratitis,⁶ with the aim of determining whether a specific deficiency in immune response plays a role in severe stromal herpetic keratitis. Kinetic studies of these parameters were made in patients with primary and recurrent disease.

Patients and methods

PATIENT SELECTION

Twenty-seven patients with dendritic ulcers were selected with and without minimal stromal involvement (17 and 10 patients respectively), and compared with a group of 30 patients with active stromal disease of disciform (10 patients), limbal (6), or diffuse type (14) (Table 1). The diagnosis of dendritic keratitis was made on the morphological appearance by a trained observer. A separate group of 12 patients

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Table 1 Details of groups of patients examined and tests performed on them

Group	Total number	Average age and age range (years)	Sex ratio M/F	Lymphocyte transformation			Total serum immunoglobulins	Macrophage migration inhibition factor	HSV complement fixing antibody
				PHA antigen (whole blood)	HSV antigen (whole blood)	HSV antigen (pure lymphocyte)			
Controls	31	45 (18-61)	18/13	21	31	23	—	12	24
Epithelial keratitis	27	42 (13-85)	22/5	19	27	12	13	9	13
Stromal keratitis	30	49 (15-79)	16/14	30	29	18	23	9	23
Herpetic keratitis and severe atopy	12	26 (18-35)	11/1	6	6	7	12	5	8

with severe atopic disease with a history of epithelial followed by stromal disease were also investigated. A control group was composed of normal subjects with or without antibody to herpes simplex virus (16 and 15 subjects respectively). Studies were made in 2 patients with keratitis (1 with a dendritic keratitis and 1 with stromal disease) who were immunosuppressed after renal transplantation.

Kinetic studies were performed in 1 patient with primary HSK, 9 patients with recurrent herpetic ulcers, 2 patients with severe kerato-uveitis and 2 atopic patients, one of whom suffered a primary attack, while the other suffered frequent recurrences of ulcerative disease.

LABORATORY METHODS

Total serum immunoglobulins. Serum immunoglobulins were assessed in 48 patients by Partigen immunodiffusion plates (Hoechst; Table 1).

Antibody against HSV. Serum titres of anti-HSV antibody were measured by complement fixation⁷ in controls and patients with herpetic keratitis (Table 1).

Lymphocyte transformation. (a) Whole blood cultures were performed in 31 controls and 62 patients with keratitis (Table 1) by the method described by Junge *et al.*⁸ (b) Isolated lymphocyte cultures: lymphocytes were obtained from peripheral blood by Ficoll-Triosil separation and cultured under the same conditions as the whole blood but with the addition of autologous serum (10%), using HSV antigen only. Cultures were performed in 23 controls, and 37 patients with keratitis (Table 1).

Macrophage migration inhibition. Lymphocytes were cultured for 7 days in Eagles minimum essential medium, with (test cultures) or without (controls) HSV antigen used at a dilution of 1:200. The supernatants were then removed and assayed for macrophage migration inhibition factor (MIF). Antigen was added to the control supernatants and fetal calf serum to both test and control media. Guinea-pig macrophages, stimulated by intraperitoneal injection of paraffin oil 5-7 days earlier,

were recovered and centrifuged into small glass tubes. These were placed in wells each of which was then filled with test or control supernatant and incubated in 5% CO₂. The areas covered by macrophages migrating on to the floors of the wells was measured 18 hours later. The percentage migration was calculated as:

$$\frac{\text{mean migration area (test supernatants)}}{\text{mean migration area (control supernatants)}} \times 100\%$$

Results

SERUM IMMUNOGLOBULINS

Serum immunoglobulin levels were measured in patients with epithelial or stromal keratitis and a group of atopics with HSK (Table 2). Serum IgE levels were raised in 6 out of 12 atopics with herpetic keratitis and were normal in patients with keratitis and a negative history of atopy. In 2 subjects with IgE levels greater than 4000 units/ml, the disease was bilateral and particularly unresponsive to treatment.

SERUM ANTIBODY AGAINST HSV

Complement-fixing antibodies measured in groups of patients with epithelial and stromal keratitis,

Table 2 Total serum immunoglobulin levels in a group of adult patients with epithelial or stromal disease, or atopic disease with associated herpetic eye disease, compared to the normal ranges of IgA, M, G, and E

Group	Number of patients	Serum immunoglobulin levels			
		IgA (G/L)	IgG (G/L)	IgM (G/L)	IgE (units/ml)
Normal range	—	1.4-4.2	8.0-17.0	0.45-1.9	60-1000.0
Dendritic ulcers	13	2.52 ±1.32	14.86 ±3.80	1.75 ±0.76	106.63 ±123.80
Stromal disease	23	2.84 ±1.16	14.53 ±2.85	1.73 ±0.76	123.52 ±160.75
Atopy with herpetic keratitis	12	2.26 ±0.77	13.25 ±3.64	1.69 ±0.78	1782.50 ±1881.19

atopy with HSK, and controls show that no differences could be distinguished between the 4 groups (Fig. 1). There was no evidence that low antibody titres are associated with an increased risk of developing stromal keratitis.

CELL-MEDIATED IMMUNITY

A. Group comparisons

The whole blood lymphocyte transformation (LT) response to phytohaemagglutinin (PHA) was tested in patients with epithelial or stromal HSK, or HSK and atopy, and in controls. Fig. 2 shows that transformation indices were the same in controls (seropositive and seronegative) and patients with epithelial disease or stromal disease and atotics with keratitis.

The concentration of herpes simplex antigen which would give optimum transformation, the 1:200 dilution of antigen, was used in whole blood cultures. The LT responses to HSV antigen in controls and patients are shown in Fig. 3. A transformation index of 8.5 separates the 2 groups of controls, with and without antibody, from each other, and therefore can be taken as an upper level of normal in the antibody-negative group and the lower level of normal in the antibody-positive group. 40% of patients with stromal disease had transformation indices lower than 8.5 compared with only 8% of patients with epithelial disease. Mean levels in seropositive controls and patients with epithelial disease were significantly higher than the mean level in the group with stromal disease

Fig. 1 Complement fixing antibody in patients with epithelial, stromal and keratitis with concurrent atopic disease, compared with controls.

Fig. 2 Lymphocyte transformation expressed as the transformation index, with the use of phytohaemagglutinin, in patients compared with controls by means of whole-blood techniques. Mean levels and standard deviations are shown.

($p < 0.05$ and < 0.01 respectively by the Wilcoxon rank sum test for 2 populations). There is therefore a tendency for patients with stromal disease to have a depression of CMI to HSV antigen when measured with the whole blood technique. Fig. 4 compares the transformation indices with HSV and PHA antigens in patients and controls and highlights the reduced transformation indices in the patients with stromal disease when compared with those with epithelial disease or the seropositive controls.

When measured in cultures of Ficoll-Triosil separated lymphocytes, however, the transformation responses to HSV antigen were similar in patients with epithelial disease and seropositive controls (Fig. 5), with slight depletion of responses in a proportion of those with stromal disease.

Macrophage migration inhibition factor (MIF) was produced in significant quantities in all 9 patients tested with stromal disease, 6 out of 9 patients with epithelial keratitis, and in 5 out of 12 controls. The test did not distinguish group differ-

Fig. 3 Lymphocyte transformation to herpes simplex antigen (whole-blood technique) in controls and patients with herpetic keratitis. Mean levels and standard deviations are shown.

ences between patients with either epithelial or stromal disease (Table 3).

B. Kinetic studies

Serial measurements of LT and MIF production were made in a 28-year-old female patient with the clinical appearance of *primary herpetic keratitis*. The ability of lymphocytes to transform to HSV antigen was first detected 10 days after the onset of clinical disease, and there was rapid resolution when the maximum response was achieved (Fig. 6). MIF was produced after a period of 10 days (Fig. 7).

In an atopic female of 18 years with a diffuse facial herpes and bilateral ulcerative keratitis there was a sharp increase in LT to HS antigen, which eventually regressed to average levels for patients with epithelial disease (Table 4). MIF was produced in substantial quantities by lymphocytes in measurements made 12 months after the original infection.

Paired measurements of lymphocyte transforma-

Fig. 4 Mean uptake of ^3H thymidine in disintegrations per minute (DPM) in patients and controls, by means of the whole-blood technique with HS antigen and PHA. Standard deviations are shown.

Fig. 5 Lymphocyte transformation to herpes antigen using separated lymphocytes in patients with herpetic keratitis, compared with the control group.

Table 3 *MIF production in a control group, compared with 3 groups of patients with HSV*

Group	Number of patients producing MIF	Mean percent inhibition $\pm$ SD
Controls	5	16.6 $\pm$ 19.9
Epithelial disease	6	34.2 $\pm$ 33.0
Stromal disease	9	64.6 $\pm$ 29.2
Atopy with herpetic keratitis	2	23.2 $\pm$ 27.9

Fig. 6 Lymphocyte transformation using whole blood technique with phytohaemagglutinin (○—○) and herpes antigen (●—●) in a patient with a primary attack of herpetic keratitis. The hatched block indicates the period of disease activity.

Fig. 7 Macrophage migration inhibition factor production and lymphocyte transformation by means of isolated lymphocytes in a patient with primary HSK tested with herpes antigen. ○—○, MIF; ●—●, LT.

tion during active recurrent ulcerative disease and convalescence in 9 individuals showed that the transformation index had an overall tendency to fall during the period of resolution (Fig. 8). Serial measurements of LT using HSV antigen and PHA were carried out in a patient with prolonged and unremitting *kerato-uveitis* (Fig. 9). Variation in these levels did not show correlation with disease activity, neither did the production of MIF.

Measurements made in a 20-year-old male with severe atopic eczema and asthma together with bilateral herpetic keratitis showed no correlation with disease activity but were nevertheless very variable: transformation indices varied between 100 and less than 2 using HSV antigen with isolated lymphocytes.

In 2 immunosuppressed renal transplant patients the LT response to HSV antigen was considerably

Table 4 *LT expressed as transformation index in an 18-year-old atopic female with primary cutaneous and epithelial herpetic disease*

Time after initiation of HSV (weeks)	Lymphocyte transformation (whole blood)	
	PHA	HS antigen
2	172.5	16.5
4	561.1	233.2
24	155.4	114.6
48	84.6	57.5

Fig. 8 Lymphocyte transformation during active disease and convalescence in patients with recurrent ulcerative herpetic keratitis.

Fig. 9 Lymphocyte transformation to PHA and HS antigen in a patient with prolonged herpetic kerato-uveitis (whole-blood technique). Disease activity is shown by the hatched blocks. The proportion of lymphocytes responding to HS antigen compared to the response to PHA is shown (○ · · · · · ○).

depressed. In 1 patient with a dendritic ulcer 4 weeks after renal transplantation the transformation index was 1.76, and in a second patient with stromal disease the index was 2.00. In spite of this, recurrent ulcerative disease does not necessarily lead to severe stromal disease, but on occasions the stromal disease can be severe.

Discussion

The object of this study was to investigate ways in which cell-mediated and humoral immune responses influenced, or were influenced by, various types of herpetic eye disease. The results show that humoral immune responses did not vary between groups of patients with either ulcerative disease or persistent stromal disease. When PHA was used as antigen there was no group difference with a whole-blood technique of LT, but there was some depression of response in stromal disease when herpes simplex antigen was used, suggesting that the lack of lymphocyte reactivity to HSV antigen was not due to general impairment or suppression of the T cell population.

Reports on the relationship between cell-mediated immunity and the disease caused by HSV give varying results.⁹⁻¹³ There is no other disease caused by HSV which parallels the 2 pathological processes which affect the cornea. Other studies concern patients with high recurrence rates or those who have developed precancerous or cancerous lesions of the oral cavity.¹⁴ The difficulties which arise when the HSV or its antigen penetrate the corneal stroma do not seem to be mirrored in other organs, and it seems that it is a phenomenon which may be unique to the eye. In contrast to the study we report,

Grabner and Jarisch¹⁵ demonstrated that leucocyte migration inhibitory factor was produced in stromal disease in similar quantities as in controls, indicating that there was not a deficit in cell-mediated immunity. However, experimentally it can be shown that stromal disease is not associated with a lympho-proliferative response in regional draining lymph nodes, but epithelial disease induces a positive response.¹⁶ The latter findings bear some relationship to the results we report.

The kinetic studies in primary herpetic keratitis show that infection of the external eye can provoke a considerable reaction in systemic cellular immunity demonstrated by the substantial rise in LT and MIF production seen during a primary HSV keratoconjunctivitis. These parameters declined after resolution of the infection. In recurrent ulcerative disease there is also an enhancement of response during the active stage, as other workers have found who used the cell-mediated cytotoxicity assay to study recurrent oral and genital herpes. Cytotoxicity was higher during recurrence than in the absence of lesions¹³ when the responses were comparable with those of seropositive individuals with a negative history of HSV infections. Similarly reinfection of immune laboratory animals produces a sharp rise in LT,¹⁷ and this is also true for reinfection of the cornea alone.⁵

Variation in the severity of stromal disease in one patient was not correlated with variations in LT or MIF production. In a severely atopic patient both recurrent epithelial and stromal disease occurred in association with extreme variability in the LT response to HSV antigen, which might be related in some way to the high recurrence rate in certain of these patients.

The considerable variability in LT and MIF production seen in the temporal studies indicates that such tests are less likely to be of clinical help if only single measurements are made. Cell-mediated immune responses have now been reported to show considerable natural variation,¹⁸ so care should be taken in the interpretation of the results from small numbers of assays.

It is concluded that there is evidence of a specific deficit in the cell-mediated immune response in patients with stromal disease, which might go some way to explain why this complication may occur, though there are other factors which include the variations in virulence between different strains of virus.¹⁹ The whole-blood technique of LT is simple to perform and therefore can be recommended for laboratory use as one index of potential risk of developing stromal disease in patients with early ulcerative disease. Kinetic studies show that there may be variation in LT and MIF production in

primary and recurrent ulcerative disease, the latter generally producing a positive response in spite of the diminutive area of tissue which is affected. The corneal surface is therefore protected during recurrence of active disease by local and systemic immunological changes, and it is when such responses are interfered with by the use of corticosteroid therapy that the more severe forms of stromal disease may be expected.

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