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

R Thomas, G Schürmann, P Lionetti, S L F Pender, T T MacDonald

Abstract
A panel of T cell receptor Vβ specific monoclonal antibodies was used to analyse Vβ 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 Vβs. Lamina propria and epithelial T cells from the same patient showed overlapping but not identical Vβ dominance. Vβ dominance was also shown in the T cells of the colon and lamina propria. Analysis of two separate regions of intestine from the same patient (5–100 cm apart) showed that T cells expressing a dominant Vβ region were often present at both sites. In most patients, however, major biases in T cell Vβ usage (two to 12-fold variation) were also apparent between the two sites. Analysis of Vβ expression in human fetal intestine also showed considerable skewing, although the most common dominant Vβ in postnatal intestine (Vβ22) was never predominant in fetal intestine. Patchy local variation in the expression of individual Vβs therefore occurs against a background of Vβ dominance over large regions of the human gut. Furthermore the results from fetal gut show that factors other than luminal antigen control Vβ expression in the gut.

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Keywords: T cell receptor, intestine.

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 Vβ 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 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 Vβ genes can be identified in vivo. Although monoclonal antibodies against all human Vβ gene products are not yet available, it is possible to obtain a panel that recognises 30–40% of T cell receptor Vβ gene products. Here we report on the distribution of 14 different T cell receptor Vβs in human gut, and show that Vβ dominance can be detected. We confirm that certain Vβ regions can be dominant over large sections of intestine, but also show that there is considerable local variation and that a Vβ dominant at one site may not be dominant at an adjacent site. Finally we also show evidence for Vβ 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 Vβ 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.
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 antismouse
IgG as described previously, except that to
enhance staining, an additional layer of perox-
idase conjugated goat antirabbit IgG (Dako
Ltd) was used. Anti-CD3δ (UCHT1, Dako
Ltd) was used to identify T cells and TcRδ1 (T
Cell Sciences, Cambridge, MA) to identify
gamma/delta T cells. Anti-Vδ antibodies used
were the following: anti-Vδ1 and 9 (Phar-
mingen, San Diego); -Vβ2, 3, 5-2, 5-3, 8, 13-3,
17, 19, 21, 22 (Immunotech, Marseille,
France); -Vβ6, 12 (T Cell Sciences).

To analyse all the Vβs in a single sample
necessitated multiple serial sections and to
avoid problems resulting from microenviron-
mental heterogeneity in T cell density, after
staining with four Vβ specific antibodies, the
next two sections were stained with CD3 and
anti-TcRδ1; the next four sections were then
stained with four more anti-Vβs, and so on.
Controls included irrelevant primary anti-
bodies (negative control) and tonsil (positive
control). All of the anti-Vβ antibodies stained
cells in the T cell zones of tonsil, except Vβ17,
which showed immunoreactivity with epithe-
elial cells. Vβ17+ cells were never seen in the
postnatal intestine and for that reason, results
for the Vβ17 antibody are not shown.

**Enumeration of Vβ expression**

*Intraepithelial lymphocytes* – the number of
immunoperoxidase stained CD3+, TcRδ1+, and
Vβ+ IEL per 100 epithelial cells was calcu-
dated by differential counts as described else-
where. At least 900 epithelial cells or 100
CD3+ cells were counted per specimen. The per
cent of γδ TcR+ IEL per 100 epithelial
sections was subtracted from the per cent of
CD3+ cells to determine the per cent of αβ
TcR+ IEL. The per cent of αβ TcR+ IEL
expressing an individual Vβ was then deter-
mised as before. To exclude the possibility
that there was microenvironmental hetero-
genicity in IEL density that might bias the
results, the per cent Vβs 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 Vβs in the lamina propria were
enumerated by computer assisted image anal-
ysis (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 Vβ+ cells per unit area gave the per
cent of cells expressing an individual Vβ.
γδ 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 Vβ+ 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 Vβ expressed at
greater than 10% of the total αβ T cells was
considered dominant. In fetal gut, an arbitrary
value of 5% was chosen. For comparison of the
expression of an individual Vβ by IEL at two
sites, statistical analysis was not possible as
each point was a single value obtained by
counting CD3+ cells and Vβ+ 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 speci-
men, Vβ 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 Vβ expression, this
proved to be statistically significant (p<0.02).

**Results**

**Use of antibodies to map T cell receptor Vβ
dominance in human intestine**

In samples from small intestine from four
subjects, and in the normal colon of six differ-
ent subjects, Vβ region dominance was seen.
In the small intestine (Fig 1A), overexpression of at least one Vβ was seen in the epithelium
of all four patients and in the lamina propria
of three of four. In some cases, the same Vβ
was dominant in the lamina propria and epithelium
(for example, Vβ8 in patients 1 and 4), but in
others there was considerable variation (for
example, Vβ13-3 in patient 2).

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

**Vβ 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 Vβ was also dominant at the second
site, even up to a metre away (Table 1). In the
other cases, no dominant Vβs were seen in
either sample and these results are not shown.
In five of six paired specimens in which \( \kappa \) dominance was seen, a \( \kappa \)B dominant at one site, was not dominant in the other (two to 12-fold less). In some cases this was spectacular as with \( \kappa \)B13 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) \( \kappa \)B dominance was seen in both sites in five of seven patients, but the \( \kappa \)B dominant at one site was not necessarily dominant at the second site (for example, \( \kappa \)B19 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 \( \kappa \)B dominance was seen and is not shown, and in another (patient 3) the numbers of \( \kappa \)B12+ 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 \( \kappa \)B 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 \( \kappa \)B6 was abundant at both sites, there was also variation in the expression of other \( \kappa \)Bs.

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 \( \kappa \)B dominance in these patients.

**\( \kappa \)B usage in fetal intestine**

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

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**Table I**

<table>
<thead>
<tr>
<th>Patient</th>
<th>V( \beta )</th>
<th>Site 1</th>
<th>Site 2</th>
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<td></td>
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<td>22-3</td>
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<td>8-6</td>
<td>14-1</td>
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<td></td>
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<td>12-8</td>
<td>5-5*</td>
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<tr>
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<td>V( \beta )22</td>
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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 \( \kappa \)B at different sites are highlighted with an asterisk.

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**Table II**

<table>
<thead>
<tr>
<th>Patient</th>
<th>V( \beta ) (of total ( \kappa )B TcR+)</th>
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<tr>
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<td>V( \beta )19</td>
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<td>10-0*</td>
</tr>
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<td>V( \beta )22</td>
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<td>16-6</td>
</tr>
<tr>
<td>3</td>
<td>V( \beta )12</td>
<td>8-1</td>
<td>10-1</td>
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<td>8-3</td>
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</tr>
<tr>
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<td>V( \beta )8</td>
<td>22-0</td>
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<td>V( \beta )19</td>
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<td>24-9*</td>
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<td>1-9</td>
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<td>V( \beta )22</td>
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<td>37</td>
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<tr>
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<tr>
<td></td>
<td>V( \beta )22</td>
<td>10-0</td>
<td>7-5</td>
</tr>
</tbody>
</table>

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 \( \kappa \)B at different sites are highlighted with an asterisk.

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*Figures 1 and 2. The frequencies of intraepithelial lymphocytes and lamina propria lymphocytes.*

*Figure 3. The frequencies of TcR+ lymphocytes in the lamina propria of normal human colon from six patients.*
sample (upper or lower bowel). There were too few positive cells in the epithelium staining with each Vβ region antibody to permit analysis of epithelial lymphocytes, however, sufficient cells were present in the lamina propria. Most of the anti-Vβ 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 Vβ usage in some samples. Vβ8 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 Vβ5-3.

**Discussion**

Previous studies have shown oligoclonality of αβ+ 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

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*Figure 2: Vβ expression by the αβ TcR+ cells in the lamina propria of human fetal small intestine of different gestational ages.*
colon. 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 Vβ along large pieces of intestine, and that in the two cases we studied where tissue was obtained from small bowel and colon, different Vβs 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 Vβ at one site, but which were not over-represented at second adjacent site between 5 and 100 cm distant. Thus for some Vβs, 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 Vβ19 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 Vβ 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 Vβ19 gene segment was used by T cells that had rearranged different D and J regions gene segments, then it would not have been apparent. Without sequence data this cannot be disproved, however, to evoke such a large expansion of polyclonal Vβ19+ 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. That T cell can make an alloresponse to CD1d on epithelial cells has been shown, however, gut epithelial cells also express abundant class I (A and B loci) and class II MHC products 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 and H-2 restricted IEL responses to reovirus have also been shown in mice. In addition, lamina propria T cells are also oligoclonal, and these are mostly CD4+ T. 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 Vβs 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. 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 population of T cells populating the gut is the restriction that places in mucosal defence to pathogens. There are numerous examples in published works where mucosal antigen specific T cell responses have been described. 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 cells and therefore an analysis of Vβ 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 Vβ 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 Vβs 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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3 Blumberg RS, Yockey CE, Gross GG, Ebert EC, Balk SP. Human intestinal intraepithelial lymphocytes are derived from a limited number of T cell clones that utilise multiple Vβ T cell receptor genes. J Immunol 1993; 150: 5144–53.
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