T cell receptor V β expression by mucosal T cells

J Spencer, M Y Choy, T T MacDonald

Abstract

Immunohistochemistry with monoclonal antibodies to the T cell receptor V β regions 5, 6, 8 and 12 was used to determine whether normal intestinal lymphocytes that are potentially exposed to many bacterially derived superantigens show any preferential expression of particular V β regions compared with the blood. No difference between V β expression in the mucosa and the blood was observed, suggesting that they share a common pool of $\alpha\beta$ T cells and that there is no expansion of $\alpha\beta$ T cells in response to bacterial "superantigens" in the gut.

The T cell receptor V β expressed by the activated T cells in the lamina propria of bowel from patients with Crohn's disease was also studied. There was no increase in V β 8 expression in these cells, suggesting that the increase in V β 8 observed in the blood and mesenteric nodes of patients with Crohn's disease is not of primary importance in the aetiology of the disease.

Finally, V β expression by mucosal T cells in coeliac disease was studied. There was no difference in V β use by T cells in coeliac disease and those in the blood and normal jejunum.

The enormous diversity of T cell receptor (TcR) specificities is created by rearrangement of germline variable (V), diversity (D), junctional (J), and constant (C) region genes. Of the many V segments available in the germline configuration, only one is incorporated into each chain of the rearranged receptor.

T cells using certain V segments of the $\alpha\beta$ T cell receptor preferentially respond to certain antigens. For example, in vitro stimulation of human and murine T cells with staphylococcal enterotoxin "superantigens" has been shown to stimulate both murine and human T cells that bear particular V β T cell receptors.^{1,2} A bias for V gene use has also been shown in vivo in man in association with certain diseases. Peripheral blood and mesenteric nodes from patients with Crohn's disease have been shown to contain a raised percentage of T cells expressing V β 8.³ Tumour infiltrating lymphocytes in uveal melanoma show increased expression of V α 7.⁴ Restricted V α use has also been shown in brain lesions in multiple sclerosis, in particular V α 10 has been implicated.⁵

The gut is potentially exposed to many bacterially derived "superantigens" which could theoretically stimulate the expansion of subpopulations of mucosal T cells. The first aim of this study, therefore, was to determine whether there is any difference in V β expression between the intraepithelial or lamina propria T cell compartments of the gut associated lymphoid tissues compared with the blood.

It has also recently been shown that the intestinal lamina propria of patients with Crohn's disease contains a population of activated T cells.⁶ Because the lesion in Crohn's disease may be due to a response to a micro-organism, the activated T cells in Crohn's disease may show restricted V β use. We therefore also examined bias in T cell receptor expression by the activated T cells in the lamina propria in Crohn's disease. The enteropathy in coeliac disease is thought to be due to an aberrant T cell response to gluten.⁷ We therefore also examined the expression of V β segments by the T cells in coeliac disease to determine whether gluten causes the expansion of a particular group of T cells.

Although there are about 20 V β families in man, only a limited number of antibodies are available. We used immunohistochemistry to study the expression of four V β segments (V β 5, V β 6, V β 8, and V β 12). Particular attention was paid to V β 8 because it has already been implicated in Crohn's disease, though not in the mucosal lesion.

Methods

All tissues were collected fresh, snap-frozen in liquid nitrogen, and stored at -70°C . The following were studied:

- 1 thirteen histologically normal jejunal biopsy specimens from patients who had clinical symptoms of coeliac disease (age range 4-55 years);
- 2 diseased and macroscopically normal tissues from five children with Crohn's disease (three from the small intestine and two from the large intestine) undergoing surgical resection (age range 10-16 years);
- 3 jejunal biopsy specimens from two children with clinical symptoms of coeliac disease who subsequently responded to a gluten free diet and gluten challenge allowing coeliac disease to be diagnosed according to the ESPGAN criteria⁸ (ages 2 and 4 years).

Previous studies quantitating V β expression in the blood and mesenteric lymph node cells

Department of
Histopathology,
University College and
Middlesex School of
Medicine, Bland
Sutton Institute,
Riding House Street,
London W1P 7PN
J Spencer
M Y Choy

Department of
Paediatric
Gastroenterology,
St Bartholomew's
Hospital, London
T T MacDonald

Correspondence to:
Dr J Spencer

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Figure 1 Percentage of T cells in normal blood, jejunal lamina propria and epithelium expressing (A) V β 5, (B) V β 6, (C) V β 8 and (d) V β 12. There is no difference in V β use between T cells in the intestinal lamina propria and epithelium and those in the blood of normal subjects.

have used immunofluorescence.²³ To verify that our results using immunoperoxidase are comparable with those using immunofluorescence and for comparison with normal bowel biopsy specimens we studied peripheral blood from eight normal subjects using immunoperoxidase on cytopsin preparations. Peripheral blood (5 ml) was taken from normal volunteers (age range 21–40 years) and the lymphocytes separated using Lymphoprep. The lymphocytes were washed and cytocentrifuged.

Murine monoclonal antibodies to human TcR variable regions (V β 5a, V β 6, V β 8, and V β 12) were obtained from T Cell Sciences Ltd (Cambridge, Massachusetts, USA). Anti-CD3 and biotinylated anti-CD25 were obtained from Dako Ltd (High Wycombe, England).

Frozen sections of normal and diseased tissues were cut (8 μ m) and stained immunohistochemically using the above monoclonal antibodies by the indirect immunoperoxidase technique. To determine whether activated T cells in diseased mucosa show any preference for a particular V β region, tissues were double stained. First the indirect immunoperoxidase technique was used with the AEC substrate (red product) (Dako Ltd) for the various V β regions. The rabbit anti-mouse immunoglobulin on the section was then blocked using normal mouse serum. Biotinylated anti-CD25 was then applied followed by avidin conjugated alkaline phosphatase and Fast Blue substrate to pinpoint the activated cells.

Single stained cells were either bright red or blue. Double stained cells were very dark red or purple.

Values of percentage of T cells expressing a particular V β were derived from counts of percentage of CD3 positive T cells and the percentage of cells expressing that V β in a serial section. Epithelium and lamina propria were counted independently. Counts of single and double stains were made using $\times 400$ total magnification.

The numbers of patients included in this study were relatively small, in particular the coeliac group of two patients. The comparisons were therefore made subjectively.

Results

EXPRESSION OF TCR V β REGIONS BY NORMAL INTESTINAL LAMINA PROPRIA AND INTRAEPITHELIAL T CELLS

The distribution of mucosal and peripheral blood T cells expressing TcR V β regions 5, 6, 8 and 12 is shown in fig 1. There was generally no difference between the expression of V β regions 5, 6, 8 and 12 in the intestinal epithelium and lamina propria and the blood. Interestingly, some histologically normal biopsy specimens had an increased percentage of cells expressing V β 6 or V β 8. In a single biopsy specimen there was a dramatic increase in V β 6 expression with about 60% of T cells in the lamina propria and epithelium expressing V β 6 (fig 2). This biopsy specimen was from a child with failure to thrive of unknown cause.

Figure 2 Histologically normal jejunal biopsy specimens stained using monoclonal antibody to V β 6. Specific staining is indicated by closed arrows, staining due to endogenous peroxidase by open arrows. (A) Biopsy specimen showing the normal distribution of V β 6 positive cells. Occasional positive cells may be observed in the lamina propria and epithelium. (B) Biopsy specimen with dramatically increased percentage of T cells expressing V β 6 (immunoperoxidase).

EXPRESSION OF TCR V β REGIONS BY MUCOSAL LYMPHOCYTES IN CROHN'S DISEASE AND COELIAC DISEASE

The distribution of mucosal T cells expressing TcR V β regions 5, 6, 8 and 12 in Crohn's disease is shown in fig 3. There was no general increase in any TcR V β expression in either of these diseases. Clusters of IL2 receptor positive cells identified in the mucosa were not mirrored by clusters of cells expressing a particular V β in a serial section. The TcR V β expressed by activated mucosal T cells was studied in detail using double staining in five cases of active Crohn's disease. The IL2 receptor positive cells counted were the intensely positive cells deep in the mucosa as the subepithelial weakly IL2 receptor positive cells are known to be activated macrophages.

In three cases IL2 receptor positive cells did not express any of the V β regions studied. In one case about 8% of IL2 receptor positive cells expressed V β 12 and in another case 9% expressed V β 5 and 7% V β 6.

EXPRESSION OF TCR V β REGIONS BY MUCOSAL LYMPHOCYTES IN COELIAC DISEASE

The distribution of lamina propria and intraepithelial T cells expressing TcRs V β 5, 6,

8 and 12 were studied in two patients with coeliac disease. In the intraepithelial compartment the percentages of T cells expressing the different V β regions in each patient were as follows: V β 5, 4.4% and 5.1%; V β 6, 1.8% and 4.4%; V β 8, 5.3% and 2.2%; B β 12, 1.4% and 1.3%. The expression of TcR V β regions by lamina propria T cells in coeliac disease is as follows: V β 5, 2.8% and 0%; V β 6, 2.4% and 1.6%; V β 8, 3.2% and 1.1%; V β 12, 0.5% and 0.4%. There was no apparent difference between TcR V β regions expressed by the intraepithelial and lamina propria T cells of the patients with coeliac disease and there was no apparent difference between V β regions expressed in patients with coeliac disease and normal controls.

Discussion

Generally there was no difference between V β use in either normal or inflamed mucosa compared with peripheral blood. This suggests that mucosal and peripheral $\alpha\beta$ T cells are part of the same T cell pool, unlike mucosal $\gamma\delta$ T cells, which differ in V region use compared with peripheral T cells.⁹ It also shows that mucosal T cells expressing V β regions 5, 6, 8 and 12 do not seem to be stimulated to proliferate in situ in response to bacterially derived "superantigens" to which they are potentially exposed in the gut.

The reason for the unusually high percentages of V β 6 or V β 8 in both the epithelium and lamina propria of some of the biopsy specimens is unclear. Although these biopsy specimens were histologically normal, the patients had intestinal symptoms at the time of biopsy. The changes were probably the result of infections which resulted in raised concentrations of TcR V β 6 or 8. The simultaneous increase in the V β regions in the epithelium and lamina propria is consistent with the hypothesis that intraepithelial lymphocytes are derived from activated lamina propria T cells.¹⁰

We failed to observe any difference between the TcR expressed by the abundant T cells that accumulate in response to gluten in the mucosa of patients with coeliac disease and the control groups. As only a group of two patients with coeliac disease were studied the results were analysed subjectively, and there may be subtle differences that we failed to detect. Similarly, although activated T cells are present in the mucosa in Crohn's disease,⁸ we failed to observe any bias towards the expression of a particular V β region in the corresponding sites. These results in Crohn's disease and coeliac disease do not, however, rule out the possibility that T cells expressing other V β s could be preferentially expressed, indeed the absence of any of the V β s studied on any of the activated T cells in three of the cases of Crohn's disease implies that in Crohn's disease another V β may be preferentially expressed by these T cells. T cells expressing increased concentrations of V β 8 have been observed by others in the blood and mesenteric nodes of patients with Crohn's disease. If the stimulation of T cells is a primary event in the aetiology of the lesions in

Figure 3 Percentage of CD3 positive cells expressing V β 5, 6, 8 and 12 in the intestinal lamina propria of patients with Crohn's disease. There is no apparent increase in any V β studied in Crohn's disease compared with normal biopsy specimens or blood.

Crohn's disease, then it would be expected that any specific subsets of T cells involved would be present in the mucosa in addition to the mesenteric nodes and blood. The failure to identify activated V β 8 positive cells in the mucosa suggests that their presence in the mesenteric nodes and blood is a epiphenomenon due perhaps to antigens which may cross the damaged mucosa and migrate via the regional lymphatics to the mesenteric nodes.

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Eponyms in pathology . . .

LANGERHANS, Paul (1847-1888) was a German physician and pathologist who was born and trained in Berlin, graduating in 1869. In the same year, while working in Virchow's laboratory, he described the cells of the pancreatic islets to which his name is now attached, calling them "clumps of epithelial-like protoplasmic cells lying in the interalveolar tissue of the pancreas". After service in a military hospital in the war of 1870, he became associate professor of pathological anatomy at Freiburg. Five years later he contracted pulmonary tuberculosis and went to convalesce in Madeira where he died in 1888 at the age of 39.