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Skin- and gut-homing molecules on human circulating $\gamma\delta$ T cells and their dysregulation in inflammatory bowel disease

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Introduction

$\gamma\delta$ T cells are innate lymphocytes that constitute only a minor T cell population in human peripheral blood, but are enriched at epithelial sites such as the skin, lung and gut [1]. Epithelial tissue is constantly exposed to pathogenic microorganisms, toxic substances and inflammatory molecules; one of the physiological functions of $\gamma\delta$ T cells may be to control excessive inflammatory responses in epithelial tissues. Indeed, murine dendritic epidermal T cells expressing a $\gamma\delta$ T cell receptor (TCR) are involved in the regulation of epidermal integrity and promote wound repair of the skin [2], whereas intestinal intraepithelial $\gamma\delta$ T ($\gamma\delta$ -IEL) cells regulate intestinal epithelial homeostasis [3,4]. $\gamma\delta$ T cells can down-regulate $\alpha\beta$ T cell responses which could otherwise result in severe immunopathology [5]. $\gamma\delta$ T cells are also important in immune surveillance of the epithelium by providing a first line of defence against infectious invading pathogens, and regulating the bridge between innate and adaptive immunity [5,6].

Summary

Changes in phenotype and function of $\gamma\delta$ T cells have been reported in inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC). Dysregulation of lymphocyte migration plays a key role in IBD pathogenesis; however, data on migratory properties of $\gamma\delta$ T cells are scarce. Human circulating $\gamma\delta$ T cells from healthy controls ($n = 27$), patients with active CD ($n = 15$), active UC ($n = 14$) or cutaneous manifestations of IBD ($n = 2$) were characterized by flow cytometry. Circulating $\gamma\delta$ T cells in healthy controls were CD3^{hi} and expressed CD45RO. They expressed gut-homing molecule $\beta 7$ but not gut-homing molecule corresponding chemokine receptors (CCR)9, or skin-homing molecules cutaneous lymphocyte-associated antigen (CLA) and CCR4, despite conventional T cells containing populations expressing these molecules. CCR9 expression was increased on $\gamma\delta$ T cells in CD and UC, while skin-homing CLA was expressed aberrantly on $\gamma\delta$ T cells in patients with cutaneous manifestations of IBD. Lower levels of CD3 expression were found on $\gamma\delta$ T cells in CD but not in UC, and a lower proportion of $\gamma\delta$ T cells expressed CD45RO in CD and UC. Enhanced expression of gut-homing molecules on circulating $\gamma\delta$ T cells in IBD and skin-homing molecules in cutaneous manifestations of IBD may be of clinical relevance.

Keywords: cell migration, cell trafficking, IBD, $\gamma\delta$ T cells

Ulcerative colitis (UC) and Crohn's disease (CD), collectively termed inflammatory bowel disease (IBD), result from a dysregulated response of the mucosal immune system to components of the luminal microbiota, and breakdown of immune tolerance in individuals who are genetically predisposed to the disease [7–9]. Many pathways are involved in the induction of chronic inflammation in the gut mucosa in IBD. A role for $\gamma\delta$ T cells in IBD was suggested originally by increased numbers of $\gamma\delta$ T cells found in inflamed areas of the gut in humans with UC and CD [10–13], but there is also growing evidence that $\gamma\delta$ T cells play a role in co-ordinating innate and acquired immune responses and maintaining the integrity of epithelial tissues [2,4–6,14]. Data from murine studies are conflicting, with some demonstrating a protective role for $\gamma\delta$ T cells in models of colitis [15–18], but others suggesting that expansion and activation of $\gamma\delta$ T cells is essential for the onset and aggravation of colitis [19–23].

Several mechanisms of immune dysregulation contribute to IBD, including disruption of lymphocyte homing and

migration patterns. Effector T cells migrating to intestinal sites express high levels of the gut-homing molecule $\alpha_4\beta_7$ [24], with its ligand human mucosal addressin cell adhesion molecule-1 (MAdCAM-1) being expressed constitutively by post-capillary endothelial cells in the small intestine [25] and colonic lamina propria [26]. Interactions between $\alpha_4\beta_7$ and MAdCAM-1 are key mediators of lymphocyte homing to intestinal sites [27]. In addition, interaction of chemokines such as CCL25 (TECK), with their corresponding chemokine receptors (CCR9), contribute to homing to the small intestine [28]. In animal models of colitis, expression of MAdCAM-1 is up-regulated in the intestinal lamina propria, and anti-MAdCAM-1 or $\alpha_4\beta_7$ treatment results in reduced lymphocyte recruitment to the intestine [29–33]. MAdCAM-1, $\alpha_4\beta_7$ and CCR9 expression are also dysregulated in humans with IBD [34–37].

Distribution patterns of circulating $\gamma\delta$ T cells in peripheral tissues have not been well described, although the major blood subset of $\gamma\delta$ T cells in macaques can enter the gut mucosa readily upon activation [38]. Although the mechanisms of specific recruitment of $\gamma\delta$ T cells to the gut mucosa are unclear, human $\gamma\delta$ T cells express CXCR3 [39], which binds chemokines CXCL9, CXCL10 and CXCL11, produced by local cells in inflammatory lesions. $\gamma\delta$ T cells may be recruited to the gut mucosa by this method under inflammatory intestinal conditions; indeed, CXCR3 expression has been implicated in coeliac disease [40]. Similarly, human circulating $\gamma\delta$ T cells can also express lymphocyte function-associated antigen (LFA)-1 [41], which recognizes intercellular adhesion molecule-1 (ICAM-1) [42], expressed by epithelial cells [43]. ICAM-1 expression is increased under inflammatory conditions [44] and tissue stress [45]. CCR2 expression has been implicated in $\gamma\delta$ T cell trafficking during inflammation [46], although $\gamma\delta$ T cells have yet to be defined for molecules involved in recruitment to the gut mucosa specifically.

IBD is associated with a variety of extra-intestinal manifestations (EIM), with up to a third of IBD patients developing cutaneous manifestations, including erythema nodosum (EN) and pyoderma gangrenosum (PG) [47]. The causes of EIM of IBD are poorly understood, but it has been suggested that compartmentalization of inflammatory processes to different organs (e.g. intestines, skin, liver) may be linked to homing and trafficking of immune cells. For example, CCL25, the ligand for gut-homing receptor CCR9, is expressed on epithelium in both the liver and the small intestine [48]. Skin T cells express E- and P-selectin ligands, including cutaneous lymphocyte-associated antigen (CLA) [49], thought to be involved in tissue-specific localization of cutaneous T cells within the skin [50]. Interaction between chemokine receptors CCR4 and CCR10 and their respective ligands CCL17 and CCL27 have also been implicated in skin-homing [51]; expression of skin-homing molecules may be dysregulated on immune cells in cutaneous manifestations of IBD.

The homing profile of $\gamma\delta$ T cells has yet to be characterized in either healthy controls or IBD patients. We aimed to characterize the homing profile of human, circulating $\gamma\delta$ T cells in healthy controls and to compare this profile to that of patients with active IBD.

Materials and methods

Human blood samples

Peripheral blood (20 ml) was collected into heparinized Vacutainer tubes (BD Biosciences, Oxford, UK) following informed consent (EC numbers 05/Q0405/71 and 08/H0717/24). Blood was obtained from healthy controls ($n = 27$; 12 female and 15 male; mean age 35 years) or from patients with active CD ($n = 15$; eight female and seven male; mean age 43 years) or UC ($n = 14$; six female and eight male; mean age 38 years). Diagnosis for patients with active CD and UC was made using clinical parameters, radiographic studies, endoscopic and histological criteria. Disease activity for UC was assessed using the UC disease activity index (UCDAI); all patients had a UCDAI > 4. Disease activity for CD was assessed using the CD activity index (CAI); all patients had a CAI > 220. All UC patients had pancolitis; CD patients were comprised of a mixture of Crohn's colitis ($n = 4$), small bowel CD only ($n = 5$), or both colonic and small bowel involvement ($n = 6$). Patients were either not receiving therapy or were on minimal treatment: 5-aminosalicylic acid (5-ASA) and/or azathioprine (AZA). Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation over Ficoll-Paque Plus (Amersham Biosciences, Chalfont St Giles, UK).

Antibody labelling

Monoclonal antibodies with the following specificities and conjugations were used: CLA-fluorescein isothiocyanate (FITC) (HECA-452), CD103-FITC (Ber-ACT8), β_7 integrin-phycoerythrin (PE) (FIB504), CD45RO-PE (UCHL1), CD3-peridinin chlorophyll cyanin 5.5 (Per-CPCy5.5) (SK7), CD3-PECy5 (UCHT1), CLA-biotin (HECA-452) and streptavidin-allophycocyanin (APC) were purchased from BD Biosciences (Oxford, UK); CCR9 (either FITC- or APC-conjugated) (112509), CCR7-PE (150503), CCR10-APC (314315), CCR7-PE (150503) and CCR4-APC (205410) were purchased from R&D Systems (Abingdon, UK); appropriate isotype-matched control antibodies were purchased from the same manufacturers. After antibody labelling, cells were fixed with 1% paraformaldehyde in 0.85% saline and stored at 4°C prior to acquisition on the flow cytometer, within 48 h.

Flow cytometry and data analysis

Data were acquired on a fluorescence activated cell sorter (FACS) Calibur cytometer (BD Biosciences) and analysed

using WinList 5.0 software (Verity, Topsham, ME, USA). T cells were identified as CD3⁺ lymphocytes within the PBMC population, and $\gamma\delta$ T cells were identified as CD3⁺ lymphocytes expressing a $\gamma\delta$ T cell receptor (TCR). The proportion of cells positive for a given marker was determined by reference to staining with an isotype-matched control antibody. WinList was used to subtract the normal cumulative histogram for isotype control staining from a similar histogram of staining with the test antibody using the superenhanced D_{max} (SED) normalized subtraction.

Statistical analyses

Statistical analyses were carried out using GraphPad Prism software (GraphPad SoftwareTM, San Diego, CA, USA). Pooled data are expressed as mean values \pm standard error; *t*-tests (paired and non-paired) were applied as stated in the figure legends. *P* < 0.05 was considered significant.

Results

Human circulating $\gamma\delta$ T cells were CD3^{hi}

Human circulating $\gamma\delta$ T cells were identified by flow cytometry as CD3⁺ lymphocytes expressing a $\gamma\delta$ TCR (Fig. 1a). Upon analysis of expression levels of CD3 per cell, measured via the mean fluorescence intensity (MFI) of CD3 staining, circulating $\gamma\delta$ T cells were CD3^{hi} in healthy controls, i.e. expressed a higher mean level of CD3 per cell than the total circulating T cell population (Fig. 1b).

Human circulating $\gamma\delta$ T cells expressed gut-homing marker β 7 but not skin-homing markers

Expression of specific tissue-homing molecules on $\gamma\delta$ T cells in peripheral blood, in particular skin- and gut-homing markers, were investigated. In healthy controls, the majority of circulating $\gamma\delta$ T cells expressed gut-homing molecule β 7 integrin, although expression of CCR9, a chemokine receptor involved in migration towards the small bowel, was minimal. β 7 was expressed by a large fraction of the total T cell population, but a significantly greater proportion of cells expressed this gut-homing marker within the $\gamma\delta$ T cell subset (Fig. 2a). Total T cells in healthy controls lacked CCR9⁺ cells (small bowel-homing) and included few CCR10⁺ cells (skin-associated migration), but incorporated significant numbers of CLA⁺ and CCR4⁺ 'skin-homing' cells. In contrast, circulating $\gamma\delta$ T cells lacked putative 'skin-homing' subsets in healthy control blood (Fig. 2b).

Levels of CD3 were decreased on $\gamma\delta$ T cells in Crohn's disease

Circulating $\gamma\delta$ T cells in healthy controls were CD3^{hi}, but circulating $\gamma\delta$ T cells in patients with active CD expressed a significantly lower level of CD3 (CD3^{med} cells; Fig. 3a,b), while $\gamma\delta$ T cells from patients with active UC expressed comparable levels of CD3 to $\gamma\delta$ T cells from healthy controls (Figs 1b and 3b).

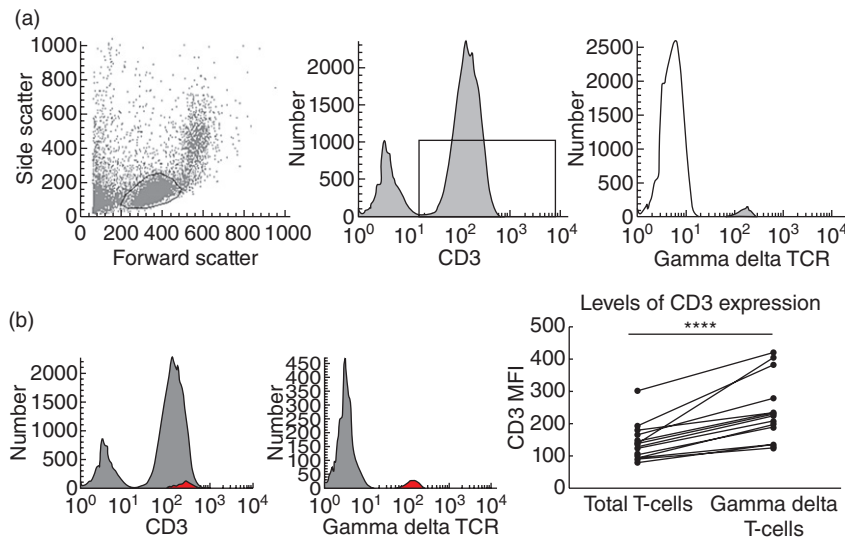


Fig. 1. Identification of human circulating $\gamma\delta$ T cells and levels of CD3 expression. (a) Identification of $\gamma\delta$ T cells from peripheral blood mononuclear cells (PBMC) according to fluorescence activated cell sorter (FACS) plots of forward- and side-scatters, and subsequent CD3 histogram and $\gamma\delta$ T cell receptor (TCR) histogram. This example is from a healthy control. $\gamma\delta$ T cells comprised $5.2 \pm 0.9\%$ ($n = 27$) of the total circulating T cell population. (b) FACS histograms demonstrating $\gamma\delta$ T cells (red) back-gated onto the CD3 peak using WinListTM software, and summary graphs demonstrating mean fluorescence intensity (MFI) of CD3 staining of total circulating T cells compared with $\gamma\delta$ T cells ($n = 27$). Histograms are representative of 27 independent experiments and were compared to isotype-matched controls. Paired *t*-test was applied. A *P*-value < 0.05 was considered statistically significant (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

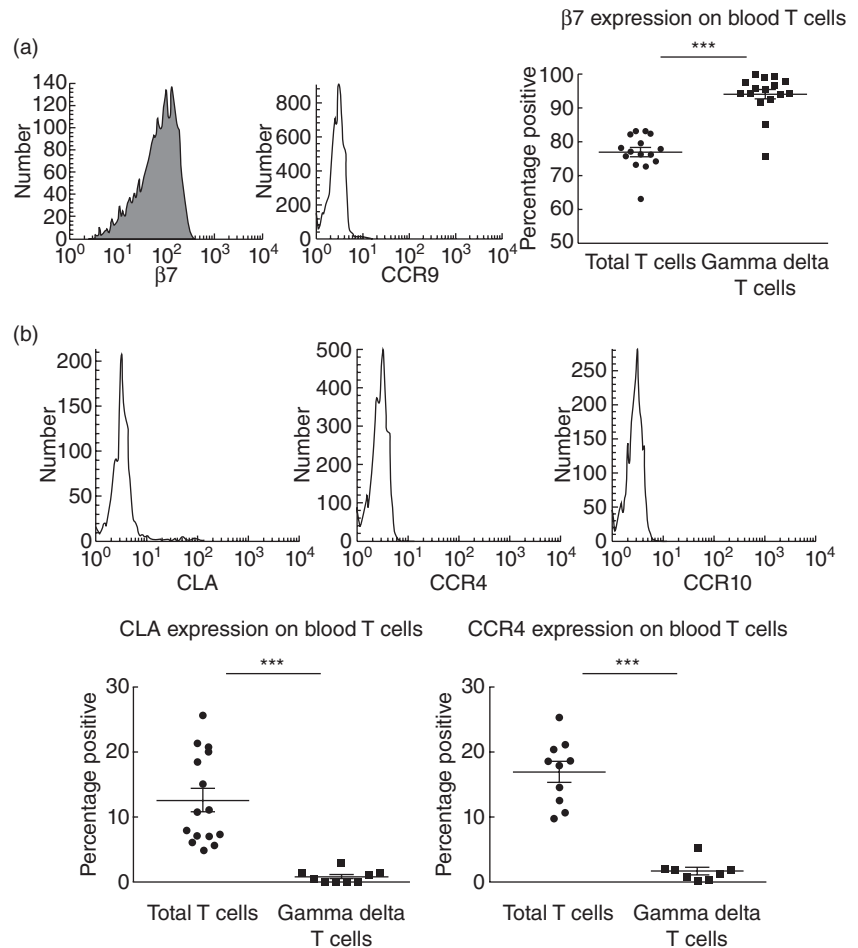


Fig. 2. Expression of gut- and skin-homing markers on $\gamma\delta$ T cells. (a) Fluorescence activated cell sorter (FACS) histograms demonstrating proportions of $\gamma\delta$ T cells expressing $\beta 7$ ($93.9 \pm 1.5\%$, $n = 16$) and corresponding chemokine receptor (CCR)9 ($3.8 \pm 1.4\%$, $n = 11$); comparison against total circulating T cells for expression of $\beta 7$ ($77.0 \pm 1.3\%$, $n = 16$). (b) FACS histograms demonstrating proportions of $\gamma\delta$ T cells expressing cutaneous lymphocyte-associated antigen (CLA) ($0.8 \pm 0.3\%$, $n = 9$), CCR4 ($1.6 \pm 0.6\%$, $n = 8$) and CCR10 ($0.2 \pm 0.1\%$, $n = 5$); comparison against total circulating T cells for expression of CLA ($12.6 \pm 1.8\%$, $n = 15$) and CCR4 ($16.9 \pm 1.6\%$, $n = 10$). Histograms are representative of several independent experiments performed with similar results and were compared to isotype-matched controls; *t*-test was applied. A *P*-value < 0.05 was considered statistically significant (**P* < 0.05 ; ***P* < 0.01 ; ****P* < 0.001). Error bars represent standard error of the mean, horizontal lines represent the mean.

Gut-homing marker CCR9 was increased on $\gamma\delta$ T cells in Crohn's disease and ulcerative colitis

$\gamma\delta$ T cells from healthy controls and patients with active CD, and active UC (no cutaneous manifestations) did not express skin-homing markers CLA, CCR4 or CCR10, and there were no differences in expression of gut-homing marker $\beta 7$ between the two groups (data not shown). However, there was a significant increase in the proportion of $\gamma\delta$ T cells expressing gut-homing marker CCR9 in both CD and UC, compared with $\gamma\delta$ T cells from healthy controls (Fig. 4). This increase in CCR9 expression was not evident in the total circulating T cell population (data not shown).

CD45RO expression was decreased on $\gamma\delta$ T cells in Crohn's disease and ulcerative colitis

The majority of circulating $\gamma\delta$ T cells display a pre-activated phenotype characterized by the expression of 'memory marker' CD45RO, allowing rapid induction of effector functions following detection of tissue stress [52]. We hypothesized that $\gamma\delta$ T cell expression of CD45RO may be altered in IBD due to the chronic inflammation and tissue stress in the

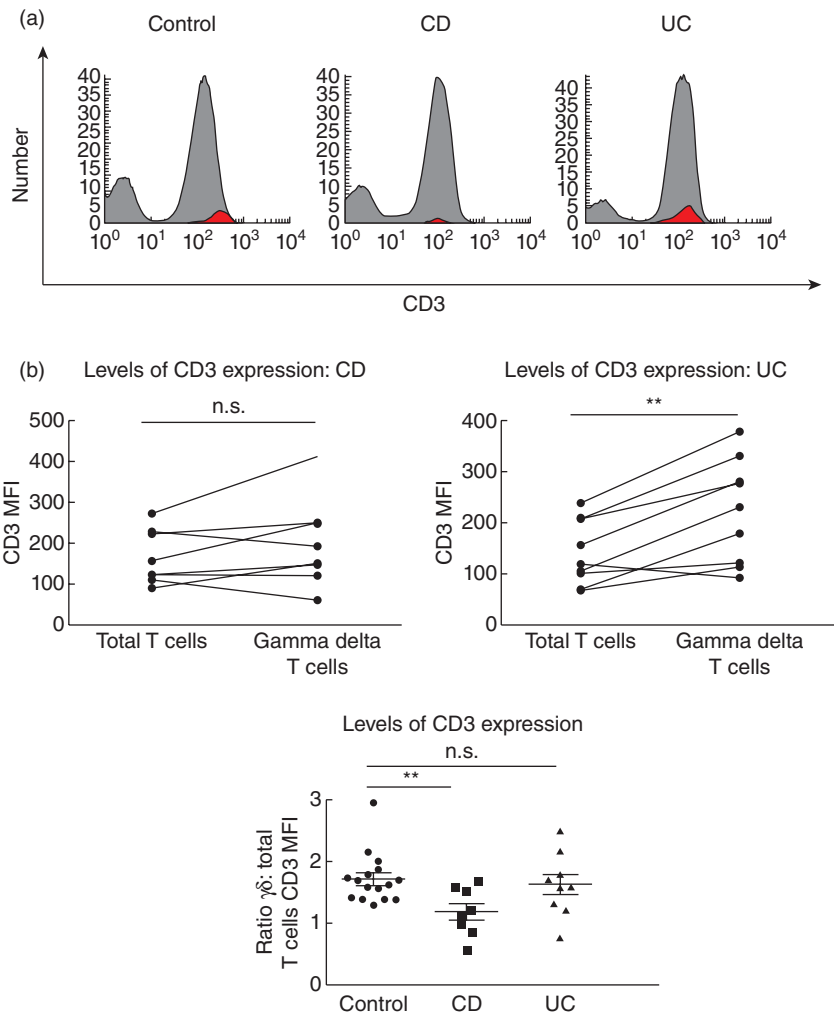
gut mucosa in IBD. Assessment of CD45RO expression on circulating T cells in healthy controls confirmed that the majority of $\gamma\delta$ T cells in blood expressed CD45RO, but CD45RO expression was reduced significantly on $\gamma\delta$ T cells from blood of patients with CD and UC without cutaneous manifestations, compared with healthy controls (Fig. 5). This change was not evident among the total circulating T cell pool (data not shown).

Because $\gamma\delta$ T cells can up-regulate expression of lymph-node homing marker CCR7 upon activation [53], we hypothesized that $\gamma\delta$ T cell expression of CCR7 may also be altered in IBD. CCR7 expression was variable on $\gamma\delta$ T cells from healthy controls (range 0–46.4%), but expression of CCR7 on $\gamma\delta$ T cells was unaltered in CD or UC without cutaneous manifestations (data not shown).

Unique population of skin-homing $\gamma\delta$ T cells in cutaneous manifestations of inflammatory bowel disease

The homing profiles of $\gamma\delta$ T cells in two patients with skin manifestations of IBD were also analysed. EN is one of the more common cutaneous manifestations of IBD, the causes

Fig. 3. Reduced CD3 levels on $\gamma\delta$ T cells in Crohn's disease but not ulcerative colitis. (a) Fluorescence activated cell sorter (FACS) histograms demonstrating levels of CD3 expression via $\gamma\delta$ T cells (red) back-gated onto the CD3 peak using WinList™ software in healthy controls, active Crohn's disease (CD) patients and active ulcerative colitis (UC) patients. Histograms are representative of several independent experiments. (b) Summary graphs demonstrating mean fluorescence intensity (MFI) of CD3 staining of total circulating T cells compared with $\gamma\delta$ T cells in active CD patients ($n = 8$) and active UC patients ($n = 9$), and summary graph demonstrating ratio of $\gamma\delta$ T cell CD3 mean fluorescence intensity (MFI): total T cells CD3 MFI in healthy controls (1.6 ± 0.1 , $n = 16$), active CD (1.2 ± 0.1 , $n = 8$) and active UC (1.6 ± 0.2 , $n = 9$). For comparison of total T cells *versus* $\gamma\delta$ T cells CD3 MFI within the same individuals, paired *t*-tests were applied. For comparison of $\gamma\delta$ T cell : total T cell CD3 MFI ratios between healthy controls, active CD and active UC patients, unpaired *t*-tests were applied. A *P*-value < 0.05 was considered statistically significant (**P* < 0.05 ; ***P* < 0.01 ; ****P* < 0.001 ; *****P* < 0.0001). Error bars represent standard error of the mean, horizontal line represents the mean.



of which are poorly understood, but the occurrence of EN is rare. In light of the extensive data collected in healthy controls and in IBD patients without EN with negligible expression of CLA and low standard errors, we considered two EN samples to be informative.

Although expression of skin-homing marker CLA was negligible on $\gamma\delta$ T cells in both healthy controls and patients with active IBD without EN, aberrant expression of CLA on $\gamma\delta$ T cells was detected in patients with IBD and EN. This unique population of CLA⁺ $\gamma\delta$ T cells in EN did not express the gut-homing marker $\beta 7$ integrin, suggesting that this small population of cells were skin-homing $\gamma\delta$ T cells (Fig. 6a). Expression of other tissue-homing markers on $\gamma\delta$ T cells was unaffected, including gut-homing markers $\beta 7$ and CCR9 and skin-homing markers CCR4 and CCR10 (data not shown).

Finally, an EN patient administered oral corticosteroids (prednisolone, 40 mg daily) underwent rapid resolution of the EN and improvement in general wellbeing (Fig. 6b). Following corticosteroid administration, the CLA⁺ $\beta 7$ ⁻ abnormal skin-homing $\gamma\delta$ T cell population was no longer detected in the circulation; circulating $\gamma\delta$ T cells were CLA⁻ $\beta 7$ ⁺ (Fig. 6a).

Discussion

We demonstrate for the first time that circulating $\gamma\delta$ T cells in the steady state express gut-homing marker $\beta 7$ but do not express molecules enabling migration towards cutaneous sites. $\gamma\delta$ T cells have not been analysed previously for expression of molecules involved in cell trafficking in the context of IBD. In this study, we identified and characterized $\gamma\delta$ T cells exhibiting dysregulation of their homing properties; a subset of altered gut-homing $\gamma\delta$ T cells was detected in both CD and UC, and a subset of skin-homing $\gamma\delta$ T cells was present in EN (a cutaneous manifestation of IBD).

In contrast to rapid recruitment of effector lymphocytes to sites of inflammation [27], the mechanisms controlling steady state traffic of T cells and their maintenance within healthy tissue are not well understood [54–57]. Although human circulating $\gamma\delta$ T cells did not express skin-homing markers or gut-homing marker CCR9 in the steady state, the majority expressed $\beta 7$ gut-homing integrin. $\beta 7$ combines with $\alpha 4$ integrin to allow leucocytes to enter intestinal tissue via interactions with MAdCAM-1 [25,26,58]. While

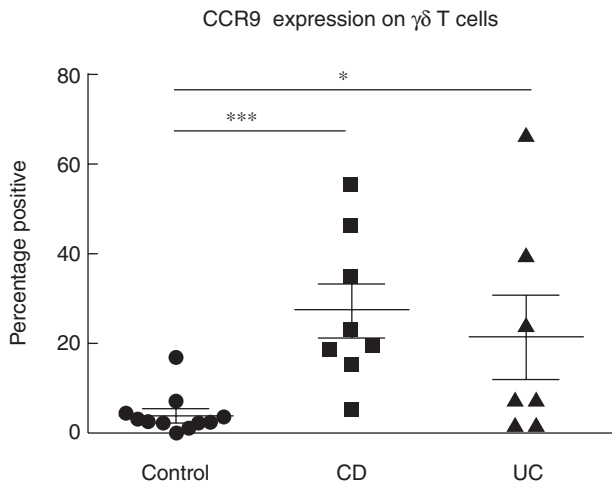


Fig. 4. Increased proportion of corresponding chemokine receptors (CCR9)⁺ $\gamma\delta$ T cells in Crohn's disease (CD) and ulcerative colitis (UC). Proportions of circulating $\gamma\delta$ T cells in healthy controls ($3.8 \pm 1.4\%$, $n = 11$), active CD patients (27.4 ± 5.9 , $n = 8$) and active UC patients ($21.4 \pm 9.2\%$, $n = 7$) expressing gut-homing marker CCR9; *t*-test was applied. A *P*-value < 0.05 was considered statistically significant (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Error bars represent standard error of the mean, horizontal lines represent mean.

circulating T cells can be either gut-homing or skin-homing [51,59], in the current report we demonstrate that circulating $\gamma\delta$ T cells are mainly gut-homing. These data may reflect the suggested role for $\gamma\delta$ T cells in intestinal homeostasis [3,4] and support studies demonstrating that the major blood subset of $\gamma\delta$ T cells in primates (V γ 2 V δ 2 T cells) can enter the gut mucosa readily upon activation [38].

The current report is consistent with previous studies demonstrating changes in the phenotype and function of human $\gamma\delta$ T cells in skin inflammation [60,61] and in IBD [11]. The increased expression of skin-homing molecule CLA on $\gamma\delta$ T cells in EN and enhanced gut-homing receptor CCR9 expression on $\gamma\delta$ T cells in CD and UC without cutaneous manifestations may reflect the recruitment of $\gamma\delta$ T cells from the circulation to the inflamed skin and gut, respectively. Indeed, homing of $\gamma\delta$ T cells to intestinal sites is impaired in CCR9-deficient mice [62] and a role for skin-homing $\gamma\delta$ T cells has been reported in inflammatory skin disease [63].

The alterations in CCR9 expression in IBD without any observed changes in gut-homing β 7 expression may be due to the inherently high expression of β 7 on $\gamma\delta$ T cells in healthy controls. Our previous studies have demonstrated that β 7 is found not only on T cells from the human gut, but also from the blood and skin. The differences in T cell homing profiles between these tissues was due to co-expression with other homing molecules and chemokine receptors [59]. Although CCR9 contributes to leucocyte homing to the small bowel in particular [28], patients exhibiting enhanced CCR9 expression on $\gamma\delta$ T cells in this study

included those with UC and Crohn's colitis, suggesting either a role for CCR9 in the colon or the existence of immunological changes in the small bowel in these diseases, despite the lack of any presenting small bowel symptoms.

The CD³^{hi} phenotype of $\gamma\delta$ T cells in healthy controls may reflect the pre-activated status of circulating $\gamma\delta$ T cells to allow rapid effector functions; indeed, CD45RO expression on $\gamma\delta$ T cells enables rapid effector functions following tissue stress and bacterial infections [52]. The reduction of CD3 levels in CD and reduced CD45RO expression in CD and UC may be a result of chronic activation of the immune system; chronic infections can reduce expression of TCR chains [64–66] likely to affect associated co-receptors such as CD3 [67]. $\gamma\delta$ T cells lost CD45RO expression following viral infection in cattle [68,69].

The population of $\gamma\delta$ T cells that we have studied, circulating $\gamma\delta$ T cells, are comprised mainly of V γ 2 V δ 2 T cells that exist only in primates and humans [70]. V γ 2 V δ 2 T cells are linked typically with anti-microbial immune responses [70,71] and IBD results from a dysregulated immune response to components of the luminal microbiota [7–9]. As V γ 2 V δ 2 cells can enter the gut mucosa readily upon activation [38], it is therefore possible that a dysregulated response of circulating V γ 2 V δ 2 T cells to the microbiota occurs in the gut and the skin in IBD and EN, respectively, or alternatively that $\gamma\delta$ T cells are recruited from the circulation to sites of inflammation to promote homeostasis at these sites.

Conclusions

Further work will be necessary to determine the functional role of $\gamma\delta$ T cells in IBD and cutaneous manifestations of

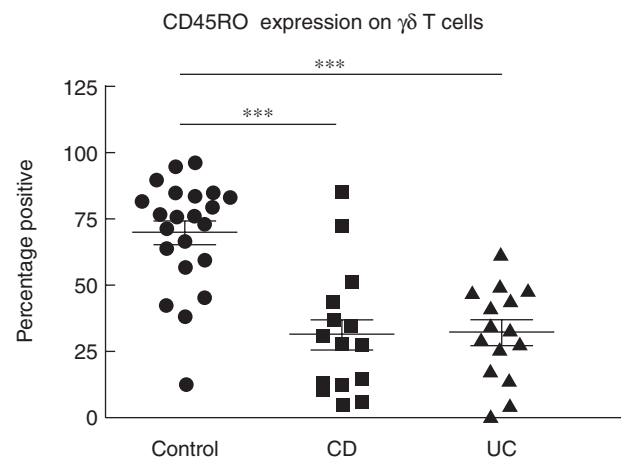


Fig. 5. Reduced proportion of CD45RO⁺ $\gamma\delta$ T cells in Crohn's disease (CD) and ulcerative colitis (UC). Proportions of circulating $\gamma\delta$ T cells in healthy controls ($69.8 \pm 4.4\%$, $n = 22$), active CD patients ($31.4 \pm 6.2\%$, $n = 15$) and active UC patients ($32.1 \pm 4.5\%$, $n = 15$) expressing CD45RO; *t*-test was applied. A *P*-value < 0.05 was considered statistically significant (*** $P < 0.001$). Error bars represent standard error of the mean, horizontal lines represent mean.

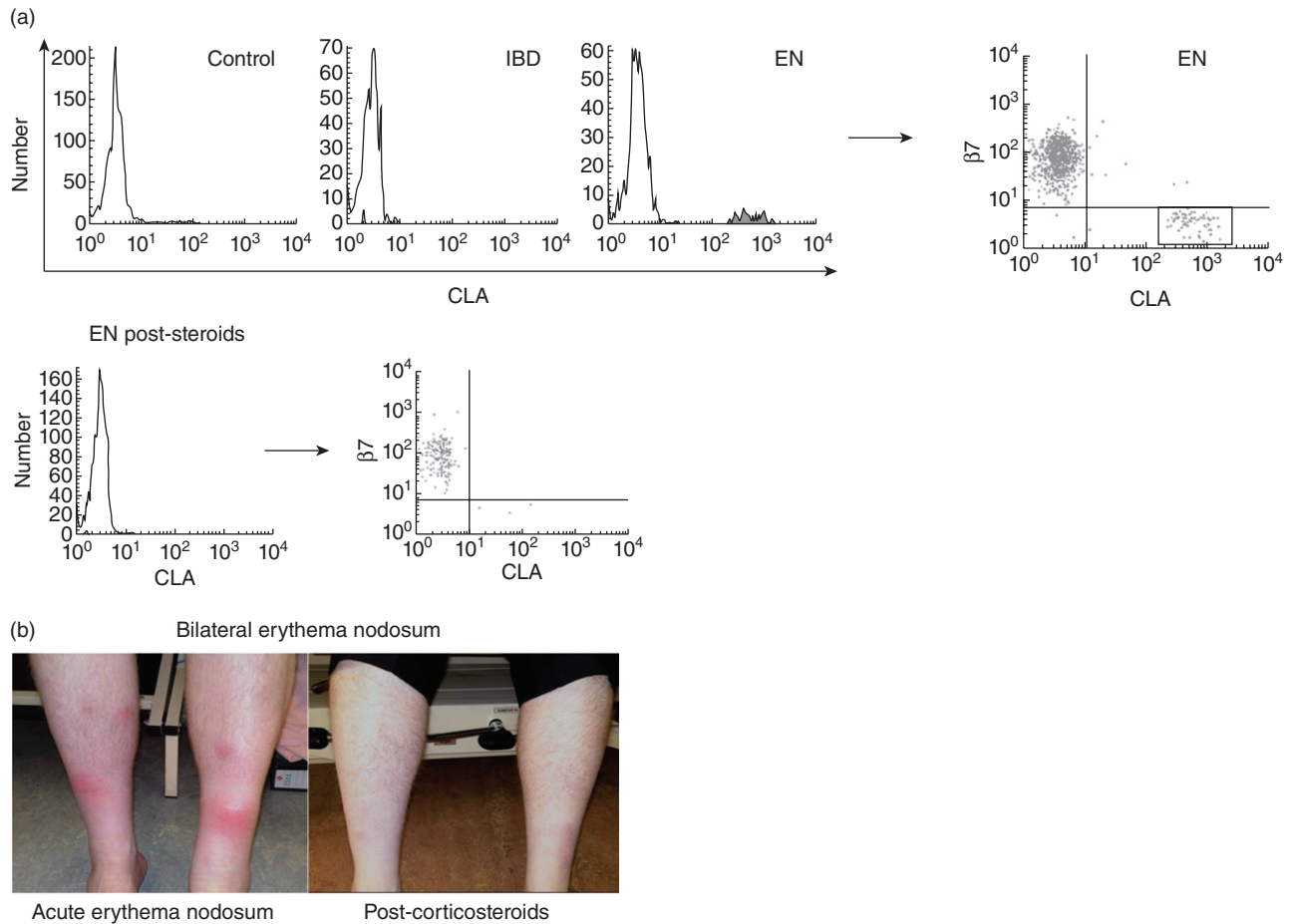


Fig. 6. Aberrant expression of cutaneous lymphocyte-associated antigen (CLA) on $\gamma\delta$ T cells in erythema nodosum. (a) Fluorescence activated cell sorter (FACS) histograms demonstrating proportions of circulating $\gamma\delta$ T cells in healthy controls ($0.8 \pm 0.3\%$, $n = 9$), active inflammatory bowel disease (IBD) ($1.2 \pm 0.4\%$, $n = 15$) and erythema nodosum (EN) with inactive IBD (9.6% and 5.7% , $n = 2$). Bottom row: EN post-steroids (1.3% , $n = 1$; pre-steroids was 9.6%) expressing CLA. Histograms are representative of several independent experiments performed with similar results, and were compared to isotype-matched controls; FACS dot-plot demonstrating proportions of circulating $\gamma\delta$ T cells in EN co-expressing gut-homing marker $\beta 7$ and skin-homing marker CLA, expressing $\beta 7$ only, or expressing CLA only. All plots were compared to isotype-matched controls. (b) Photographs of shins of EN patient pre- and post-corticosteroids.

IBD, but the dysregulation of $\gamma\delta$ T cell homing profiles in these diseases is likely to be clinically relevant. Oral corticosteroids led to rapid resolution of EN, accompanied by removal of the unique skin-homing population of $\gamma\delta$ T cells from the circulation. Targeting tissue-homing pathways provides a more specific approach to IBD therapy, and allows for selective anti-inflammatory treatments. For instance, $\alpha_4\beta_7$ inhibitor MLN-O2 [72] and CCR9 inhibitor Traficet-EN [73] have efficacy in IBD. These data suggest that homing profiles could potentially be used as markers for active inflammation at particular sites in IBD and in cutaneous manifestations of IBD.

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Disclosure

Authors have no disclosures to report.

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