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Meenan, J.K.P.; Spaans, J.; Grool, T.A.; Pals, S.T.; Tytgat, G.N.J.; van Deventer, S.J.H.

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# Altered expression of $\alpha_4\beta_7$ , a gut homing integrin, by circulating and mucosal T cells in colonic mucosal inflammation

J Meenan, J Spaans, T A Grool, S T Pals, G N J Tytgat, S J H van Deventer

## Abstract

**Background and Aims**—Expression of  $\alpha_4\beta_7$  on memory T lymphocytes identifies a cell population that preferentially migrates to the gut. Detection of  $\alpha_4\beta_7$  on circulating lymphocytes may permit the identification of specific subsets trafficking between the circulation and the gut in inflammatory bowel diseases.

**Patients**—Samples and clinical details were taken from patients with Crohn's disease (CD), ulcerative colitis (UC), diverticulitis/infectious colitis, and healthy controls.

**Methods**—Peripheral blood and lamina propria mononuclear cells were isolated. Cells were labelled with CD3, CD4, CD25, CD45RO or  $\alpha_4\beta_7$ .

**Results**—Median levels of circulating total memory T cells (CD4<sup>+</sup>CD45RO<sup>+</sup>) were increased in CD ( $p < 0.01$ ) and UC ( $p < 0.05$ ). However, the proportion of systemic gut homing T cells (CD4<sup>+</sup>CD45RO<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup>) was decreased in CD ( $p < 0.05$ ), UC ( $p < 0.002$ ), and inflammatory controls ( $p < 0.05$ ). Levels of activated gut homing T cells (CD4<sup>+</sup>CD25<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup>) were increased in CD ( $p < 0.01$ ) and UC ( $p < 0.05$ ). For both CD4<sup>+</sup>CD45RO<sup>+</sup> and CD4<sup>+</sup>CD25<sup>+</sup> cells, the proportion of lymphocytes coexpressing  $\alpha_4\beta_7$  was decreased compared with controls. In small and large intestine lamina propria, expression of  $\alpha_4\beta_7$  on CD3<sup>+</sup> cells was extensive, although it was decreased in CD ( $p < 0.03$ ), UC ( $p < 0.05$ ), and inflammatory controls ( $p < 0.05$ ).

**Conclusions**—Circulating and mucosal gut homing lymphocyte populations are changed in patients with colonic inflammation. This may arise due to a dilution effect from recruited naive T cells, or from integrin down regulation. Changes in general CD4<sup>+</sup> lymphocyte populations mask more subtle variations in those cells with gut homing potential.

(*Gut* 1997; 40: 241-246)

Keywords: inflammatory bowel disease, lymphocyte, integrin, gut homing.

Mucosal recruitment of leucocytes during an inflammatory response involves the expression and activation of several families of molecules. The local accumulation of soluble mediators and the sequential engagement of adhesion molecules (selectins, integrins, and im-

munoglobulin gene superfamily), as described by the 'area-code' paradigm, permits the differential recruitment of acute and chronic inflammatory cells to sites of injury.<sup>1</sup>

Under non-inflammatory conditions, lymphocytes continuously traffic between the circulation and gut associated lymphoid tissue (GALT), permitting the exposure of naive cells to gut antigens and the accumulation of those with antigen specificity. Consequently, immunocompetent cells resident in the mucosa coexist with transiting specialised lymphocytes. This recirculation is central to normal immune surveillance and is mediated in an analogous fashion to the recruitment of leucocytes during inflammation. Specificity, in respect of organ, tissue, and cell type, is lent to this homing process by the engagement of distinct integrins and addressins at the lymphocyte and endothelial surfaces respectively.<sup>1</sup>

The GALT, comprising Peyer's patches, lamina propria, and intraepithelial lymphocytes, is populated by lymphocytes that migrate from the circulation. The  $\beta$  integrin family would seem to play a significant part in trafficking and localisation to the gut:  $\alpha_E\beta_7$  (intraepithelial lymphocytes), and  $\alpha_4\beta_7$  (GALT).<sup>2</sup> The importance of such a homing mechanism is illustrated by the inability of  $\beta_7$  integrin gene knockout mice to form Peyer's patches and decreased numbers of lamina propria CD4<sup>+</sup> and antibody secreting cells.<sup>3</sup> These mice develop a spontaneous form of enteritis.  $\alpha_4\beta_7$  is widely expressed on T and B lymphocytes.<sup>4</sup> Expression of this molecule on memory T cells, however, delineates a specific cell population that preferentially migrates to the gut through interaction with the mucosal addressing cell adhesion molecule-1 (MAdCAM-1) on high endothelial venules; fibronectin and VCAM-1 also act as ligands, though to a lesser extent.<sup>5,6</sup> Further specificity is given to this process by the expression of MAdCAM-1 and a relative paucity of VCAM-1, which favours  $\beta_1$  integrin binding, at the gut endothelium.<sup>7</sup> It is uncertain whether expression of  $\alpha_4\beta_7$  influences lymphocytic function within the gut mucosa.

The close association between mucosal and circulating lymphocyte populations may be of particular significance in diseases marked by chronic mucosal inflammation with persistent adhesion molecule up regulation, leucocyte recruitment, and antigen stimulation. Specifically, variation both in the proportion of circulating gut homing cells and their degree of activation may occur. Altered expression of

## Departments of Experimental Internal Medicine

J Meenan  
J Spaans  
S J H van Deventer

## Pathology

S T Pals

## and Gastroenterology and Hepatology

G N J Tytgat

## Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands

Correspondence to:  
Dr J Meenan,  
Department of  
Gastroenterology,  
Rayne Institute,  
St Thomas's Hospital,  
Lambeth Palace Road,  
London.

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$\alpha_4\beta_7$  has already been described under pathological conditions, in gut related non-Hodgkin's lymphoma.<sup>8</sup>

The overlapping clinical, radiological, and histological features of the idiopathic inflammatory bowel diseases (IBD) belie distinct immunological differences between Crohn's disease (CD) and ulcerative colitis (UC), in particular, with respect to the role of mucosal lymphocytes. The importance of circulating CD4<sup>+</sup> T cells in the development of mucosal inflammation is underscored by lymphocyte transfer studies and the ameliorating effect of HIV disease progression on co-existing IBD.<sup>9-10</sup> Data on circulating leucocyte phenotypes in IBD are conflicting, possibly a reflection of the inability to define subsets with gut specificity.<sup>11</sup> The aim of this study was to define in patients with colonic mucosal inflammation, the prevalence and activation level of circulating lymphocytes with gut homing potential. Additionally, we wished to investigate the expression of  $\alpha_4\beta_7$  on T cells that are resident in the colonic mucosa.

### Methods

Blood for flow cytometry, full blood count and differential count, erythrocyte sedimentation rate, and serum albumin values was obtained from patients with a diagnosis of CD (n=46) and UC (n=23), as defined by endoscopy, histopathology or radiology; diverticulitis and infectious colitis (n=5) and from 25 healthy, age and sex matched volunteers with no history of IBD. Patient details including current disease activity and medication were recorded. The clinical activity of the patients' disease was scored using the criteria of de Dombal (CD) and of Truelove and Witts (UC).<sup>12-13</sup>

Endoscopic colonic biopsy specimens were taken from patients undergoing colonoscopy for the investigation of CD (n=6) or UC (n=13). Patients (n=17) undergoing endoscopy for the investigation of altered bowel habit, or rectal blood loss, with a normal mucosal appearance and histology, served as a non-inflammatory disease control group. Patients with infectious colitis (*Campylobacter jejuni* (1) and *Salmonella* (1)) and diverticulitis (n=3) served as an inflammatory disease control group. Areas of mucosal inflammation were scored by the criteria of Baron.<sup>14</sup>

### Peripheral blood leucocyte isolation

Peripheral blood mononuclear cells (PBMNC) were isolated from heparinised blood diluted 1:1 with Hank's buffered salt solution (HBSS; Life Technologies, Paisley, Scotland), layered over Ficoll Hypaque (Pharmacia, Uppsala, Sweden), and centrifuged at 1500 g for 15 minutes at 22°C. Cells at the HBSS/Ficoll interface were removed by pipette, depleted of platelets by repeated low speed centrifugation, and resuspended in FACS buffer (phosphate buffered saline, sodium azide 0.01% and bovine serum albumin 0.1% (Sigma, St Louis, USA)) to a concentration of 5×10<sup>6</sup> cells/ml.

Cell viability was consistently >98% as assessed visually by trypan blue exclusion.

### Biopsy mononuclear cell isolation

Four endoscopic biopsy specimens were taken into RPMI 1640 supplemented with fetal calf serum (FCS) 10% and gentamicin (Life Technologies). Biopsy specimens were teased apart, added to a 14 ml tube (Falcon, Cambridge, USA) containing RPMI 1640/FCS 10%/gentamicin with collagenase type IV 50 IU/ml (Sigma) and placed on a mixing table (Multi-purpose rotator, Scientific Industries, New York, USA) at 37°C. After one hour, the supernatant was pelleted, washed and cells resuspended in FACS buffer to a concentration of 2×10<sup>6</sup> cells/ml. Cell viability was >90%.

### Cell labelling

Cell suspensions were double, or, triple labelled with previously determined optimal concentrations of a panel of monoclonal antibodies: CD3<sub>FITC</sub> (Becton-Dickinson (BD)); CD4<sub>biotin</sub> (BD); CD25<sub>PE</sub> (Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam, the Netherlands); CD45RO<sub>FITC</sub> (CLB); Act-1 (a kind gift of Dr AI Lazarovits, University Hospital, Robarts Research Institute, University of Western Ontario, London, Ontario, Canada<sup>6</sup>), directed against human  $\alpha_4\beta_7$ ; rabbit antimouse-PE (Dako, Glostrup, Denmark); streptavidin-Cy5 (Dako). IgG<sub>1</sub> and IgG<sub>2a</sub> (BD) with appropriate fluorescent conjugates served as isotype control antibodies. Cells were incubated with antibodies at 4°C for 30 minutes and washed three times in FACS buffer between each step. Non-specific binding of antibodies was minimised by a 10 minute incubation of cell suspensions with pooled human serum 10% (CLB) in FACS buffer before the addition of the first antibody. Furthermore, mouse serum 5% (CLB) and human serum 10% was added to monoclonal and conjugate antibody solutions respectively.

### Flow cytometry

Flow cytometry was performed with a FACScan (BD) using Lysys II software (BD). Lymphocyte populations were gated using Forward scatter/Side scatter and 2-3×10<sup>4</sup> events saved. The lymphocyte gate in biopsy suspensions was drawn using CD3<sup>+</sup> as a marker.

### Statistics

Results were entered in SPSS for Windows 6.0 (SPSS Inc, USA). Data are expressed as median (range). The Mann-Whitney U test was used to compare groups. Correlations are presented as the Pearson correlation coefficient. The influence of disease activity was tested using one way analysis of variance followed by a modified Bonferroni test for significance. Alpha was set at 0.05.

## Results

Disease activity was similar between patients with CD (3 (1–4)) and UC (1 (1–4)), however, there was a trend towards greater clinical activity in the CD group. Overall proportions of circulating memory T helper cells ( $CD4^+CD45RO^+$ ) were increased (Fig 1) in both CD (50% (12–84),  $p<0.01$ ) and to a lesser extent in UC (46% (21–72),  $p<0.05$ ) compared with non-inflammatory controls (36% (16–71)). This population of cells was slightly increased in the inflammatory disease control group (42% (25–80);  $p>0.05$ ).

Expression of  $\alpha_4\beta_7$  on systemic  $CD4^+$  T cells was consistent across CD (47% (27–72)), UC (50% (32–66)) and inflammatory (49% (30–60)) or non-inflammatory controls (55% (31–65)) groups. However, the proportion of T helper cells with gut homing potential ( $CD4^+CD45RO^+\alpha_4\beta_7^+$ ) was decreased (Fig 2) in CD (27% (11–70),  $p<0.05$ ), UC (18% (6–32),  $p<0.002$ ) and inflammatory controls (23% (8–30),  $p<0.05$ ) in comparison with non-inflammatory control values (37% (16–87)). The difference between the two IBD

groups was not significant ( $p=0.08$ ).  $CD4^+CD45RO^+$  cells correlated closely with  $CD4^+CD45RO^+\alpha_4\beta_7^+$  cells ( $r=0.45$ ,  $p<0.01$ ). In CD and UC, in addition to an overall decrease in gut homing lymphocytes, the proportion of circulating  $CD4^+CD45RO^+$  cells expressing  $\alpha_4\beta_7$  was reduced (both  $p<0.01$ ).

Percentages of circulating  $CD4^+\alpha_4\beta_7^+$  expressing the IL2 $\alpha$  receptor (CD25), a marker of activation, were increased from 4.1% (2.5–6.5) in controls to 7.0% (1.6–18.4,  $p<0.01$ ) in CD and 5.2% (2.2–9.1,  $p<0.05$ ) in UC patients (Fig 3). The difference between CD and UC groups was significant ( $p<0.05$ ). As with CD45RO, the proportion of  $CD4^+CD25^+$  cells (data not shown) with coexpression of  $\alpha_4\beta_7$  (ratio of triple to double stained cells), correlated closely ( $r=0.76$ ,  $p<0.001$ ) and once again, was reduced in CD and UC.

$CD4^+$  cell expression of CD45RO ( $p<0.04$ ), CD45RO/ $\alpha_4\beta_7$  ( $p<0.04$ ), CD25 ( $p<0.01$ ), and CD25/ $\alpha_4\beta_7$  ( $p<0.05$ ) reflected clinical disease activity in the IBD group. CD25 expression inversely correlated with serum albumin concentrations ( $-0.52$ ,  $p<0.02$ ). Erythrocyte sedimentation rate correlated directly with platelet counts ( $r=0.41$ ,  $p<0.03$ ) and inversely with serum albumin ( $r=0.69$ ,  $p<0.002$ ). No difference in results was noted between patients using mesalamine, immunosuppressive treatment (corticosteroids, corticosteroids and azathioprine) or those taking no medication.

The proportion of colonic mucosal T cells ( $CD3^+$ ) expressing  $\alpha_4\beta_7$  (Fig 4) was reduced in CD (47% (34–76),  $p<0.03$ ), UC (57% (38–88),  $p<0.05$ ) and inflammatory controls (49% (29–80),  $p<0.05$ ) as compared with non-inflammatory controls (72% (48–89)). The degree of mucosal inflammation in IBD patients observed at endoscopy, was not associated with the proportion of  $CD3^+\alpha_4\beta_7^+$  cells found.

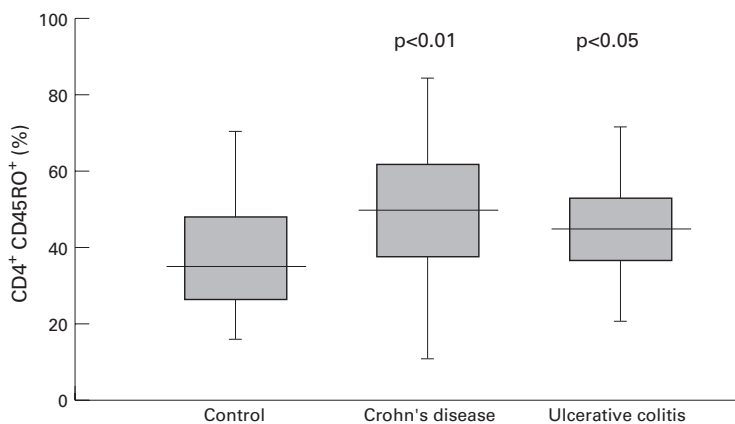


Figure 1: Proportion of circulating  $CD4^+$  lymphocytes displaying memory ( $CD45RO^+$ ) phenotype (control,  $n=25$ ; Crohn's disease,  $n=46$ ; ulcerative colitis,  $n=23$ ). Range, median, and 25th–75th centiles are represented by error bars, heavy line, and shaded box respectively.

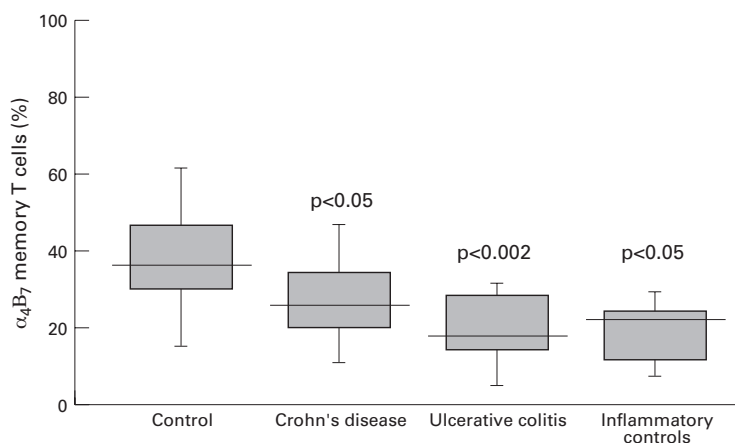


Figure 2: Decreased proportion of  $CD4^+$  T lymphocytes displaying markers of gut tropism ( $CD4^+CD45RO^+\alpha_4\beta_7^+$ ) in patients with inflammatory bowel disease (Crohn's disease,  $n=30$ ; ulcerative colitis,  $n=17$ ) and inflammatory controls,  $n=6$ . Non-inflammatory control (Control),  $n=25$ . Range, median, and 25th–75th centiles are represented by error bars, heavy line, and shaded box respectively.

## Discussion

Dysregulation of soluble (IL2, IL10) and cellular (T lymphocyte subsets) elements of the immune system, in the presence of a normal gut flora, leads to the development of chronic gut inflammation.<sup>9 15 16</sup> Whether such inflammation truly mimics the conditions giving rise to CD and UC, or merely reflects the limited ability of the gut to respond to injury is unclear. Studies detailing the degree of lamina propria lymphocyte activation, however, suggest that T cells may play a pivotal part in both the initiation and propagation of mucosal inflammation in murine models and in IBD, particularly CD.<sup>17 18</sup> Accumulating evidence suggests that  $CD4^+$  lymphocytes may be pivotal on account of their defined ability to recirculate to the gut; evidence for  $CD8^+$  and B cells possessing a similar capability being less certain.<sup>19</sup> Consequently, we have focused on variations in  $CD4^+$  phenotype in colonic mucosal inflammation.

In line with previous studies, we have shown a rise in circulating  $CD4^+$  cells of memory

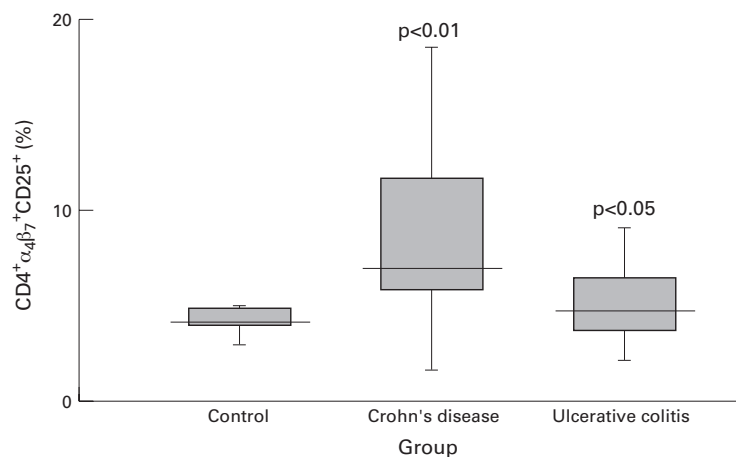


Figure 3: Increased proportions of circulating activated (CD25<sup>+</sup>) CD4<sup>+</sup> lymphocytes coexpressing  $\alpha_4\beta_7$  in inflammatory bowel disease. Control, n=15; Crohn's disease, n=30; ulcerative colitis, n=17. Range, median, and 25th–75th centiles are represented by error bars, heavy line, and shaded box respectively.

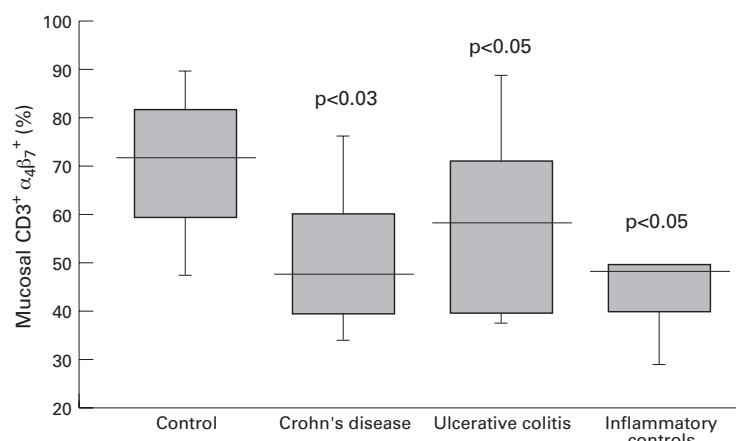


Figure 4:  $\alpha_4\beta_7$  expression on large bowel lamina propria CD3<sup>+</sup> cells is extensive, though reduced in patients with inflammatory disease. Non-inflammatory control, n=17; Crohn's disease, n=6; ulcerative colitis, n=13; inflammatory control, n=6. Range, median, and 25th–75th centiles are represented by error bars, heavy line, and shaded box respectively.

phenotype in patients with IBD.<sup>20</sup> This finding mirrors the chronic nature of the mucosal inflammatory response in IBD and similar findings have been reported for gluten sensitive enteropathy.<sup>21</sup> In contradistinction, the acute inflammation associated with appendicitis is associated with a fall in the proportion of systemic CD4<sup>+</sup>CD45RO<sup>+</sup> cells.<sup>22</sup> The increased prevalence of these cells in IBD indicate an ongoing process of differentiation from naive to memory phenotypes occurring in the mucosal perivascular area,<sup>23</sup> or, accepting the possibility of bidirectional CD45 isoform exchange,<sup>24</sup> persistent antigen rechallenge of circulating cells.

Maintenance of the integrity of distinct lymphoid compartments, for example GALT, is dependent on selective recirculation and homing of lymphocytes. Expression of the integrin  $\alpha_4\beta_7$ , defines a specific T cell subset with gut tropism.<sup>5</sup> However, this specificity requires the co-expression of other surface membrane molecules, functioning in a manner analogous to a postcode.<sup>1</sup>  $\alpha_4\beta_7$  is widely expressed on circulating lymphoid cells.<sup>4</sup> The equal expression of  $\alpha_4\beta_7$  on CD4<sup>+</sup> cells across

all the groups under study, emphasises the non-specific nature of this integrin. We have found that the proportion of circulating cells displaying the markers of gut tropism (CD4<sup>+</sup>CD45RO<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup>) is lower in patients with colonic mucosal inflammation. The previously defined increase in lamina propria memory cells in IBD implies that sequestration of this cell population may occur.<sup>25–26</sup> The disparity between CD4<sup>+</sup>CD45RO<sup>+</sup> and CD4<sup>+</sup>CD45RO<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup> populations within each inflammatory group suggests the possibility of integrin down regulation, either following recruitment to the mucosa, or subsequent to their return to the circulation. It seems that the altered expression of  $\alpha_4\beta_7$  on circulating lymphocytes does not represent a disease specific process, but reflects the presence of mucosal inflammation in general. This finding is in keeping with a previous report detailing changes in B cell populations of patients with infectious colitis.<sup>27</sup>

The function of  $\alpha_4\beta_7$  on cells other than CD4<sup>+</sup>CD45RO<sup>+</sup> is uncertain, although a gut homing role for circulating macrophages has been ascribed.<sup>28</sup> Whether expression of this molecule on naive T cells identifies a subset of lymphocytes that is exposed to antigen preferentially in the mucosal perivascular space,<sup>23</sup> rather than in regional lymphoid tissue remains speculative.

The presence of circulating activated lymphocytes, as defined by expression of the IL2 receptor  $\alpha$  chain (CD25), in IBD is well recognised and it is probable that activation of T cells in IBD occurs within the mucosa.<sup>29</sup> Consequently, the presence of these cells in the intravascular compartment may represent recirculation of lymphocytes from the mucosa.

However, the reduced proportion of CD4<sup>+</sup>CD25<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup> cells in the IBD groups, although probably due to mucosal recruitment or integrin down regulation, raises the possibility of cellular activation occurring within the circulation.<sup>30</sup> One mechanism might be through the action of circulating IL2, levels of which are known to mirror disease activity in IBD.<sup>31</sup> Should such activation occur, integrin conformational change may allow for further lymphocyte migration to the gut mucosa.<sup>32</sup>

We have identified significant numbers of large bowel lamina propria T cells to express  $\alpha_4\beta_7$ , though the proportions are reduced in CD, UC, and inflammatory controls. The degree of integrin expression by colon T cells in our study (29–89%) is much greater than that previously reported (2–5%) but, is consistent with the level of staining that we have seen using immunohistochemistry (unpublished data) and the levels that might be expected.<sup>33</sup> The difference in observed expression may be explained by the lack of uniformity of action between the different collagenase solutions used in these studies.

The reduced numbers of mucosal CD3<sup>+</sup> $\alpha_4\beta_7$ <sup>+</sup> cells in IBD may result from non-specific recruitment of lymphocytes, or from integrin down regulation. Under inflammatory conditions, cytokine induced up regulation of

vascular adhesion molecules, as seen in IBD,<sup>34</sup> might make the endothelium more permissive to lymphocyte subsets that do not home normally to the gut. Irani has demonstrated that blocking of lymphocyte homing molecules in vivo fails to influence early inflammation in rodent viral encephalitis.<sup>35</sup> In mucosal inflammation, VCAM-1, a secondary ligand for  $\alpha_4\beta_7$ , is an unlikely candidate for this role as gut endothelial expression of VCAM-1 is low or non-existent and does not alter in active IBD.<sup>36–38</sup> The extent of expression of the human ligand corresponding to the murine molecule MAdCAM-1, under normal or inflammatory conditions, is unknown.

Altered regulation of  $\alpha_4\beta_7$  may be a more plausible explanation for our results; T cell activation in vitro is associated with decreased expression of both  $\alpha_4\beta_7$  and  $\alpha_4\beta_7$ .<sup>39</sup> This mechanism may influence the propagation of mucosal inflammation as the integrins demonstrate two key functions: regulation of signals from within the cell and modulation of cellular behaviour by the extracellular matrix. A functional consequence of prolonged CD4<sup>+</sup> cell activation is the release of proinflammatory cytokines including IFN $\gamma$  and TNF $\alpha$ .<sup>40</sup> It can be postulated that lymphocyte interaction with molecules such as fibronectin, a ligand of  $\alpha_4\beta_7$ ,<sup>41</sup> or indeed shedding of this integrin influences cell function. Teague has demonstrated  $\alpha_4\beta_7$  to be a costimulatory molecule for anti-CD3 induced IL2 production, expression of CD25 and DNA synthesis in peripheral blood T cells.<sup>42</sup>

In conclusion, circulating numbers of gut homing lymphocytes are decreased in diseases associated with colonic mucosal inflammation, although the proportion of activated cells is increased. In these conditions, the mucosa shows a distinct decrease in the proportion of cells expressing  $\alpha_4\beta_7$ . This may arise due to a dilution effect from recruited naive T cells, or from integrin down regulation. These data suggest that the altered lymphocyte subsets previously reported in IBD, mask variations specific to gut homing populations.

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