Oral Diseases (2006) 12, 271–277. doi:10.1111/j.1601-0825.2005.01185.x

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ORIGINAL ARTICLE

Diversity of $\gamma\delta$ T cells in patients with Behcet's disease is indicative of polyclonal activation

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OBJECTIVE: Behcet's disease (BD) is a multisystemic disease, with vasculitic lesions in the oral and genital mucosa, eyes, joints, skin and brain. We have previously found that $\gamma\delta$ T cells are increased in peripheral blood of BD patients. The aim of this study was to investigate the extent of $\gamma\delta$ T cells in oral biopsies from BD patients with special emphasis on the restriction of Vγ and Vδ usage.

PATIENTS AND METHODS: Expression of Vγ and Vδ chains on peripheral blood $\gamma\delta$ T cells from 31 BD patients and 19 healthy controls was analysed by flow cytometry and the expression of Vγ and Vδ chains in nine ulcerated and eight non-ulcerated oral mucosa from BD patients and non-ulcerated oral mucosa from three healthy controls was analysed by immunohistochemistry.

RESULTS: Vγ9 and Vδ2 were the predominant chains expressed in peripheral blood of BD patients, although other Vγ and Vδ chains were also expressed. The presence of $\gamma\delta$ T cells was only observed in the ulcerated oral mucosa but not in the non-ulcerated mucosa from the BD patients, and not in the non-ulcerated mucosa from the healthy controls. These $\gamma\delta$ T cells showed no preferential expression of any of the Vγ or Vδ chains.

CONCLUSION: These data suggest a polyclonal rather than oligoclonal activation of the $\gamma\delta$ T cells. This may indicate that during repeated inflammation of the oral mucosa, the $\gamma\delta$ T cells are responding to a wide variety of antigenic stimuli with consequent expansion of $\gamma\delta$ T cells expressing various Vγ and Vδ chains and that different antigenic stimuli or responses may be responsible for the clinical heterogeneity of the disease.

Oral Diseases (2006) **12**, 271–277

Keywords: aphthous stomatitis; Behcet's disease; TCR$\gamma\delta$ T cells; oral ulcer

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Received 13 December 2004; revised 25 June 2005; accepted 30 June 2005

Introduction

Behcet's disease (BD) is a multi-systemic disease with recurrent ulceration affecting both the oral and genital mucosa. Furthermore, patients with BD suffer from lesions affecting the eye, skin, joints, gastro-intestinal tract, central nervous, vascular and respiratory systems. However, the aetiopathogenesis of BD remains unknown. In humans only a small fraction of peripheral T cells expresses the $\gamma\delta$ T-cell receptor (TCR). However, these cells are present in relatively high proportions at mucosal sites. We have previously demonstrated an increased proportion of $\gamma\delta$ T cells in peripheral blood in patients with BD (Fortune *et al*, 1990) and an increased percentage of these cells are in an active stage, capable of secreting pro-inflammatory cytokines (Freysdottir *et al*, 1999). Very little is known about the role of $\gamma\delta$ T cells in the immune system, although an immune regulatory role has been suggested (Janeway *et al*, 1988). $\gamma\delta$ T cells have been associated with several functions not directly related to antigen recognition, such as producing a tissue-specific growth factor, regulating the development of epithelial cells, stimulating production of nitric oxide by epithelial cells controlling $\alpha\beta$ T-cell responses, and promoting isotype switching in B lymphocytes (Boismenu and Havran, 1994; McMenamin *et al*, 1994; Jones-Carson *et al*, 1995; Komano *et al*, 1995; Chien *et al*, 1996). Recently they have been shown to be regulatory for the induction of mucosal IgA responses (Fujihashi *et al*, 1996; Yamashita *et al*, 1997). Furthermore, they seem to recognize structures presented by micro-organisms as well as by stressed cells but not normal cells (Tanaka *et al*, 1995; Porcelli *et al*, 1996).

At least eight functional Vγ genes have been described and eight Vδ transcripts have been identified (Kabelitz, 1992). The majority of peripheral blood $\gamma\delta$ T cells express Vγ9 in combination with Vδ2 (Moretta *et al*, 1991). Furthermore, the Vγ9 subset has been shown to be easily stimulated *in vitro* by bacterial and mycobacterial antigens as well as with some tumour cell lines (Kabelitz, 1992). It is, therefore, not surprising that Vγ9 and/or

Vδ2 are reported to be the main γδ subtype found in peripheral blood of patients with various infectious diseases (Jouen-Beades *et al*, 1997; Schneider *et al*, 1997).

A second major subset of peripheral γδ T cells expresses Vδ1. These γδ T cells are found in the normal intestine (Groh *et al*, 1998); however, only a few are found within the healthy oral mucosa and skin in humans (Liavaat *et al*, 1994; Bramanti *et al*, 1995; Nordlind and Liden, 1995). The percentage of γδ T cells expressing Vδ1 is increased in inflamed tissues, such as in inflamed intestinal mucosa in coeliac disease, in the synovium in patients with rheumatoid arthritis and in the lung of patients with sarcoidosis (Halstensen *et al*, 1989; Keystone *et al*, 1991; Forrester *et al*, 1993).

Increased frequency of Vγ9 and/or Vδ2 positive γδ T cells have been found in peripheral blood of BD patients (Yamashita *et al*, 1997; Triolo *et al*, 2003), although in another study the highest restriction of Vδ3 usage was found in BD patients (van Hagen *et al*, 2003). Furthermore, an increase in Vδ1 positive γδ T cells in cerebrospinal fluid of BD patients with active neurological disease has also been demonstrated (Hamzaoui *et al*, 1991). These results suggest that the restriction of Vδ usage may vary amongst BD patients.

We hypothesized that the oral mucosa, which is the commonest (and frequently the earliest) site affected by BD, may reflect subsequent changes in Vγ and Vδ usage in both the peripheral blood and distant sites of active disease. The objective of this study was to analyse both the Vγ and Vδ expression on γδ T cells in both peripheral blood and oral mucosa simultaneously in BD. Both normal mucosa (non-lesional) and ulcerated mucosa (lesional) from patients with BD were included in the study.

Patients and methods

Patients and control subjects

Thirty-nine patients with BD were recruited from outpatient clinics at GKT Dental Institute (Guy's Hospital), London, and the Jordan Hospital, Amman, Jordan. All the BD patients fulfilled the International Criteria (International study group for Behcet's disease, 1990). For comparison, 19 healthy individuals were recruited into the study. Both patients and controls gave their informed consent, and the study was approved by the respective Local Research Ethics Committees. The patients were classified as having active clinical disease if they had active oral and/or genital ulceration and evidence of having clinical signs of more than one other organ system involved at the time of the analysis. The predominant clinical feature in the BD patients was oral ulceration. In the patient group, 15 patients had mucocutaneous disease, of which 10 also had arthritis; 14 patients had mucocutaneous lesions with ocular disease and 10 patients had any of the above including central nervous system (CNS) involvement.

Peripheral blood was collected from 31 patients, 18 with active disease and 13 with inactive disease, and 19 healthy control subjects, matched for ethnicity. Biopsy specimens were collected from 17 patients, of which nine were from ulcerated (lesional) and eight from non-ulcerated (non-lesional) sites in the mouth, and healthy oral mucosa from three control subjects.

Antibodies

Monoclonal mouse antibodies (MoAbs) against human Vγ2,3,4 (VγI.2VγI.3VγI.4, clone 23D12), Vγ4 (VγI.4, clone 4A11), Vγ9 (VγII, clone 7A5), Vδ2 (clone 15D) and Vδ3 (clone P11.5B) were purchased from Serotec (Oxford, UK) and MoAbs against human pan TCRγδ (clone IMMU510), Vγ8 (VγI.8, clone R4.5) and Vδ1 (clone R9.12) were obtained from Immunotech (Beckman Coulter, High Wycombe, UK). Fluorescein isothiocyanate (FITC)-labelled goat anti-mouse Ig, the $F(ab)_2$ fragments, biotin-labelled rabbit anti-mouse Ig and peroxidase (Px)-labelled StreptABComplex were purchased from DakoCytomation (Ely, UK). MoAbs against human CD3 (clone OKT3) was tissue culture supernatant produced in our own laboratory.

Immunofluorescence staining and flow cytometry

Peripheral blood was collected from 31 patients and 19 controls. Mononuclear cells were separated on histopaque (Sigma, Poole, UK) and stored in liquid N_2 until they were used. The day before staining the cells were thawed and allowed to recover overnight in RPMI medium (Gibco^{TM}, Invitrogen, Paisley, UK), supplemented with 10% foetal calf serum (FCS; Gibco^{TM}), at 37°C, 5% CO_2 and 100% humidity. The following day, 1.5×10^5 cells in 100 μl of phosphate-buffered saline (PBS) containing 1% FCS and 0.02% NaN_3 (staining buffer) were incubated for 45 min on ice with 10 μl of anti-pan TCRγδ (at 1/30), anti-Vγ4 (at 1/12) anti-Vγ8 (at 1/3), anti-Vγ9 (at 1/12), anti-Vδ1 (at 1/3), anti-Vδ2 (at 1/12) or anti-Vδ3 (at 1/3), or with 50 μl of staining buffer (negative control) or anti-CD3 (positive control). Then the cells were washed twice with 1 ml of staining buffer at 250 ***g*** for 5 min at 4°C and subsequently incubated with 100 μl of FITC-labelled goat anti-mouse Ig, diluted 1/100, for 30 min on ice. Cells were washed twice with staining buffer and fixed in 400 μl of 1% paraformaldehyde in PBS and were then analysed by flow cytometry (Epics XL, Beckman Coulter). Ten thousand cells were collected and the lymphocyte population [peripheral blood lymphocytes (PBL)] was selected on a dot plot showing size and granularity and the percentage of positive cells determined by setting the negative control as 0.5%. The results were expressed as the ratio of the percentage of cells expressing a Vγ or a Vδ chain to the percentage of cells expressing the γδ TCR. This allowed detection of absolute increase or decrease of cells expressing the Vγ or Vδ chains analysed. The mean ± standard deviation (s.d.) was calculated for each group and the significance evaluated using Student's *t*-test for non-paired samples with unequal variance, with $P < 0.05$ being regarded as significant.

Immunohistochemical staining

Fresh frozen tissue sections at 5 μm were incubated with 50 μl of PBS (negative control), anti-CD3 (positive

control), anti-pan TCR$\gamma\delta$ (1/20), or antibodies against the miscellaneous Vγ or Vδ chains (at 1/10 dilution) at room temperature for 1 h. The sections were washed with PBS and then incubated for 30 min at room temperature with 50 μl of biotin-labelled rabbit anti-mouse Ig, diluted 1/20. After PBS wash the sections were incubated with 50 μl of Px-labelled StreptABComplex (prepared according to the manufacturer's directions) for 30 min at room temperature, washed again in PBS and then incubated for 10 min at room temperature with 3,3′-diaminobenzidine tetrahydrochloride (DAB). After a PBS wash the sections were counterstained for few seconds with haematoxylin and rinsed with tap water. Then the sections were dehydrated with xylene and ethanol and mounted with DPX mountant. The staining was evaluated under a light microscope with results expressed as 0 (no staining), 1+ (<5 positive cells), 2+ (6–20 positive cells), 3+ (21–40 positive cells), 4+ (41–60 positive cells), or 5+ (>60 positive cells). The sections were evaluated by two independent individuals.

Results

Expression of Vγ and Vδ chains in peripheral blood

Expression of Vγ4, Vγ8, Vγ9, Vδ1, Vδ2 and Vδ3 chains was analysed on PBL from patients with BD and from healthy controls, with results expressed as percentage of positive cells expressing the Vγ or Vδ chains as a ratio of percentage of positive cells expressing TCR $\gamma\delta$.

As can be seen in Figure 1, the most commonly expressed Vγ chain was the Vγ9 chain, observed in BD patients (active or inactive) and healthy controls, whereas Vδ2 was the most commonly expressed Vδ chain. There was no difference in Vγ9/$\gamma\delta$ ratio between BD patients with active (85.0 ± 30.8%) or inactive disease (83.9 ± 29.3%) and healthy controls (89.9 ± 19.0%; Figure 1). However, when the patients were grouped according to disease type it was noted that the CNS patients had slightly lower Vγ9/$\gamma\delta$ ratio (64.3 ± 43.0%) than the ocular (87.9 ± 29.0%) and mucosal (91.5 ± 16.3%) patients, although this difference did not reach significant levels (Figure 2).

A higher percentage of $\gamma\delta$ T cells expressing the Vγ8 and Vδ3 chains was found in peripheral blood from BD patients with active disease (23.8 ± 24.3%, $P = 0.20$ and 38.6 ± 51.8%, $P = 0.05$, respectively) compared with controls (14.7 ± 17.3% and 12.9 ± 9.0%, respectively), and to a lesser extent for Vδ3 chain in patients with inactive disease (24.5 ± 31.2%; Figure 1). This increase in Vγ8/$\gamma\delta$ and Vδ3/$\gamma\delta$ ratios was mainly detected in the mucosal patient group (36.9 ± 34.3%, $P = 0.17$ and 36.9 ± 34.3%, $P = 0.04$, respectively; Figure 2). The mucosal patients also had a raised Vδ1/$\gamma\delta$ ratio (80.9 ± 69.5%) compared to the ocular (49.9 ± 40.1%) and CNS (39.5 ± 24.9%) patients and the healthy controls (61.9 ± 43.8%), although this difference did not reach a significant level (Figure 2).

A lower percentage of $\gamma\delta$ T cells from patients with active disease was expressing the Vγ2,3,4, Vγ4 and Vδ2 chains (21.9 ± 15.9%, $P = 0.08$, 12.8 ± 15.0%, $P = 0.11$ and 63.9 ± 27.7%, $P = 0.07$, respectively) compared with controls (34.0 ± 24.2%, 22.4 ± 20.8% and 78.6 ± 18.9%, respectively; Figure 1). This was also observed for patients with inactive disease for Vγ2,3,4 and Vγ4 chains (21.3 ± 14.3%, $P = 0.07$ and 11.8 ± 12.1%, $P = 0.08$, respectively). When the

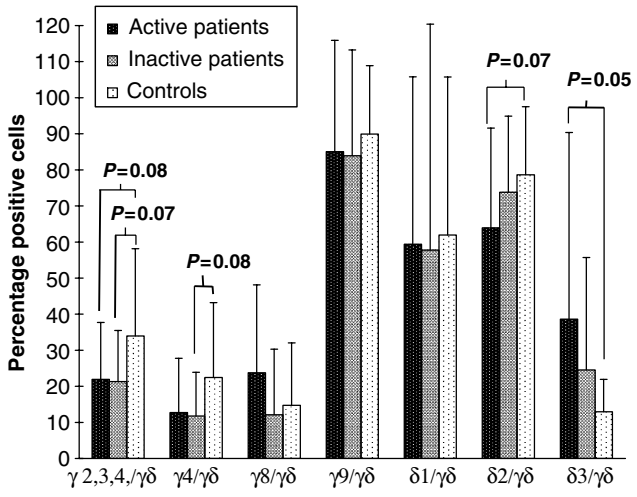

Figure 1 Vγ/TCR$\gamma\delta$ and Vδ/TCR$\gamma\delta$ ratios in peripheral blood from patients with active or inactive Behcet's disease (BD) and healthy controls. Immunofluorescence staining and flow cytometric analysis of peripheral blood lymphocytes from 18 BD patients with active disease (black bars), 13 BD patients with inactive disease (grey bars) and 19 healthy controls (white bars). The bars show the calculated mean of the percentage of Vγ or Vδ positive cells as a ratio of the percentage of TCR$\gamma\delta$ positive cells for each group with standard deviation indicated. Significant difference between groups is shown

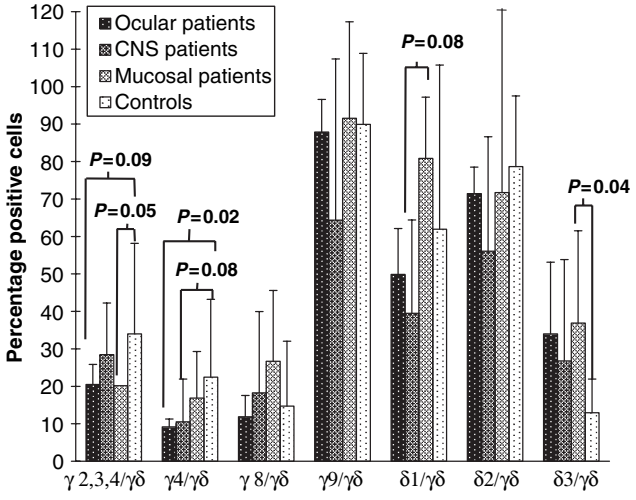

Figure 2 Vγ/$\gamma\delta$ and Vδ/$\gamma\delta$ ratios in peripheral blood from patients with ocular, central nervous system (CNS) or mucosal Behcet's disease (BD) and healthy controls. Immunofluorescence staining and flow cytometric analysis of peripheral blood lymphocytes from 11 BD patients with ocular disease (black bars), 7 BD patients with CNS disease (dark grey bars), 12 BD patients with mucosal disease (light grey bars), and 19 healthy controls (white bars). The bars show the calculated mean of the percentage of Vγ or Vδ positive cells as a ratio of the percentage of $\gamma\delta$ positive cells for each group with standard deviation indicated. Significant difference between groups is shown

Table 1 Expression of CD3, TCRγδ, Vγ and Vδ chains in ulcerated oral mucosa from patients with BD or in non-lesional oral mucosa from patients with BD and from healthy controls*

	Behcet's patients		*Healthy individuals*
Monoclonal antibodies	*Lesional (n = 9)*	*Non-lesional (n = 8)*	*Non-lesional (n = 3)*
CD3	9/9 (100)	8/8 (100)	3/3 (100)
pan TCRγδ	8/9 (89)	2/7 (28)	1/3 (33)
Vγ4	7/9 (78)	0/7 (0)	0/3 (0)
Vγ8	5/9 (56)	0/8 (0)	0/3 (0)
Vγ9	7/9 (78)	2/7 (28)	0/3 (0)
Vδ1	5/8 (62)	0/8 (0)	0/3 (0)
Vδ2	7/9 (78)	1/7 (14)	0/3 (0)
Vδ3	6/9 (67)	0/8 (0)	0/2 (0)

Percentage values are given in parentheses.
*Percentage of positive staining in oral biopsies.

patients were grouped according to clinical disease type, the ocular patients had a significantly lower Vγ4/γδ ratio than the controls ($P = 0.02$) and the mucosal patients had a significantly lower Vγ2,3,4/γδ ratio than the controls ($P = 0.05$; Figure 2). In addition to a lower Vγ9/γδ ratio, the CNS patients also had decreased Vδ2/γδ ratio 56.1 ± 30.6%) compared with ocular (71.4 ± 23.5%) and mucosal patients (71.7 ± 24.6%), and healthy controls (78.6 ± 18.9%), although this difference did not reach significant levels (Figure 2).

Expression of Vγ and Vδ chains in oral mucosal tissues
Table 1 and Figure 3 summarize the results of immunohistochemical staining of oral biopsies from three healthy individuals and 17 patients with BD. The mucosal biopsies from non-ulcerated mucosa (non-lesional) were taken from both healthy individuals, and from patients with BD. The biopsies from ulcerated mucosa (lesional) were only taken from the BD patients. Table 1 shows the number of individuals within each group expressing CD3, γδ TCR, γ4, γ8, γ9, δ1, δ2 and δ3, whereas Figure 3 shows the distribution of the scoring for each biopsy.

The healthy individuals had few infiltrating CD3 positive cells, located in the basal epithelial layer and just under the basement membrane. These cells did not express the γδ TCR. Only one out of three individuals had a single positive γδ T cell. Similar results were obtained from the BD biopsies from non-lesional sites, although infiltration of CD3 positive cells was slightly more common than in the healthy individuals, with only a few γδ T cells observed in two out of seven samples analysed. However, in the BD biopsies from lesional sites, infiltration of CD3 positive cells was prominent, with T cells expressing the γδ TCR detected in all biopsies but one.

In the non-lesional mucosa from the BD patients and from the normal individuals there was no expression of Vγ4 or Vγ8. However, seven of the nine BD patients with ulcerated mucosa expressed Vγ4 and Vγ9, and five out of nine patients expressed Vγ8. One patient expressed all three Vγ chains analysed (Figure 4). Similarly, the healthy mucosa from the normal individuals did not express any Vδ chains. In the healthy (non-lesional) mucosa from the BD patients only one out of seven patients expressed Vδ2, whereas both Vδ1 and Vδ3 were negative in all the samples. In comparison, most of the BD patients with mucosal ulceration expressed more than one type of Vδ, with Vδ2 being the most commonly used chain. Four patients expressed all three Vδ chains analysed. This is shown in Figure 4 using a representative patient sample.

Discussion

The role of γδ T cells in the oral mucosa is not clear. We have previously observed that γδ T cells were increased in peripheral blood of patients with BD (Fortune *et al*, 1990). These cells are in an activated stage and can be induced to produce the pro-inflammatory cytokines IFN-γ and TNF-α (Freysdottir *et al*, 1999), suggesting that they may contribute to the pathogenesis of BD. In other immune mediated diseases, such as in rheumatoid arthritis, where depletion of synovial γδ T cells *in vivo* was performed before the onset of systemic disease, the severity of the clinical disease was significantly reduced (Peterman *et al*, 1993).

The discovery of the structure of the γδ TCR showing extensive junctional diversity suggested that γδ T cells recognized antigens in a similar manner as αβ T cells. However, γδ T cells are able to recognize antigens without MHC restriction (Holoshitz *et al*, 1989). Amongst the possible ligands for the γδ TCR are self

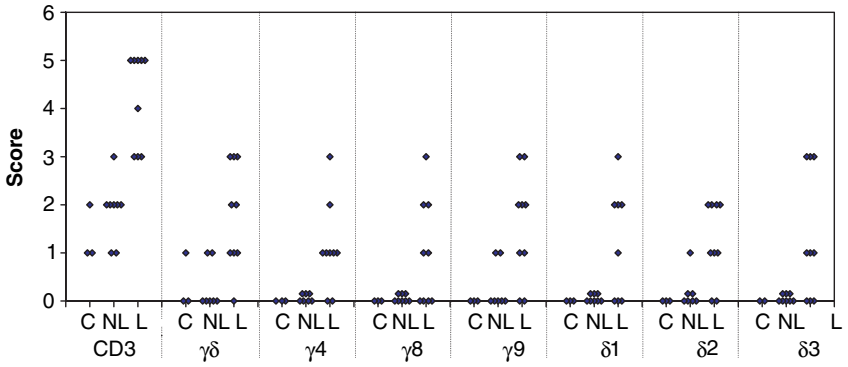

Figure 3 Distribution of CD3, TCRγδ, Vγ and Vδ scoring in an oral mucosa of Behcet's disease (BD) patients and healthy controls. Oral mucosa from healthy controls (C), BD patients without lesion (NL), or BD patients with lesion (L) were immunoenzyme stained with antibodies against CD3, TCRγδ, Vγ4, Vγ8, Vγ9, Vδ1, Vδ2 and Vδ3. Results were expressed as 0 (no staining) or 1+ (< 5 positive cells), 2+ (6–20 positive cells), 3+ (21–40 positive cells), 4+ (41–60 positive cells), or 5+ (> 60 positive cells)

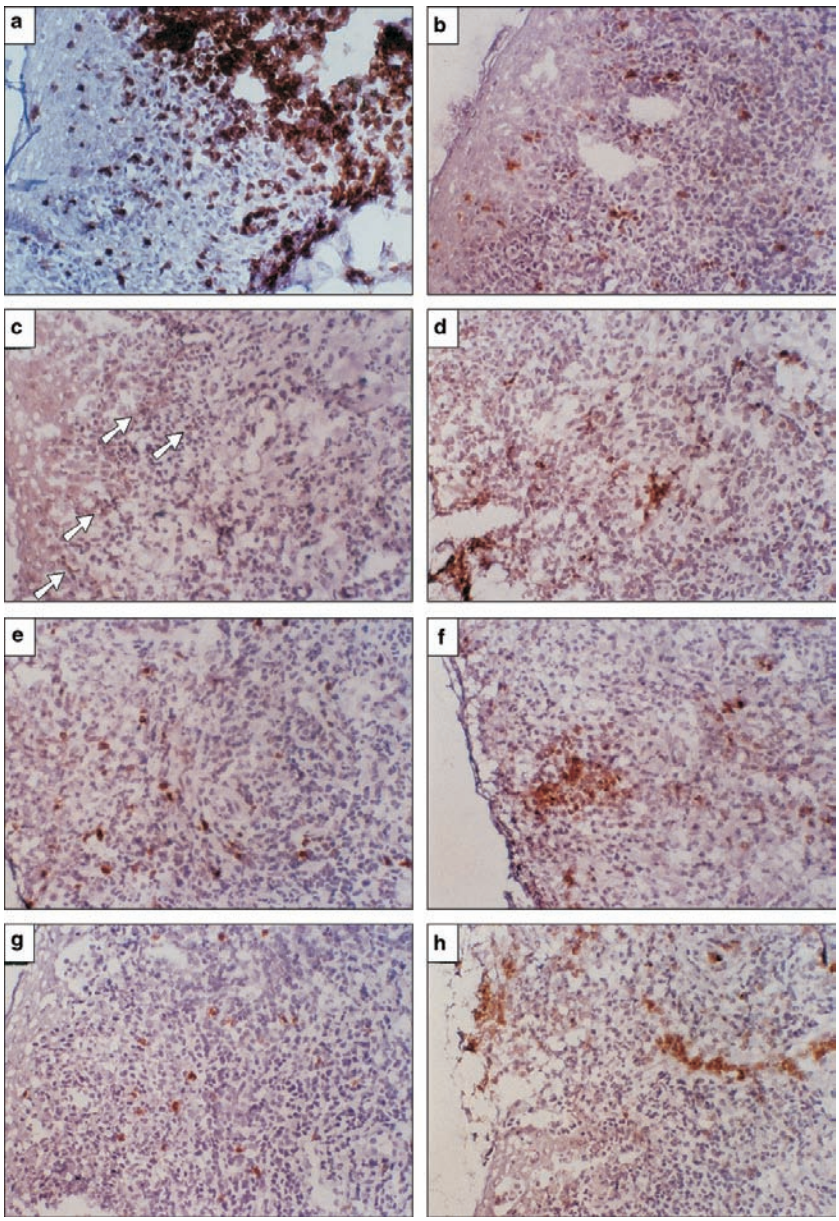

Figure 4 CD3, TCR$\gamma\delta$, Vγ and Vδ staining in an oral mucosal lesion of a Behcet's disease (BD) patient. Oral mucosal lesion from a patient with BD immunoenzyme stained with antibodies against CD3 (**a**), TCR$\gamma\delta$ (**b**), Vγ4 (**c**), Vγ8 (**d**), Vγ9 (**e**), Vδ1 (**f**), Vδ2 (**g**), and Vδ3 (**h**). Arrows point at some of the Vγ4 positive cells. Magnification ×274

determinants expressed on proteins induced by stress, such as the heat shock proteins (HSP), microbial antigens and other phosphorylated ligands, with $\gamma\delta$ T cells expressing Vγ9Vδ2 being the main subset responding to the phosphoantigens (Kabelitz, 1992; Burk *et al*, 1995; Tanaka *et al*, 1995; Porcelli *et al*, 1996). We have previously demonstrated that patients with BD respond to four HSP peptides (Hasan *et al*, 1996), suggesting a role for HSP in the pathogenesis of BD as a possible candidate ligand for the $\gamma\delta$ TCR.

The aim of this study was to analyse the restriction of the Vγ and Vδ usage by $\gamma\delta$ T cells in peripheral blood and oral mucosa in patients with BD and healthy controls. This might help to clarify the nature of the antigenic determinants present in the ulcerated mucosa of the BD patients, and the role of the $\gamma\delta$ T cells in the local immune response.

Vγ9 was the predominant chain expressed in peripheral blood, although all the Vγ chains were also expressed in the BD patients and healthy controls. The high frequency of the Vγ9 chain expression would predict an abundant expression of Vδ2, as this has been the Vδ chain reported to be expressed with the Vγ9 chain on PBL (14). Indeed, this was the most frequent Vδ chain expressed in peripheral blood $\gamma\delta$ T cells in BD patients and healthy controls. However, the frequency of Vδ2 was lower than that of Vγ9, indicating that chains other than Vδ2 pair with the Vγ9 chain.

There was no difference in Vγ9 chain expression between patients with active or inactive BD or between BD patients and healthy controls. However, a reduction in both Vγ9/$\gamma\delta$ and Vδ2/$\gamma\delta$ ratios was observed in the CNS disease group, indicating a different $\gamma\delta$ T-cell response in this group compared to the other disease

groups. This may be because of a difference in response to antigenic stimulus, such as phosphoantigens, in these patients resulting in activation of different γδ T-cell subsets or that the Vγ9Vδ2 T cells are reduced in the peripheral blood because of their homing from the blood to the CNS.

A surprising finding was the high frequency of Vδ1 expressed in the peripheral blood γδ T cells as well as the increase in the Vδ1/γδ ratio in the mucosal disease group. The Vγ8/γδ ratio was the only Vγ/γδ ratio raised in the mucosal group. The concurrent increase in the Vδ1/γδ and the Vγ8/γδ ratio suggest that these chains may be paired in the mucosal patients. The Vδ1 chain is preferentially expressed in the intestinal epithelium (Groh *et al*, 1998), suggesting a role in epithelial surveillance. The presence of all three Vδ chains analysed in the lesional oral mucosa does, however, not indicate a preferential role of Vδ1 in the oral mucosa. The ligand(s) for Vδ1 are not known and it needs to be elucidated whether the Vγ8Vδ1 T cells play a specific role in the mucosal BD.

Interestingly, although Vγ9 and Vδ2 were the major chains expressed in peripheral blood γδ T cells, other Vδ and Vγ chains were also expressed in the BD patients, suggesting that significant recombinational diversity exists in the γδ T-cell pool. This diversity was also reflected in the oral biopsies of ulcerated mucosa, where the γδ T cells showed no preferential usage of Vγ or Vδ chains, with most biopsies expressing at least two Vγ and Vδ chains. Some of these variable γδ T cells were expanded in the peripheral blood in BD patients with an increase in γδ T cells expressing Vγ8 and Vδ3 chains in both active and inactive disease; however a decrease in γδ T cells expressing Vγ4 chain was observed in BD patients with active disease and to a lesser extent in BD patients with inactive disease. This suggests that although there is receptor heterogeneity in BD patients, there is a shift in the γδ T-cell pool during the active stage of the disease. Whether this is driven in an antigenic, superantigenic or a bystander manner needs to be elucidated.

In the biopsy specimens from healthy individuals with normal mucosa, γδ TCR positive cells were virtually absent. Similarly, in BD patients with healthy non-ulcerated mucosa there was minimal expression of γδ T cells. It is not known whether the γδ T cells accumulate at site of the oral lesions before or during mucosal ulceration. When the patients were grouped according to clinical disease expression, it was clear that the patient groups differed in their preferential Vγ and Vδ chain expression, suggesting that different antigenic stimuli or antigenic responses may be responsible for the involvement of different tissues in clinical expression of BD.

The presence of all three Vγ and all three Vδ chains analysed within the oral lesions is suggestive of a polyclonal rather than oligoclonal activation of the γδ T cells, which is in contrast with what has been described for αβ T cells (Direskeneli *et al*, 1999). The diversity in Vγ and Vδ usage by the γδ T cells may indicate that during repeated inflammation of the oral mucosa, the γδ T cells are responding to a wide variety of antigenic and/or non-antigenic stimuli.

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