T cell receptor V β expression in human intestine: regional variation in postnatal intestine and biased usage in fetal gut

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Abstract

A panel of T cell receptor V β specific monoclonal antibodies was used to analyse V β gene usage at different sites in human postnatal and fetal intestine. In normal small intestine, at a single site, different patients showed expansion of T cells expressing individual V β s. Lamina propria and epithelial T cells from the same patient showed overlapping but not identical V β dominance. V β dominance was also shown in the T cells of the colonic lamina propria. Analysis of two separate regions of intestine from the same patient (5-100 cm apart) showed that T cells expressing a dominant V β region were often present at both sites. In most patients, however, major biases in T cell V β usage (two to 12-fold variation) were also apparent between the two sites. Analysis of V β expression in human fetal intestine also showed considerable skewing, although the most common dominant V β in postnatal intestine (V β 22) was never predominant in fetal intestine. Patchy local variation in the expression of individual V β s therefore occurs against a background of V β dominance over large regions of the human gut. Furthermore the results from fetal gut show that factors other than luminal antigen control V β expression in the gut.

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show that by and large, those clones dominant at one site in the gut, are also dominant at a second distant site.⁴ Immunohistochemistry on the other hand offers the advantage that microenvironmental heterogeneity in expression of different V β genes can be identified *in vivo*. Although monoclonal antibodies against all human V β gene products are not yet available, it is possible to obtain a panel that recognises 30-40% of T cell receptor V β gene products.

Here we report on the distribution of 14 different T cell receptor V β s in human gut, and show that V β dominance can be detected. We confirm that certain V β regions can be dominant over large sections of intestine, but also show that there is considerable local variation and that a V β dominant at one site may not be dominant at an adjacent site. Finally we also show evidence for V β skewing in human fetal gut.

Methods

Clinical material

This study received ethical approval from the Hackney and District Health Authority. Four samples of normal ileum, all from men aged between 20-49, were studied. Three were from patients undergoing resection for right side colonic carcinoma and the other was from a cadaveric organ donor. Six samples of histologically normal colon were also studied (four men and two women aged between 19 and 72). Four were from elderly patients undergoing resection for colonic carcinoma and two were from patients (aged 19 and 25) undergoing resection for polyposis coli.

For studies of V β expression at two sites in the intestine, material was obtained from 11 patients with inflammatory bowel disease. Eight patients had Crohn's disease and were undergoing resection because of strictures. Four of these were male adults aged between 29 and 35, and four were children aged between 13 and 16. Macroscopically and histologically normal tissue between the strictures was sampled.

Ten samples of human fetal intestine aged between 16 and 24 weeks were also studied. These were obtained from the MRC Tissue Bank, The Brompton Hospital, London.

Immunohistochemistry

One cm square pieces of intestinal mucosa were dissected out of the bowel within minutes

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Normal human small intestine contains many T cells. A surprising and as yet unexplained recent finding is that the T cells of the intestinal epithelium and lamina propria are oligoclonal.¹⁻³ Sequencing of T cell receptor gene segments used by isolated human intraepithelial and lamina propria T cells (IEL and LPL) has shown unequivocally that a large proportion of the expressed T cell V β receptors have identical D and J regions with the same N-region insertions, with each subject showing a unique pattern of oligoclonal expansion.¹⁻³ Clonal dominance demonstrated by sequencing has also been confirmed *in situ* by immunohistochemistry.²

Polymerase chain reaction and sequencing methodology to unequivocally identify V region dominance in the gut requires comparatively large pieces of gut to permit the isolation of IEL and LPL from which sufficient mRNA can be extracted. Nevertheless, using this technique, evidence has recently been provided to

of resection and immediately snap frozen in liquid nitrogen. At a second (or multiple) macroscopically normal site(s), tissue was also obtained and the distance noted. Tissues were stored at -70°C until analysis.

Seven μm frozen sections were cut and stained by the indirect peroxidase method using peroxidase conjugated rabbit antimouse IgG as described previously,⁵ except that to enhance staining, an additional layer of peroxidase conjugated goat antirabbit IgG (Dako Ltd) was used. Anti-CD3 (UCHT1, Dako Ltd) was used to identify T cells and TcR $\delta 1$ (T Cell Sciences, Cambridge, MA) to identify gamma/delta T cells. Anti-V β antibodies used were the following: anti-V $\beta 1$ and 9 (Pharmingen, San Diego); -V $\beta 2$, 3, 5.2, 5.3, 8, 13.3, 17, 19, 21, 22 (Immunotech, Marseille, France); -V $\beta 6$, 12 (T Cell Sciences).

To analyse all the V β s in a single sample necessitated multiple serial sections and to avoid problems resulting from microenvironmental heterogeneity in T cell density, after staining with four V β specific antibodies, the next two sections were stained with CD3 and anti-TcR $\delta 1$; the next four sections were then stained with four more anti-V β s, and so on. Controls included irrelevant primary antibodies (negative control) and tonsil (positive control). All of the anti-V β antibodies stained cells in the T cell zones of tonsil, except V $\beta 17$, which showed immunoreactivity with epithelial cells. V $\beta 17$ + cells were never seen in the postnatal intestine and for that reason, results for the V $\beta 17$ antibody are not shown.

Enumeration of V β expression

Intraepithelial lymphocytes – the number of immunoperoxidase stained CD3+, TcR $\delta 1$ +, and V β + IEL per 100 epithelial cells was calculated by differential counts as described elsewhere.⁶ At least 900 epithelial cells or 100 CD3+ cells were counted per specimen. The per cent of $\gamma\delta$ TcR+ IEL per 100 epithelial cells was subtracted from the per cent of CD3+ cells to determine the per cent of $\alpha\beta$ TcR+ IEL. The per cent of $\alpha\beta$ TcR+ IEL expressing an individual V β was then determined as before.⁷ To exclude the possibility that there was microenvironmental heterogeneity in IEL density that might bias the results, the per cent V β s were calculated from the closest adjacent section stained with CD3 (maximally 14 μm away). In fact, however, there was less than 10% variation in the number of CD3+ cells per 100 epithelial cells between the different sections cut at different levels in the tissue.

Lamina propria T cells – T cells expressing individual V β s in the lamina propria were enumerated by computer assisted image analysis (SeeScan, Cambridge, England). Briefly sections of lamina propria were visualised on a colour monitor and an area of lamina propria outlined using a mouse controlled cursor; the area within the outline was then calculated. The positively stained cells within this area were flagged on the visual display. At least six areas of lamina propria containing minimally

500 T cells were counted. Dividing the number of CD3+ cells per unit area by the number of V β + cells per unit area gave the per cent of cells expressing an individual V β . $\gamma\delta$ TcR+ T cells made up only around 1% of the CD3+ cells in the lamina propria and were not taken into account. As with IEL counts, the per cent V β + cells was calculated from the closest section stained with CD3 and there was less than 10% variation in the number of CD3+ cells per unit area between the different sections stained with CD3. Most of the counts were made by one of the authors (RT). The reproducibility and accuracy of the counts were confirmed in selected samples by SP.

Data analysis

In postnatal intestine any V β expressed at greater than 10% of the total $\alpha\beta$ T cells was considered dominant. In fetal gut, an arbitrary value of 5% was chosen. For comparison of the expression of an individual V β by IEL at two sites, statistical analysis was not possible as each point was a single value obtained by counting CD3+ cells and V β + cells per 900 epithelial cells (see above). For this reason a twofold variation in expression was considered meaningful. However, for comparison of lamina propria T cells where at least six measurements were made on a single specimen, V β expression at different sites could be compared using the Mann-Whitney U test. In all cases, however, where there was at least a twofold difference in V β expression, this proved to be statistically significant ($p < 0.02$).

Results

Use of antibodies to map T cell receptor V β dominance in human intestine

In samples of normal small intestine from four subjects, and in the normal colon of six different subjects, V β region dominance was seen. In the small intestine (Fig 1A), overexpression of at least one V β was seen in the epithelium of all four patients and in the lamina propria of three of four. In some cases, the same V β was dominant in the lamina propria and epithelium (for example, V $\beta 8$ in patients 1 and 4), but in others there was considerable variation (for example, V $\beta 13.3$ in patient 2).

V β region usage in the colonic epithelium could not be quantified because in a single section stained with an anti-V β antibody, there were too few T cells in the epithelium for reliable measurement. However, lamina propria T cells were more abundant and showed clear V β skewing (Fig 1B), with different V β s dominant in different subjects.

V β dominance at different sites in the intestine

In the small bowel epithelium, in six of seven cases in which paired tissue was available, a dominant V β was also dominant at the second site, even up to a metre away (Table I). In the other case, no dominant V β s were seen in either sample and these results are not shown.

Figure 1: (A) Vβ expression by the αβ TcR+ cells in the epithelium and lamina propria of normal human small bowel from four patients. (B) Vβ expression by the αβ TcR+ cells in the lamina propria of normal human colon from six patients.

TABLE I Variation in Vβ expression by αβ TcR+ intraepithelial lymphocytes at different sites in the gut

Patient	Vβ	% Vβ IEL (of total αβ TcR+ IEL)	
		Site 1	Site 2
1	Vβ3	1.0	12.0*
	Vβ8	12.0	17.0
	Vβ19	1.3	12.5*
	Vβ21	1.3	10.4*
	Vβ22	6.2	12.2
2	Vβ19	15.6	6.7*
	Vβ22	24.1	22.3
	Vβ8	8.6	14.1
3	Vβ13.3	2.3	27.0*
	Vβ19	11.0	1.9*
	Vβ21	10.0	2.2*
	Vβ22	29.2	19.1
4	Vβ8	12.8	5.5*
	Vβ12	42.5	22.6
	Vβ19	10.5	9.5
5	Vβ22	23.0	27.0
	Vβ8	1.1	12.1*
6	Vβ19	1.2	13.1*
	Vβ22	40.0	44.3

Patients 1–5, tissues sampled between 5 and 15 cm apart. Patient 6, samples 100 cm apart. Instances where there is at least a twofold variation in expression of an individual Vβ at different sites are highlighted with an asterisk.

In five of six paired specimens in which Vβ dominance was seen, a Vβ dominant at one site, was not dominant in the other (two to 12-fold less). In some cases this was spectacular as with Vβ13.3 in patient 3, which comprised 2.3% of the T cells at one site and 27% at a second site 10 cm away.

In paired samples from the small intestinal lamina propria (Table II, patients 1–7) Vβ dominance was seen in both sites in five of seven patients, but the Vβ dominant at one site was not necessarily dominant at the second site (for example, Vβ19 in patient 5, which made up only 6.5% of the T cells at one site but 24.9% at a second site 1 metre distant). In one patient no Vβ dominance was seen and is not shown, and in another (patient 3) the numbers of Vβ12+ cells was only moderately increased. In patients 7 and 8 where samples were taken from the small bowel and colon, there were appreciable differences and the Vβ dominant at one site was not detected at the other. In patients 9–11, where both samples were from the colon, regional variation was seen in two (patients 9 and 10) and in the other, although Vβ6 was abundant at both sites, there was also variation in the expression of other Vβs.

These tissues were obtained from patients with Crohn's disease, and although histologically normal, there is the possibility that the underlying disease might affect the results. However, we do not feel that this is the case because, as in the true normal patients, we had no difficulty in observing Vβ dominance in these patients.

Vβ usage in fetal intestine

We also analysed Vβ expression in samples of fetal human intestine. As this was a retrospective study, we did not know the location of the

TABLE II Variation in Vβ expression in αβ TcR+ cells in the lamina propria at different sites in the gut

Patient	Vβ	% Vβ cells (of total αβ TcR+)	
		Site 1	Site 2
1	Vβ8	22.2	19.2
	Vβ19	6.3	13.7*
	Vβ22	4.4	10.7*
2	Vβ8	16.6	5.4*
	Vβ19	5.0	10.0*
	Vβ22	12.0	16.6
3	Vβ12	8.1	10.1
	Vβ8	6.5	11.3
4	Vβ22	19.5	22.7
	Vβ6	8.3	15.0
	Vβ8	22.0	12.5
5	Vβ19	6.5	24.9*
	Vβ8	4.5	12.0*
	Vβ19	1.9	14.1*
6	Vβ22	16.5	32.1
	Vβ19	4.8	14.3*
	Vβ21	3.7	10.5*
7	Vβ1	11.0	0.9*
	Vβ3	11.4	3.3*
	Vβ8	10.4	6.0
8	Vβ8	6.4	11.8
	Vβ22	17.6	8.2*
9	Vβ6	16.4	18.5
	Vβ8	7.6	10.6
	Vβ19	7.2	17.8*
10	Vβ22	10.0	7.5
	Vβ6	16.4	18.5
11	Vβ8	7.6	10.6
	Vβ19	7.2	17.8*
	Vβ22	10.0	7.5

Patients 1–4, small intestine tissues sampled between 5 and 15 cm apart. Patients 5 and 6, small intestine, samples 100 cm apart. Patients 7 and 8, site 1 ileum, site 2 colon, 40 cm away. Patients 9–11 colon, between 5 and 25 cm apart. Instances where there is at least a twofold variation in expression of an individual Vβ at different sites are highlighted with an asterisk.

Figure 2: V β expression by the $\alpha\beta$ TcR $^{+}$ cells in the lamina propria of human fetal small intestine of different gestational ages.

sample (upper or lower bowel). There were too few positive cells in the epithelium staining with each V β region antibody to permit analysis of epithelial lymphocytes, however, sufficient cells were present in the lamina propria. Most of the anti-V β antibodies stained only 1 or 2% of the T cells in the lamina propria (Fig 2). However, to our surprise, there was considerable skewing of V β usage in some samples. V β 8 was commonly overexpressed, however, as with postnatal gut there was appreciable variation between specimens;

notably as in foetus number 9 where 31% of the lamina propria T cells expressed V β 5-3.

Discussion

Previous studies have shown oligoclonality of $\alpha\beta^{+}$ T cells in the human gut epithelium and lamina propria.¹⁻³ In a more recent study of intestine obtained at two sites from seven subjects, the same dominant clones were seen at both sites in all cases except one, where the tissue was taken from the small intestine and

colon.⁴ Based on this, the authors suggested that a small number of T cell clones comprised the majority of T cells in the human gut. In this study, using immunohistochemistry, we can confirm this finding in that there is dominance in the expression of a single V β along large pieces of intestine, and that in the two cases we studied where tissue was obtained from small bowel and colon, different V β s were expressed. Where our results differ from those of others, is that we were also able to consistently identify large numbers of T cells expressing a single V β at one site, but which were not over-represented at second adjacent site between 5 and 100 cm distant. Thus for some V β s, dominance is patchy.

There are several explanations for this discrepancy. The first is that in situations where major differences were seen (for example, patient 5, Table II) where T cells expressing V β 19 made up 6.5% of the T cells in the lamina propria at one site and 24.5% at a second site one metre away), that the V β dominance at one of the sites does not represent a single clone. By the polymerase chain reaction methodology used by Blumberg and colleagues, if the V β 19 gene segment was used by T cells that had rearranged different D and J regions gene segments, then it would not have been apparent.⁴ Without sequence data this cannot be disproved, however, to evoke such a large expansion of polyclonal V β 19+ T cells would probably require local superantigen induced T cell activation.

A second and in our opinion more probable explanation, is that the differences reflect the sensitivities of the different methodologies used.

It has been suggested that perhaps one of the reasons for oligoclonality of IEL is that they are preferentially selected because they recognise peptides in the context of CD1d, which is non-polymorphic.¹ That T cell can make an alloresponse to CD1d on epithelial cells has been shown,⁸ however, gut epithelial cells also express abundant class I (A and B loci) and class II MHC products⁹⁻¹¹ and there is no a priori reason why these molecules should not function as antigen presenting molecules. Indeed class II restricted T cell responses using enterocytes as antigen presenting cells has been shown^{12,13} and H-2 restricted IEL responses to reovirus have also been shown in mice.¹⁴ In addition, lamina propria T cells are also oligoclonal,³ and these are mostly CD4+.¹⁵ There is no CD1d expressed in the lamina propria and so the lamina propria oligoclonal cells are either derived from IEL or are responding to different stimuli. Finally the idiosyncratic way in which patterns of certain V β s are expressed in different subjects, seems to suggest that the stimulus for the expansion varies. In this context, the recent finding that there is oligoclonality in mouse IEL is particularly relevant. Even in inbred mice from the same cage, there is considerable variation in the clones present.¹⁶ This could be caused by the massive expansion of a few T cells that react with antigens of the flora, the interaction

between the bacterial antigens and the T cells present in the gut being probably due to chance and therefore unpredictable. What is unclear is, if this is the case, why it is not an ongoing process so that the early dominant clones become diluted by further clones responding to other antigens, so that with time, mucosal T cells should evolve from oligoclonal to a polyclonal population.

One of the difficulties of oligoclonal populations of T cells populating the gut is the restriction that places in mucosal defences to pathogens. There are numerous examples in published works where mucosal antigen specific T cell responses have been described.^{14,17-21} It seems intuitive that the capacity to generate polyclonal responses to different pathogens in the gut would be of some advantage. If the T cell populations really are functionally restricted, this is best interpreted as showing that pathogens induce some kind of stress response by epithelial cells and that the effector path of immunity is not directed against the pathogen, but against the epithelial cells. We see no reason why these two findings cannot be reconciled, however, with clonal dominance over large regions of the gut, forming a background on which antigen specific responses to gut pathogens can be generated and expressed.

Previous work in this laboratory has shown that human fetal gut contains T cells²² and therefore an analysis of V β expression was carried out, with the view that this tissue should be largely free from exposure to exogenous antigens. To our surprise, there was skewing of V β expression in fetal gut lamina propria in most of the specimens. There is little evidence in humans for waves of T cells with restricted TcRs leaving the thymus and populating peripheral tissues. Until we analyse in detail other tissues besides the intestine, however, we cannot find out if our data point to a local expansion of a precursor T cell that migrates into the gut wall, or is merely a reflection of certain V β s being overexpressed in all of the tissues of the foetus at a certain stage of development. It would, however, be attractive to speculate that the first is true, and is responsible for the oligoclonality seen in postnatal intestine, if it persists. It has not yet been established, however, whether dominant clones persist in the postnatal intestine over long periods of time, or whether new clones arise as others disappear.

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