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Immunohistology of oral lesions from patients with recurrent oral ulcers and Behçet's syndrome

L. W. POULTER* & T. LEHNER† *Department of Immunology, Royal Free Hospital and School of Medicine; and †Department of Immunology, United Medical and Dental Schools of Guy's and St Thomas', Guy's Hospital, London, England

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SUMMARY

A qualitative and quantitative immunohistological investigation was performed on biopsies of oral ulcers from patients with Behçet's syndrome (BS) and those with recurrent oral ulcers (ROU). The results were compared with control oral biopsies from patients with other diseases and normal oral mucosa. The expression of HLA-DR on the cell membrane of keratinocytes was found in 13 out of 15 lesions from patients with BS and ROU, as compared with only one out of 15 controls. The relative density of HLA-DR was investigated quantitatively by microdensitometry and this confirmed that DR expression in the epithelial cells of patients with BS and ROU was significantly greater than in diseased and normal control oral tissues. A prominent mononuclear cell infiltration consisted predominantly of T lymphocytes and mature macrophages. Analysis of the CD4 and CD8 subsets of T cells failed to show significant differences between BS, ROU and control diseased tissues. Increased numbers of Langerhans cells were found in the epithelium by morphometric analysis with the CD1 monoclonal antibody in BS and ROU but an increased number was also found in lichen planus. The results suggest that the immunohistological changes in oral lesions of BS and ROU manifest an enhanced immune response in the epithelium, keratinocytes express HLA-class II antigen and increased number of Langerhans cells as well as in the lamina propria with a prominent infiltration of CD4, CD8 and macrophage-like cells. The characteristic pattern of exacerbations and remissions of oral ulceration can be interpreted by the hypothesis that an initiating microbial agent may induce a mononuclear cell infiltration, with the release of cytokines, expression of class II antigen in keratinocytes and causing ulceration, followed by down-regulation of immunity by tolerant T cells induced by the class II positive keratinocytes, leading to a remission.

Keywords recurrent oral ulcers Behçet's syndrome immunohistology epithelial HLA-DR

INTRODUCTION

Recurrent oral ulcers (ROU) are a common condition which varies greatly in severity from transient soreness to prolonged ulceration, with difficulties in eating and speaking. Behçet's syndrome (BS) however, is less common in this country, but its significance has been enhanced by the frequency with which it enters the differential diagnosis of ocular, rheumatological, neurological, dermatological and gastro-intestinal diseases. The pathogenesis of both diseases involves autoimmune (Oshima *et al.*, 1963; Lehner, 1967; Dolby, 1969; Rogers, Samo & Shorter, 1974), immunogenetic (Ohno *et al.*, 1973; Lehner *et al.*, 1979), immune complex (Williams & Lehner, 1977; Gupta *et al.*, 1978), chemotactic (Matsumura & Mizushima, 1975; Sobel *et al.*, 1977) and immuno-virological (Denman *et al.*, 1980; Eglin, Lehner & Subak-Sharpe, 1982; Bonas *et al.*, 1986) mechanisms. Whereas systemic studies have demonstrated a variety of immunological processes taking part in ROU and BS, local histological investigations have suggested an early cell-mediated response (class IV), followed by an immune complex (class III) response (Lehner, 1969; Yamana *et al.*, 1982; Muller & Lehner, 1982).

The objectives of this investigation were to explore the local immune response, with particular reference to the expression of HLA class II in the epithelial cells. This has been reported in a variety of autoimmune diseases (Bottazzo *et al.*, 1982) and keratinocytes might acquire the functions of antigen presentation (Stingl *et al.*, 1978) and interleukin-1 (IL-1) production (Luger *et al.*, 1981). The proportion of the CD4 and CD8 T cell subsets, their state of activation and the proportion of macrophages, dendritic cells and Langerhans cells were also investigated. The results suggest that HLA-DR expression in the keratinocytes of oral mucosa in patients with ROU or BS is a significant feature of these diseases. The increased proportion of

Correspondence: Professor T. Lehner, Department of Immunology, Guy's Hospital, Guy's Tower, Floor 28, London Bridge, London SE1 9RT, England.

T cells, mature macrophages and Langerhans cells are also prominent features in ROU and BS and are consistent with a local cell-mediated immune reaction. In addition we searched for the presence of herpes simplex virus (HSV) antigens, as the virus was implicated in the aetiology of BS and ROU (Eglin *et al.*, 1982).

MATERIALS AND METHODS

Biopsy specimens

Biopsy examination was carried out from oral ulcers of 30 patients attending the Immunological Clinic at Guy's Hospital. The diagnoses are indicated in Table 1 and were based on conventional clinical criteria, confirmed by biopsy examination in erythema multiforme, systemic lupus erythematosus, lichen planus and Sjögren's syndrome. The control lesions were selected so as to examine the specificity of the findings, both in local and systemic diseases, associated with oral ulcerating and non-ulcerating lesions. The ulcers were biopsied 2–4 days after the patient had noticed the lesion in all but four patients in whom the lesions were more than 4-days old. Normal oral mucosa was obtained from two patients who were having their wisdom teeth removed. The biopsies were carried out from the lips, cheeks and tongue. Local-infiltration anaesthesia was used, with 2% lignocaine hydrochloride and containing 1:100000 adrenaline.

All biopsies were embedded in OCT medium and frozen in isopentane, cooled in a bath of liquid nitrogen; 6 μm frozen sections were cut in a cryostat maintained below −25°C, placed onto glass slides, air dried for 30 min and fixed in a 1:1 mixture of chloroform/acetone for 10 min. These sections were wrapped in cling film and stored at −20°C. The frozen biopsies were orientated so that the resulting sections contained clearly definable epithelium and lamina propria. Intermittent sections were stained with 0·1% toluidine blue to ensure orientation and sections of all samples were stained with haematoxylin and eosin to reveal the general histology of the sample.

Immunohistology

A panel of monoclonal antibodies was used to identify subsets of lymphocytes and macrophages within the tissues (Table 2). An indirect immunoperoxidase method was used in which the monoclonal antibody first layer was applied at a dilution of either 1:5 or 1:10 of the culture supernatant for 90 min. The

Table 1. Diagnosis and clinical manifestations of 30 patients investigated by immunohistology of oral biopsies

		Clinical Manifestations					
Diagnosis	*n*	Oral	Genital	Skin	Joints	Eyes	Others
Recurrent oral ulcers*	9	(9)	—	—	—	—	—
Behçet's syndrome	6	(6)	(5)	(3)	(5)	(3)†	1 thrombophlebitis
Erythema multiforme	4	(4)	—	(1)	—	—	—
Lichen planus	6	(6)	—	—	—	—	—
Systemic lupus erythematosus and Sjögren's syndrome	2	(1)	—	(1)	(1)	—	—
Non-specific ulcers	3	(3)	—	—	—	—	—

*Patients with minor (one), major aphthous ulcers (three) and herpetiform ulcers (five).
†Two uveitis and one iritis.

Table 2. Monoclonal antibodies used to identify subsets of lymphocytes and macrophages within tissues

Name	Cluster designation	Class	Mol. wt antigen (kD)	Source	Specificity in normal tissue
T mix	CD 2, 5, 8	IgG	50, 67, 32–33	RFHSM	All T cells
B mix	CD 19, 22	IgG1	95/135	RFHSM	All B cells
RFT8	CD 8	IgM	32–33	RFHSM	Cytotoxic/suppressor type T cells
Leu 3a	CD 4	IgG1	55	Beckton Dickinson	Helper/inducer type T cells
RFT2	CD 7	IgG2	40	RFHSM	T blasts
Tac	CD 25	IgG1	55	Dr T. Uchyama	IL-2 receptors
RFDR1	—	IgM	28/33	RFHSM	HLA-DR
RFD7	—	IgG1	77	RFHSM	Mature macrophages
RFD1	—	IgM	28/33	RFHSM	Interdigitating cells (20% of B cells)
NA1/34	CD1	IgG1	49	Dr A. McMichael	Thymocytes/Langerhans cells
αDQ	—	IgG1	28–33	Beckton Dickinson	HLA-DQ
αDP	—	IgG1	28–33	Dr C. Navarette	HLA-DP

RFHSM, Royal Free Hospital and School of Medicine.

sections were then washed in phosphate-buffered saline (PBS) and a second layer goat anti-mouse immunoglobulin conjugated to horseradish peroxidase was applied for 60 min. The sections were washed again and then placed in a development solution containing hydrogen peroxide and diaminobenzidine for 10 min. After washing the sections were counterstained with Harris's haematoxylin, dried and stored.

Other studies involved the indirect immunofluorescence method in which combinations of two monoclonal antibodies of different classes were applied as a first layer for 45 min. After washing, a mixture of class-specific goat anti-mouse IgM and IgG antibodies were applied. The anti-mouse reagent was conjugated to fluorescein isothyocyanate (FITC) and the anti-mouse IgM was conjugated to tetra rhodamine isothyocyanate (TRITC). After 60-min incubation the sections were washed and stored in PBS/glycerol. Negative controls included omitting the first layer reagents, and for positive controls normal tonsil tissue was stained in parallel with all monoclonal antibodies used.

Microscopy

A standard Zeiss microscope was used with planapochromatic objectives. Immunoperoxidase preparations were examined using Kohler illumination. Immunofluorescence preparations were examined using a mercury vapour lamp with epi-illumination. Appropriate barrier filters were used to visualize FITC and TRITC. Photomicroscopy was performed, using a Zeiss M65 automatic camera. Histological and immunohistological preparations of all specimens were examined independently by both authors. The proportion of $CD4^+$ and $CD8^+$ cells were determined by counting the number of each of these subsets and expressing them as a proportion of the total T cells, identified in the same field using double immunofluorescence.

Morphometric analysis of Langerhans cells

Morphometric studies were performed using computer assistance with Medical Research Council (MRC) software (SSRCONV 3.15). Sections were treated with the monoclonal antibody CD1 (NA1/34), followed by the immunoperoxidase method, and examined by a Zeiss standard microscope with drawing attachment through which a scribe was visible. The area of epithelium was traced with the scribe in at least five fields from duplicate sections of each specimen. After tracing the area of the epithelium the position of all Langerhans cells (identified by a definable cell body) were marked within this area. The mean numbers of Langerhans cells within each specimen were then computed and expressed as the mean number of Langerhans cells per unit area.

Quantitative immunocytochemistry

The expression of HLA-DR molecules in the epithelium and within the lamina propria of selected specimens was performed using glucose oxidase conjugated RFDR1 monoclonal antibody as described previously (Poulter *et al.*, 1987). Briefly, the enzyme glucose oxidase was conjugated to RFDR1 and the sections were incubated for 1 h. The presence of enzyme was then visualized using a developing solution containing substrate and nitro blue tetrazolium as the capture reagent. The density of the resulting reaction product (a formazan) was then quantified using an integrating microdensitometer (Vickers M85) set at 585 nm with a spot size of 1 and slit width of 20. Using an A6 aperture, the density of formazan reaction product was measured in random areas of positive staining within the epithelium and lamina propria. A minimum of five readings per area in each specimen was taken after 'blank' readings (off the specimen) were adjusted to zero, using an appropriate threshold setting. The results were expressed as mean relative absorption per unit area.

Identification of HSV antigens

Heteroantiserum to HSV antigen (Dako) was used in indirect immunoperoxidase and indirect immunofluorescence studies of four biopsies. Monolayers of fibroblasts infected with HSV (kindly supplied by Dr Jane Grundy) were used as positive controls. The control preparations consistently gave positive results with these methods. Stained sections or monolayers were examined microscopically as detailed above.

Statistical analysis

Student's *t*-test for non-paired data was used to determine statistical significance of some of the results.

RESULTS

Histology

The biopsies of oral lesions from BS and ROU revealed a mononuclear cell infiltrate in all sections. Although the ulcer itself contained and was surrounded by polymorphonuclear cells and contained cell debris, outside this zone and extending diffusely throughout the lamina propria large numbers of infiltrating lymphocytes and macrophage-like cells were seen. No histological difference was found between the lesions in BS and those with ROU. Furthermore, no obvious difference was found in the inflammatory cell infiltrate associated with control samples taken from cases of erythema multiforme, systemic lupus erythematosus and non-specific ulcers. In lichen planus, however, there was an exclusive mononuclear cell infiltration. Samples of normal oral mucosa showed only an occasional mononuclear cell in the lamina propria.

Lymphocyte populations

All the lesions contained significant numbers of T lymphocytes. A small proportion of B cells was also seen. The proportions of T cells in the infiltrates were significantly greater in cases of ROU (range 50–73% of total infiltrating mononuclear cells), compared with BS (range 25–58%), and erythema multiforme (range 23–52%) ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively). Within the T cells the CD4 subset varied from 10 to 50% and the CD8 subset from 10 to 30%. The ratio of $CD4^+$ cells to $CD8^+$ cells was also variable with no significant difference between the groups of patients (Fig. 1). Most cases in BS and ROU (eight out of 11) showed only a slightly lower ratio than that seen in normal peripheral blood (i.e. $< 1{\cdot}8:1$).

In BS and ROU T cells were distributed in clusters within the dense infiltrate surrounding the ulcerated area, as well as adjacent to the basal layer of the epithelium (Fig. 1b). Some T cells infiltrated the epithelial cell layers. There were also clusters of T cells around some blood vessels. No T cells were found in normal control tissue (Fig. 1a). About 30% of the T cells (assessed subjectively) expressed CD7 antigen, suggesting blastogenesis, and a smaller number were HLA-DR positive. However, only very few cells expressed IL-2 receptors (CD25).

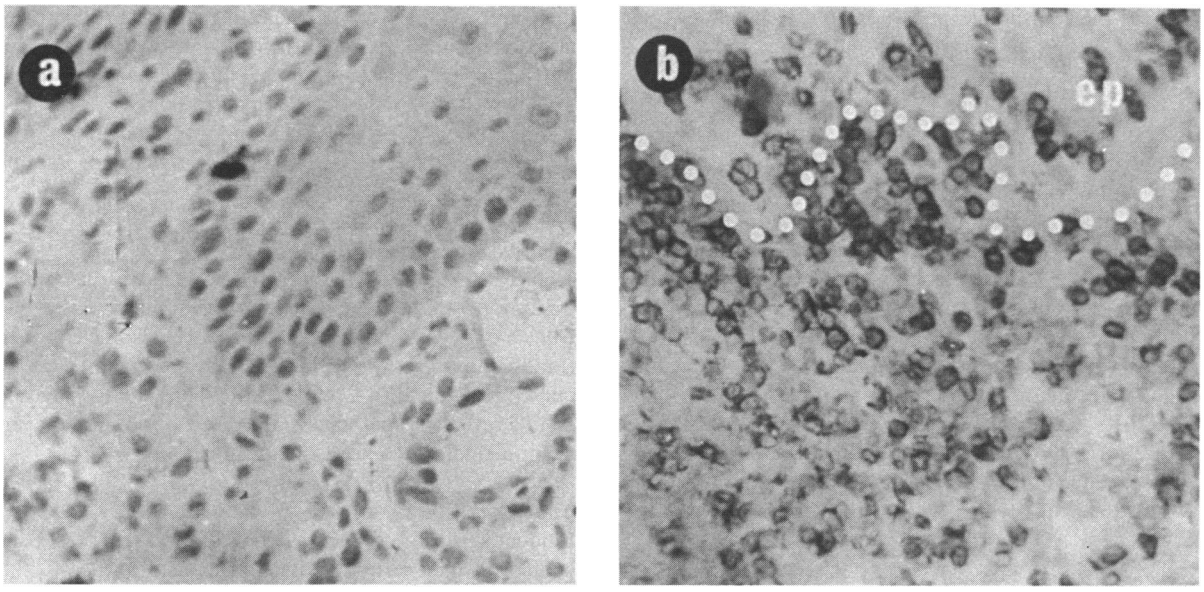

Fig. 1. Normal oral mucosa showing no T cells (a); and (b) infiltration of T lymphocytes in the lamina propria of the ulcerated oral mucosa in a patient with Behçet's Syndrome. Some clustering of T cells below the epithelium (ep) and cells infiltrating the epithelium can be seen; T mix immunoperoxidase, original magnification × 250.

Table 3. HLA class II expression in epithelial cells (given as number of class II positive biopsies/number of biopsies examined); CD4:CD8 ratios and Langerhans cells

Diagnosis	DR	DP	DQ	CD4:CD8*	Langerhans* cells
1 Recurrent oral ulcers (ROU)	8/9	0/3	2/4	2·2±1·4	11·1±3·6
2 Behçet's syndrome (BS)	5/6	0/3	1/3	1·8±1·2	6·9±3·1
3 Erythema multiforme	1/4	0/3	0/3	2·1±0·6	4·1±2·1
4 Lichen planus	0/6	—	—	1·7±0·6	12·2±3·3
5 Lupus erythematosus, Sjögren's syndrome	0/2	0/2	0/2	—	—
6 Non-specific ulceration	0/3	0/1	0/1	—	—
7 Normal tissue	0/2	0/2	0/2	1·35±0·4	5·5±0·7
ROU,BS 3–6	13/15 1/15	0/6 0/6	3/7 1/6		

*Mean ± s.d.

In BS and ROU biopsies those T cells expressing CD7 antigen were mostly distributed in small clusters adjacent to the basal layer of the epithelium or actually present within the epithelium. These observations were not made in diseased or healthy control tissues.

Macrophage-like cells

The majority of macrophage-like cells in BS and ROU were RFD7$^+$ mature tissue macrophages. These were diffusely distributed throughout the lamina propria, with groups clustered in some of the papillae and around small blood vessels. No RFD7$^+$ cells were seen within the epithelium. RFD1$^+$ dendritic cells were also present, but to a lesser extent, representing < 20% (subjective assessment) of the non-lymphoid mononuclear cells. Only a few RFD1$^+$ cells were found in the diseased control biopsies and an occasional RFD1$^+$ cell was found in normal oral mucosa. Langerhans cells were identified in the epithelium in some samples of ROU and a few CD1$^+$ cells with the morphology of Langerhans cells were also found within the infiltrating T cells in the lamina propria. Morphometric analysis revealed a significantly larger number of epithelial Langerhans cells in ROU than in normal controls ($P < 0{\cdot}01$) or erythema multiforme ($P < 0{\cdot}01$) (Table 3). A slightly higher number of Langerhans cells was also found in BS ($P < 0{\cdot}05$), but in lichen planus the number of Langerhans cells was also increased to a level similar to that found in ROU ($P < 0{\cdot}01$).

Distribution of HLA-DR molecules

In the sections from ROU and BS the most striking feature was

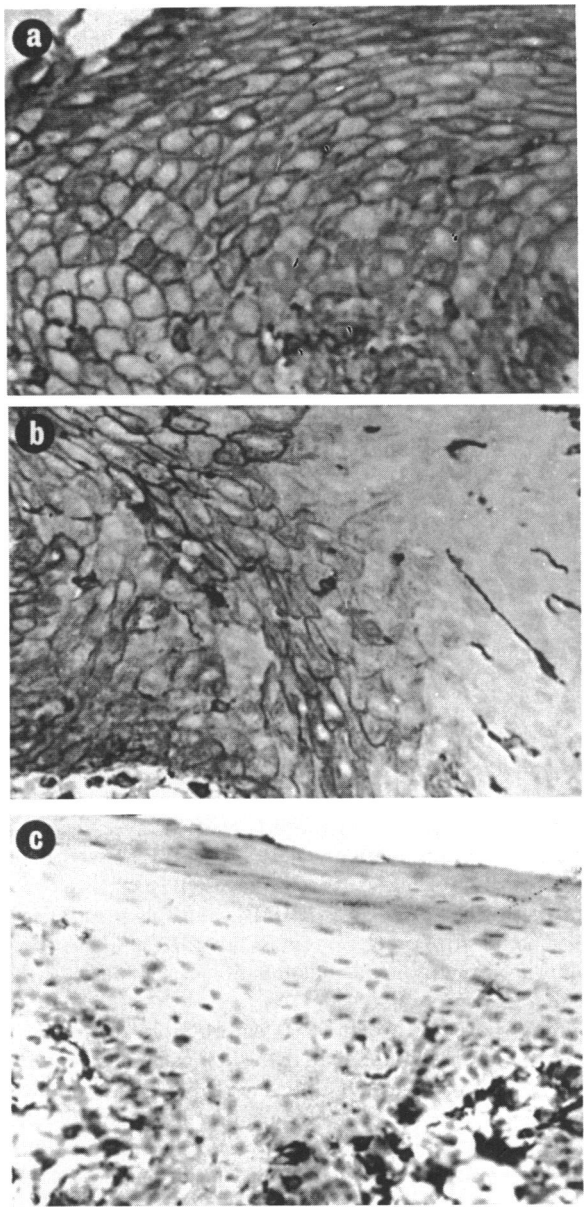

Fig. 2. Immunoperoxidase staining of HLA-DR expression on the oral epithelial cells in Behçet's syndrome (a); Behçet's syndrome showing example of 'cut off' of HLA-DR expression (b); and in Lichen planus (c) (showing no epithelial staining but DR positive cells in the lamina propria). Original magnification × 400.

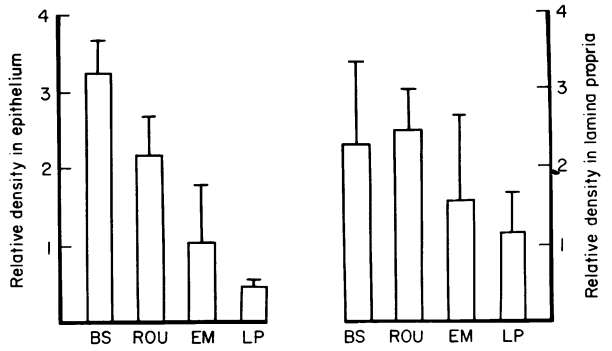

Fig. 3. Microdensitometric measurement of HLA-DR expression in the epithelium and lamina propria of sections taken from cases of Behçet's syndrome (BS), recurrent oral ulcers (ROU), erythema multiforme (EM) and lichen planus (LP). Mean ± s.d. of 4–6 specimens in each group. The two specimens of normal oral mucosa showed no HLA-DR expression and the relative density was $<0{\cdot}1$ per unit area (not indicated).

the expression of HLA-DR antigen on the keratinocytes of the epithelium (Fig. 2a). This was most evident in the epithelium immediately adjacent to the ulcerated site and in some cases extended throughout the epithelium of the specimen. In other cases there was an abrupt 'cut-off' of the expression of class II major histocompatibility complex (MHC) antigen, distal to the damaged surface (Fig. 2b). In such cases, however, class II expression of keratinocytes often reappeared in the epithelium adjacent to T cell infiltration beneath the basement membrane or in the epithelium. Most of the HLA-DR expression was found on the cell membrane of keratinocytes, but occasionally the cytoplasm was also involved in more diffuse staining. The epithelium of all the diseased and normal controls showed either little or no expression of HLA-DR (Fig. 2c).

A striking difference was found between the three types of class II expressions (Table 3). HLA-DR was expressed in the keratinocytes of most patients with ROU (eight out of nine) and BS (five out of six). In contrast, DP was not expressed in any of the six biopsies examined and DQ was detected in the epithelium of three out of seven biopsies. However, we were rather surprised to find that of the 15 controls only one biopsy (from erythema multiforme) expressed DR in the keratinocytes (Table 3); DP was not detected, and only one out of six cases expressed DQ. It therefore appears that considerable degree of specificity of DR expression was found in oral mucosa from patients with ROU or BS.

In view of the potential significance of this finding we determined quantitatively by microdensitometry the relative density of HLA-DR expression. In BS ($P<0{\cdot}005$) and ROU ($P<0{\cdot}01$) this was significantly higher than in normal controls, erythema multiforme or lichen planus (Fig. 3). Furthermore, HLA-DR expressions in the epithelium of patients with BS was greater than that in ROU ($P<0{\cdot}05$). Quantification of HLA-DR in the lamina propria also showed a higher density of DR expression in the infiltrating cells in the lesions of BS and ROU than those in erythema multiforme and lichen planus (Fig. 3), although there was no significant difference between them. HLA-DR was detected in endothelial cells of the small blood vessels in eight out of nine biopsies from patients with ROU or BS, but also in five out of nine other lesions. Minor salivary glands were also examined for expression of HLA-DR and this was found in acinar or duct cells of most biopsies containing salivary gland tissue: four from ROU, one from erythema multiforme and one from Sjögren's syndrome.

Presence of HSV antigens

Both indirect immunoperoxidase and indirect immunofluorescence methods with heterologous anti-HSV antisera failed to detect HSV antigen in the biopsies examined. Control staining of fibroblast cultures infected *in vitro* with HSV were consistently positive with the anti-HSV antisera.

DISCUSSION

The most important finding has been the expression of HLA-DR on the cell membrane of keratinocytes, often adjacent to activated T cells, in 13 out of 15 patients with BS or ROU (Fig. 2, Table 3). This is unlikely to be secondary to the damage or inflammation induced by ulceration, as we have found class II expression only in one out of 15 biopsies from non-specific ulceration, systemic lupus erythematosus, Sjögren's syndrome, erythema multiforme or lichen planus (Table 3). We were surprised by the degree of specificity for HLA-DR expression, as tested by the monoclonal antibody RFDR1, and suggest that this be explored with other antibodies. In particular, keratinocytes in lichen planus have been reported to express HLA class II antigen (Volk-Platzer, Groh & Wolff, 1987; Valsecchi *et al.*, 1988). However, these investigated erosive lichen planus, as well as cutaneous lesions. Furthermore, we have used the direct (single layer) method, in contrast to the multiple amplification method used by Volk-Platzer *et al* (1987). As far as we are aware these workers did not quantify their results, and this is particularly significant as the glucose oxidase method used is designed to give limited reaction product to allow accurate measurements by microdensitometry. HLA class II expression can be induced in a variety of pathological conditions that are characterized by lymphoid cell infiltrations. This has been first demonstrated in graft-*versus*-host reaction (Breathnach & Katz, 1983), allograft rejection (Dallman & Mason, 1983), cell-mediated immunity in the Mantoux reaction of the skin (Collings, Waters & Poulter, 1985), in inflammatory bowel disease in thyrocytes of autoimmune thyroiditis (Bottazzo *et al.*, 1983) and in keratinocytes of oral ulcers in BS and ROU (Poulter, Lehner & Duke, 1986). A common mechanism for the large number of diseases with HLA-DR expression in non-lymphoid tissue may well be interferon production which can induce class II expression *in vitro* (Pober *et al.*, 1983). In ROU or BS interferon could be produced by the activated T cells or by some viral infections, such as HSV (Eglin *et al.*, 1982; Bacon *et al.*, 1984). In the lesions in this investigation HSV was not detected.

The function of class II expression in keratinocytes is open to two interpretations. One possibility is that it is involved in autoantigen presentation to T cells (Bottazzo *et al.*, 1983). This offers a basis for the early observations of humoral and cellular autoimmune responses to oral mucosa (Oshima *et al.*, 1963; Lehner, 1967; Dolby, 1969; Rogers *et al.*, 1974). Keratinocytes are also capable of producing IL-1 (Luger *et al.*, 1981) and this might be enhanced by activated keratinocytes expressing DR antigen. The second interpretation is that the function of class II positive keratinocytes is to induce unresponsiveness. The concept is based on the recent reports that I-E$^+$ keratinocytes induce antigen specific unresponsiveness in T cell proliferation (Gaspari, Jenkins & Katz, 1988). Similarly, I-E$^+$ pancreatic islet B cells induce specific unresponsiveness in T cells and grafts of I-E$^+$ transgenic islets into I-E$^-$ hosts are not rejected (Markmann *et al.*, 1988). Indeed, contrary to the hypothesis that non-lymphoid cells expressing Ia are capable of presenting autoantigens, keratinocytes and pancreatic islet cells have either poor or no accessory cell function (Gaspari *et al.*, 1988; Markmann *et al.*, 1988).

We therefore favour the interpretation that keratinocytes in oral epithelium express HLA-DR under the influence of cytokines released from T cells and that these keratinocytes may in time induce T cell unresponsiveness, leading to down-regulation of the immune response and clinical remission. Hence, the recurrent clinical pattern of mucosal ulceration can be interpreted as initial up-regulation or augmentation of immunity, associated with the mononuclear cell infiltration, class II expression in keratinocytes and ulceration, followed by down-regulation of immunity due to class II positive keratinocytes inducing T cell unresponsiveness and leading to a remission.

Characterization of the lymphoid cell infiltration is consistent with the hypothesis that the histological changes associated with ROU and in patients with BS represent manifestations of a cell-mediated hypersensitivity reaction. The predominance of mononuclear cells (mainly T lymphocytes), with a small proportion of B cells but not plasma cells confirm previous findings in ROU and BS (Lehner, 1969; Muller & Lehner, 1986; Yamana *et al.*, 1986). Although there was considerable variation in the proportion of CD4 cells, the CD4:CD8 ratio in the lesions was below that found in normal peripheral blood, suggesting that CD8 cells may be recruited to the site of damage. The presence of mature macrophages as the dominant non-lymphoid accessory cells has also been emphasized in the past (Lehner, 1969; Saito *et al.*, 1971; Muller & Lehner, 1982). These features are commonly seen in the classical Mantoux reaction (Poulter *et al.*, 1982). The presence of a small proportion of RFD1$^+$ dendritic cells and the expression of HLA-DR in the epithelial cells adjacent to T cell infiltration in the lamina propria are also consistent with the immunopathology of delayed-type hypersensitivity reactions (Collings *et al.*, 1985). It is noteworthy that the proportion of mature macrophages (RFD7$^+$) was greater than that of dendritic cells (RFD1$^+$) and this may reflect the transient nature of oral lesions in ROU or BS, unlike the reverse finding in chronic synovial inflammation in rheumatoid arthritis (Poulter & Janossy, 1985).

Langerhans cells are a normal feature of oral epithelium, but increased numbers were found in the lesions of ROU and BS, as compared with erythema multiforme (Table 3). It is noteworthy that antigen binding, processing and presenting cells of the macrophage, dendritic and Langerhans cell series are found in relatively large numbers in ROU and BS. Whether this reflects the high load of microbial and antigenic material in the oral mucosa associated with recurrent ulceration or a failure to deal effectively with the pathogen is not yet clear. However, an increase in the number of Langerhans cells was also found in lichen planus which was not of the erosive type and therefore offered less chances of direct entry of microbial antigens. That a cell mediated immune mechanism is the basis for the immunopathology of BS and ROU is reinforced by the association between activated T cells and the expression of HLA class II antigens.

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