# Expression of CD45RB functionally distinguishes intestinal T lymphocytes in inflammatory bowel disease

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**Abstract: The importance of CD45RB expression on T cells was already shown in mice where $CD45RB^{high}$ expression determines pathogenic potential. In this study, we analyzed the expression of CD45RA, CD45RB, and CD45RO on $CD4^+$ T lymphocytes in the intestinal mucosa and in the circulation of patients with inflammatory bowel disease (IBD). In addition, we studied the cytokine profile of these cells. In the circulation, virtually all $CD4^+CD45RB^{high}$ T cells expressed the naive marker CD45RA, and circulating $CD4^+CD45RB^{low}$ cells expressed the memory marker CD45RO in IBD patients and a control patient population. In contrast, the intestinal $CD4^+$ $CD45RB^{high}$ T cells are in normal controls for 90% $CD45RO^+$. However, in IBD, 27.7% [Crohn's disease (CD)] and 49% [ulcerative colitis (UC)] of the intestinal $CD4^+$ $CD45RB^{high}$ T cells are $CD45RA^+$. This special CD4CD45RA$^+$ T cell in IBD can be found in the lamina propria as well as in lymphoid follicles (confocal laser-scanning microscopy). The $CD4^+CD45RB^{high}$ T lymphocytes produce significantly less interleukin (IL)-10 and IL-4 and produce more tumor necrosis factor α than $CD45RB^{low}$ T lymphocytes in control patients. $CD4^+CD45RB^{low}$ T cells from IBD patients produced less IL-10 than $CD4^+CD45RB^{low}$ T lymphocytes of controls, and interferon-γ production by both T lymphocyte subsets was decreased in IBD. These data indicate that CD and UC are characterized by an influx of $CD4^+CD45RB^{high}$ T lymphocytes. These $CD4^+CD45RB^{high}$ T lymphocytes seem to be important in the pathogenesis of IBD, as they produce more proinflammatory cytokines and less anti-inflammatory cytokines compared with $CD4^+CD45RB^{low}$ T lymphocytes. *J. Leukoc. Biol.* 75: 1010–1015; 2004.**

***Key Words:*** *cytokines · inflammation · human*

## INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are complex, multifactorial human inflammatory bowel diseases of which the etiology remains poorly understood. Recently, a genetic defect in the gene encoding a protein involved in the recognition of microbes, NOD2, was identified [1]. This links a dysregulated immune response to enteric bacteria to disease development in the immune susceptible host. Pathogenic $CD4^+$ T lymphocytes are believed to play a major role in the pathogenesis of inflammatory bowel disease (IBD).

To study the mechanism of disease development, several mouse models of experimentally induced or spontaneously occurring colitis are available (for review, see ref. [2]). One of these models involves the adoptive transfer of a pathogenic $CD4^+CD45RB^{high}$ T cell subpopulation into an immuno-deficient recipient. Transfer of $CD4^+CD45RB^{high}$ T cells from donor mice into genetically immuno-deficient (recombination activating gene-2−/−) mice or severe combined immunodeficiency mice results in a spontaneous chronic and severe inflammation of the large intestines [3]. In contrast, cotransfer of the $CD4^+CD45RB^{low}$ T cell subset protects against disease [4] or results in a later onset of disease [5].

The level of expression of CD45RB on mouse $CD4^+$ T cells is believed to distinguish naïve ($CD45RB^{high}$) from activated/memory ($CD45RB^{low}$) cells [6].

In humans, two other isoforms of CD45, CD45RA and CD45RO, are used to distinguish naïve and primed/memory T cells, respectively [7, 8]. However, there is also a reverse CD45RB expression on CD45RA and CD45RO cells in human peripheral blood [9]. In the peripheral blood, CD45RA-positive T cells express high levels of CD45RB, whereas CD45RO cells express little CD45RB. Hitherto, the role of CD45RB expression in human intestinal inflammation has not been evaluated in detail.

The expression of CD45 is essential for the activation of T lymphocytes via the T cell receptor. The intracellular domain of CD45 contains a protein tyrosine phosphatase that regulates the intracellular proteins $p56^{lck}$ and $p59^{fyn}$ on T cell activation [10]. T lymphocytes express different isoforms of the extracellular domain of CD45 [7]. The expression of the isoforms varies between subpopulations of T lymphocytes and changes upon stimulation and differentiation. The CD45 isoforms display different abilities to support T cell activation [11].

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Conflicting reports exist concerning the distribution of memory and naïve cells in the peripheral blood cells of IBD patients. The number of memory cells was reported to be elevated in the peripheral blood of CD patients [12], but these observations were not confirmed in another report [13]. In the mucosa of the gut, almost all CD4-positive cells express the CD45RO marker and are therefore considered memory cells.

In view of the functional differences of T lymphocytes that express the various CD45 isoforms, we investigated the expression of CD45RA, CD45RO, and CD45RB by peripheral- and gut-derived T lymphocytes from IBD patients and controls. To further study the functional difference between $CD45RB^{high}$ and $CD45RB^{low}$ cells, peripheral- and gut-derived T lymphocytes were sorted and stimulated with CD3/CD28. The pro- and anti-inflammatory cytokine profile of these subsets was determined.

## MATERIALS AND METHODS

### Patients

Mucosal lymphocytes were isolated from surgical resection specimens from patients with active CD or UC who underwent partial resection of the intestines. Eight CD patients (two male and six female) with a mean age of 34.8 years (range, 27–44 years) and median disease duration of 6.25 years (range, 1–12 years) were included. Four patients had ileal disease, and four had colonic disease. All patients were on corticosteroids. The mean age of the eight UC patients (four males and four females) was 34.8 years (range, 22–48 years), and mean disease duration was 4 years (range, 1–9 years). Six of these patients were on corticosteroids. Control mucosal lymphocytes were obtained from six patients undergoing a resection for cancer (three males and three females). Mean age of this group was 61.8 (range, 40–78 years). The disease was located in the rectum (two) and in the colon (four), and noninvolved bowel segments were used for this study.

### Cell preparation of mucosal specimen

Freshly obtained mucosal specimens (3 cm×0.5 cm) were homogenized using an automated, mechanical tissue desecration device (Medimachine system, Dako, Danmark). Cell suspensions were centrifuged with Ficoll (Pharmacia, Uppsala, Sweden), and the mononuclear cells were subsequently passed through a 40-μM filter cell strainer (Becton Dickinson Transduction Laboratories, Franklin Lakes, NJ). Subsequently, cells were washed with fluorescence-activated cell sorter buffer (phosphate-buffered saline, containing 0.5% bovine serum albumin, 0.3 mmol/L EDTA, and 0.01% sodium azide) and were kept on ice for the rest of the procedure.

### Flow cytometry

Four color FACSCalibur analysis was used to determine the expression of CD45RA and CD45RO within the $CD45RB^{high}$ and $CD45RB^{low}$ subgroups. One million cells per well (96-well microplate, Greiner B.V. Labor Techniek, Alphen aan de Rijn, The Netherlands) were incubated with anti-CB45RB (Southern Biotechnology Associates, Birmingham, AL) and anti-immunoglobulin G–peridinin chlorophyll protein (Per CP) as secondary antibody. Subsequently, cells were incubated with anti-CD4–allophycocyanin (APC)-, anti-CD45RO–phycoerythrin (PE)-, and anti-CD45RA–fluorescein isothiocyanate (FITC)-labeled antibody (Immunotech, Marseille, France). Isotype controls were used to determine the negative gates.

Analysis was done on a FACSCalibur in conjunction with the Cellquest Pro (BD Biosciences, Pharmingen, San Diego, CA) software, and 5000 cells were counted. $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ subsets were defined as 40% of the highest and 40% of the lowest $CD4^+$CD45RB-expressing cells.

### Sorting of peripheral blood mononuclear cells (PBMC) and gut-derived T cells

Blood was drawn into a tube containing heparin (final concentration, 10 U/ml). PBMC were obtained using Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) density gradient centrifugation (400 *g*, 20 min). Subsequently, the cells residing at the interface were washed three times and resuspended in Iscove's medium supplemented with 10% pooled human serum (BioWhittaker, Belgium) and ciproxin (10 μg/ml; Gibco-BRL, Life Technologies, Rockville, MD). Gut-derived T cells were isolated as described above under sterile conditions and resuspended in Iscove's medium supplemented with 10% pooled human serum and ciproxin. For staining, per $1 \times 10^7$ cells, 20 μl unlabeled mouse anti-human-CD45RB antibody was added, and cells were incubated for 30 min on ice. After washing with sterile medium [Iscove's medium supplemented with 10% pooled human serum (BioWhittaker)] and ciproxin (10 μg/ml), a secondary goat anti-mouse–FITC-labeled antibody (Immunotech) was added and incubated for 20 min on ice in the dark. Subsequently, cells were washed, and a 10-min blocking step with normal mouse serum (CLB, Amsterdam, The Netherlands) without preservative was performed before staining with 10 μl mouse anti-human–CD4–PE–Cy5-labeled antibody (Immunotech) per $1 \times 10^7$ cells for 20 min on ice in the dark. Cells were sorted by use of the FACS-Vantage (BD Biosciences, Pharmingen) under sterile conditions. $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ subsets were sorted and defined as 40% of the highest and 40% of the lowest $CD4^+$CD45RB-expressing cells. After sorting, the subpopulations were reanalyzed on a FACScan or FACS-Calibur (BD Biosciences, PharMingen), and only the populations more than 95% pure were used. The sorted cells were washed and resuspended in RPMI 1640 (BioWhittaker), supplemented with 5% pooled human serum (BioWhittaker) and antibiotics (Gibco-BRL, Life Technologies). Sorted cells were stimulated (concentration, $1\times10^5$/well) with anti-human anti-CD3 and anti-CD28 antibodies (both 1:1000, CLB) for 72 h at 37°C.

### Cytokines

Supernatant of the stimulated peripheral blood cells was aspirated, and the concentration of interleukin (IL)-4, IL-10, interferon-γ (IFN-γ), and tumor necrosis factor α (TNF-α) was analyzed using enzyme-linked immunosorbent assay (CLB), according to the instruction of the manufacturer. For cytokine determination in the supernatants of the stimulated gut-derived T cells, the Cytometric Bead Array kit (BD Biosciences, PharMingen) was used, according to the manufacturer's instructions.

### Immunofluorescence staining

Double-staining was performed as described previously with some modifications [14]. Briefly, cryostat fragments of colon tissue were cut into 4–6 μm sections, air-dried overnight, and fixed in acetone for 10 min at room temperature. The slides were first incubated with 5% (vol/vol) normal goat serum (CLB), then with optimal dilutions of CD45RA–FITC (BD Biosciences, PharMingen), CD4–PE (BD Biosciences, PharMingen), rabbit anti-PE (Biogenesis, UK), and Cy3-conjugated goat anti-rabbit (Jackson Immunoresearch Laboratories, West Grove, PA). For each fluorochrome label, negative-control antibodies were included. Fluoresence was analyzed using a Leica TCS SP (Leica Microsystems, Heidelberg, Germany) confocal system, equipped with an Ar/Kr laser combination.

### Statistics

Data are provided as mean and SEM. The Mann-Whitney U-test and the Wilcoxon test were used where appropriate (SPSS for Windows, version 7.5). A double-sided $P < 0.05$ was considered to represent a significant difference.

## RESULTS

### Differential expression of CD45RA and CD45RO within the mucosal $CD4^+CD45RB^{high}$ T cell subpopulation

To examine the distribution of naïve (CD45RA) and memory cells (CD45RO) within the $CD45RB^{high}$ and the $CD45RB^{low}$ subgroups, four-color flowcytometric analysis was performed on peripheral blood and mucosal lymphocytes of controls, CD, and UC patients (**Fig. 1A**). In peripheral blood of controls,

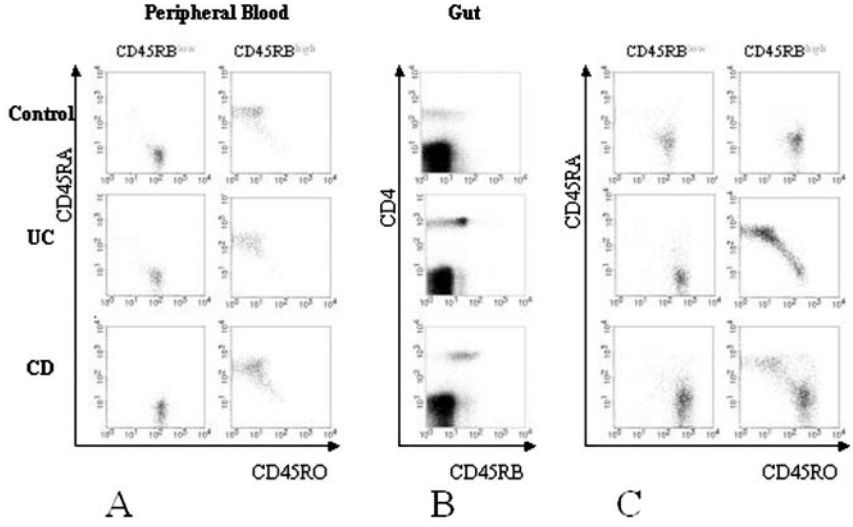


**Fig. 1.** Distribution of CD45RA and CD45RO within $CD45RB^{high}$ and $CD45RB^{low}$ subpopulations in the gut mucosa and peripheral blood. (A) Expression of CD45RO (*x*-axis) and CD45RA (*y*-axis) in $CD4^+$ peripheral blood lymphocytes in $CD45RB^{low}$ (left) and $CD45RB^{high}$ (right) cells in control, UC, and CD. (B) CD45RB and CD4 expression on gut-derived lymphocytes in control, UC, and CD. (C) Expression of CD45RO (*x*-axis) and CD45RA (*y*-axis) in $CD4^+$ gut-derived lymphocytes in $CD45RB^{low}$ (left) and $CD45RB^{high}$ (right) cells in control, UC, and CD.

99.6 ± 0.4% of the $CD45RB^{high}$ cells were $CD45RA^+$, whereas the $CD45RB^{low}$ subgroup almost exclusively contained CD45RO cells (93.4±1.2%). No differences were observed among UC, CD, and controls (**Table 1**).

Within the gut mucosa, the number of $CD4^+CD45RB^{high+}$ T cells in IBD is significantly increased compared with controls (Fig. 1B). In controls, 2.5 ± 1.2% of the $CD4^+$ T cells are $CD45RB^{high}$ compared with 18 ± 5% in CD and 41 ± 9.3% in UC. Also, the distribution of CD45RO and CD45RA within the $CD45RB^{high}$ and $CD45RB^{low}$ $CD4^+$ populations differed from peripheral blood (Fig. 1C). The $CD4^+CD45RB^{low}$ cells from the gut, in contrast, were almost exclusively CD45RO-postive, and no differences were seen among CD, UC, or normal, control, gut-derived T cells (Table 1). However, within the $CD4^+CD45RB^{high}$ subpopulation of gut-derived $CD4^+$ T cells, CD45RA-positive and CD45RO-positive cells were found (Table 1). In CD patients, the CD45RA/RO ratio was 27.7/59.7 and in UC, 49.0/34.8, compared with 4.5/90.0 in controls. This indicates that in IBD, the majority of the gut-derived $CD4^+$ T cells is in fact $CD45RB^{high}$ $CD45RA^+$.

To determine the localization of these $CD4^+$/CD45RA cells in the intestinal mucosa, cryostat sections of IBD patients were stained for CD45RA and prepared for confocal laser-scanning microscopy (**Fig. 2**). In IBD patients, CD45RA-positive cells were found in the lamina propria and in follicular structures.

## Cytokine profile of CD45RB cells in peripheral blood cells

To investigate functional differences between the $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ T cell subsets, PBMC were sorted for the expression of this marker. After sorting, cells were stimulated for 72 h by anti-CD3/anti-CD28 double-stimulation, and the production of IFN-γ, IL-10, TNF-α, and IL-4 was determined (**Fig. 3**). $CD4^+CD45RB^{low}$ T cells of normal controls produced fivefold more IL-10 than $CD4^+CD45RB^{high}$ T cells (330.5 pg/ml±42.1 and 65.9 pg/ml±29.1, respectively). Furthermore, IL-4 production was significantly increased in $CD4^+CD45RB^{low}$ T cells (952.7 pg/ml±187.8 vs. 226.4 pg/ml±65.9 in $CD4^+CD45RB^{high}$ T cells). In contrast, $CD4^+CD45RB^{high}$ T cells produced more TNF-α (1122.3 pg/ml±198.1 and 2617.9 pg/ml±482.6). Both CD45RB subpopulations produced large amounts of IFN-γ ($P$=0.064; 6718.0 pg/ml±1761.3 and 11981.6 pg/ml±3439.4).

Stimulated $CD4^+CD45RB^{low}$ T cells of CD and UC patients produced significantly less IL-10 than cells isolated from normal controls (200.6 pg/ml±58.6 in CD patients and 147.9 pg/ml±93.9 in UC patients compared with 330.5 pg/ml±42.1 in controls). IFN-γ production of peripheral blood $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ T cells was signifi-

TABLE 1. CD45RA, CD45RB, and CD45RO Expression on Gut- and Peripheral Blood Derived $CD4^+$ T Lymphocytes in UC or CD and Control Patients

| | $CD4^+$ lymphocytes | $CD45RB^{low}$ CD45RO | $CD45RB^{high}$ CD45RA | $CD45RB^{high}$ CD45RO |
|---|---|---|---|---|
| Control | Gut-derived | 98.1 ± 0.7% | 4.5 ± 1.0% | 90.0 ± 3.6% |
| N = 6 | Peripheral blood | 93.4 ± 1.2% | 96.5 ± 0.4% | |
| UC | Gut-derived | 94.8 ± 5.6% | 49.0 ± 9.2%* | 34.8 ± 7.9% |
| N = 8 | Peripheral blood | 90.1 ± 2.6% | 92.6 ± 3.4% | |
| CD | Gut-derived | 95.4 ± 1.5% | 27.7 ± 6.6%* | 59.7 ± 8.1% |
| N = 8 | Peripheral blood | 97.9 ± 1.5% | 86.4 ± 4.2% | |

From patients with UC or CD and control patients, the gut-derived and peripheral blood lymphocyte, $CD4^+$ lymphocytes were gated. From this population, the 40% $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ T cells were gated as described in Materials and Methods. In both subgroups, the percentages of CD45RA and CD45RO expression were determined. Data are presented as mean and SE; * significant as compared with controls ($P < 0.05$).

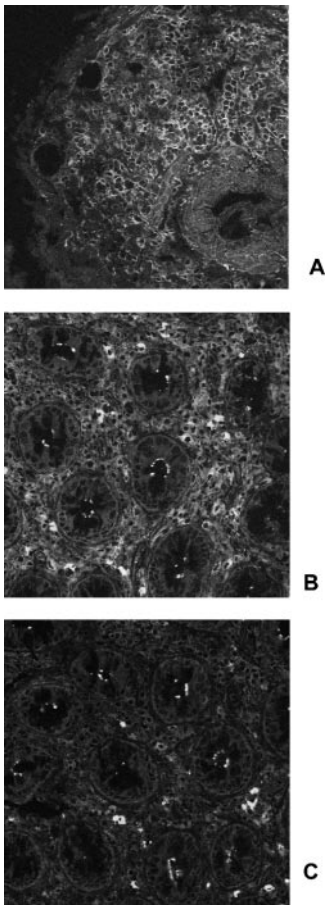


**Fig. 2.** Confocal laser-scanning microscopy of CD45RA (green) and CD4 (red) in cryosections of intestinal tissue of a CD patient. (A) CD45RA/CD4 staining in follicular structure. (B) CD45RA/CD4 staining in lamina propria. (C) Negative control. Intense green cells are considered to be nonspecific. Original magnification, 200×.

cantly reduced in CD patients compared with normal controls. In addition, the IFN-γ production of $CD4^+CD45RB^{high}$ cells of UC patients was significantly decreased. There was a trend toward higher production of IL-4 in CD patients ($P=0.083$), whereas in UC patients, the IL-4 production was significantly decreased (398.1 pg/ml±116.2 in CD and 162.1 pg/ml±78.8 in UC). No differences in TNF-α production were found among the three patient groups. The lymphocytes isolated from the gut of UC and CD patients demonstrated an enhanced CD69, CD134, CD44, and human leukocyte antigen-DR expression compared with control lymphocytes (results not shown), indicating an activated phenotype. This did not result in an enhanced, spontaneous cytokine secretion, as in the absence of CD3 and CD28, these cells did not secrete detectable levels of cytokines.

## Cytokine profile of CD45RB gut-derived T cells

Cells were isolated from surgical resection specimen and sorted for the expression of the $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ T cells. After sorting, the cells were stimulated for 72 h by anti-CD3/anti-CD28 double-stimulation, and the production of IFN-γ, IL-10, TNF-α, and IL-4 was determined (**Fig. 4**). The results were similar to those found in peripheral blood. $CD4^+CD45RB^{low}$ T cells produced more IL-10 and less TNF-α as compared with the $CD4^+CD45RB^{high}$ subgroup. $CD4CD45RB^{high}$ T cells isolated from a normal, control patient produced very little. TNF-α ($CD4^+CD45RB^{low}$ 48.4 pg/ml and $CD4^+CD45RB^{high}$ 320.4 pg/ml in controls). $CD4^+CD45RB^{low}$ T cells from CD patients produced 504.0 ± 226.1 pg/ml and $CD4^+CD45RB^{high}$ 1933 ± 1128 pg/ml. In contrast to the low TNF-α production, control-derived cells produced high amounts of IL-10 compared with cells derived from CD patients. Control $CD4^+CD45RB^{low}$ T cells produced 691.3 pg/ml, as compared with 219.0 ± 73.8 pg/ml in CD patients. The $CD4^+CD45RB^{high}$ and $CD4^+CD45RB^{low}$ subgroups produced similar amounts of IFN-γ (mean production in CD: 5051±2002 pg/ml in $CD45RB^{high}$ and 4444±1419 pg/ml in $CD45RB^{low}$).

## DISCUSSION

Transfer of $CD4^+CD45RB^{high}$ expressing T lymphocytes into an immunodeficient mouse induces severe intestinal inflammation [2, 3], and cotransfer with lymphocytes expressing $CD4^+CD45RB^{low}$ T cells prevents disease development [4–6]. Because of this functional importance of CD45RB expression in mouse intestinal inflammation, we studied the role of intestinal $CD4^+CD45RB$ T cell expression in patients with IBD. In normal human mucosa, all $CD4^+$ T cells have the memory

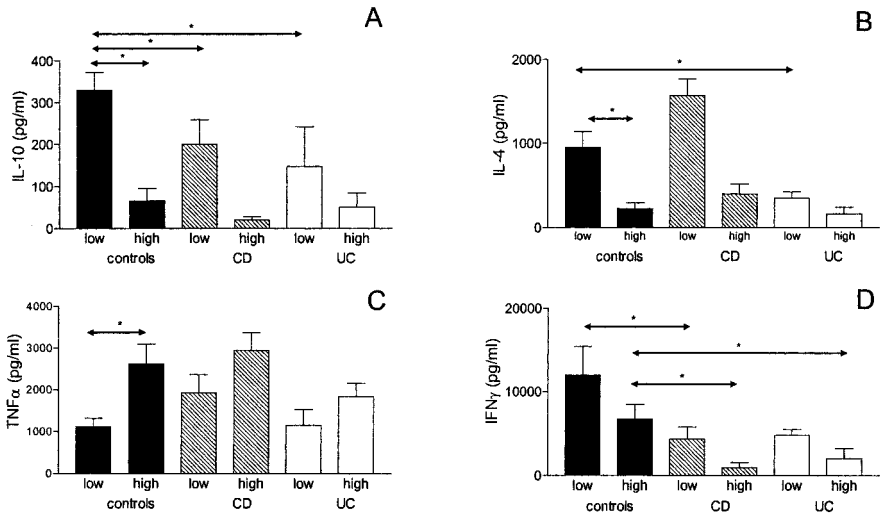


**Fig. 3.** Cytokine production of peripheral blood cells sorted for $CD4^+CD45RB^{high}$ (high) and $CD4^+$-$CD45RB^{low}$ (low) expression and subsequently stimulated with CD3/CD28 for 72 h. (A) IL-10 production. (B) IL-4 production. (C) TNF-α production. (D) IFN-γ production. Solid bars represent normal controls (n=12); hatched bars represent CD patient (n=6); and open bars represent UC patient (n=5). *, Significant difference ($P<0.05$).

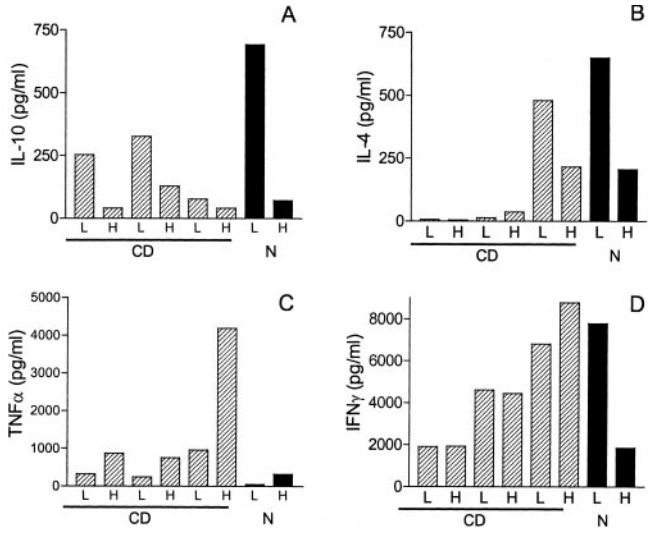


**Fig. 4.** Cytokine production of gut-derived T cells sorted for $CD4^+CD45RB^{high}$ (H) and $CD4^+CD45RB^{low}$ (L) expression and subsequently stimulated with CD3/CD28 for 72 h. (A) IL-10 production. (B) IL-4 production. (C) TNF-α production. (D) IFN-γ production. Solid bars represent a normal control; hatched bars represent CD patients.

phenotype CD45RO. We here report that in UC and CD, the percentage of $CD4^+CD45RB^{high}$ $CD45RA^+$ T cells is significantly increased in the mucosal compartment. These $CD4^+CD45RA^+$ T cells were found mainly in the follicular structures that are prominent in chronic inflammation.

The cytokine profile differs from $CD45RB^{high}$ and $CD45RB^{low}$. The same profile was found in intestines and peripheral blood, independent of CD45RA or CD45RO expression; thus, the level of CD45RB expression may be more important than that of CD45RA or CD45RO.

In the peripheral blood of CD or UC patients and normal controls, all $CD4^+$ $CD45RB^{high}$ T cells were CD45RA-positive, and $CD4^+$ $CD45RB^{low}$ T cells were CD45RO-positive, which is in agreement with previous reports [7, 8]. Analysis of cells derived from the intestines revealed that in normal controls, the majority of the $CD45RB^{low}$ and $CD45RB^{high}$ cells of control appeared to be memory cells ($CD45RO^+$). However, in IBD patients, a significant part of the $CD4^+CD45RB^{high}$ T cells were also $CD45RA^+$, and even a higher percentage was found in UC compared with CD. Further studies will be needed to elucidate the reason why and how these naïve cells migrate to the gut mucosa in IBD patients. The preferred presence of memory cells in the gut observed in this study is in concordance with the finding that T lymphocytes within the synovial compartment of rheumatoid arthritis patients are almost exclusively $CD45RO^+$ [13].

$CD45RB^{high}$ cells switch to $CD45RB^{low}$ within the tissue [13, 15, 16], and when $CD45RB^{high}$ cells become $CD45RB^{low}$, the capacity to produce IL-2 is significantly reduced. The $CD45RB^{low}$ cells will undergo apoptosis as a result of the loss of IL-2 [17, 18]. This apoptotic process is mediated by a gradual loss of Bcl-2 and gain of CD95 [9, 19]. One possible explanation for the lower percentage of $CD4^+CD45RB^{high}$ T cells in CD compared with UC is that the mechanism that controls T lymphocyte activation and survival probably differs. In CD, but not in UC, lamina propria T lymphocytes are less sensitive to apoptosis as a result of a change in the Bax/Bcl-2 ratio [20, 21]. Our data suggest that this resistance to apoptosis in CD is not caused by a defective switch from $CD45RB^{high}$ to $CD45RB^{low}$ and probably is a result of events further downstream.

Sorting experiments further emphasized the functional significance of the $CD45RB^{high}$ and $CD45RB^{low}$ expression in human intestinal inflammation. Peripheral blood and intestinal T cells were sorted for CD45RB expression, and subsequently, the cytokine pattern was analyzed. In peripheral blood and intestinal cells, different cytokine profiles were observed between $CD4^+CD45RB^{low}$ and $CD4^+CD45RB^{high}$. $CD4^+CD45RB^{low}$ cells produce high amounts of IL-4 and IL-10, whereas the $CD4^+CD45RB^{high}$ T cells produce high amounts of TNF-α. The markedly different cytokine profile of the CD45 subsets is reminiscent of analog mouse T lymphocyte subsets. In the mouse transfer model of colitis, the $CD45RB^{low}$ subset protects against development of colitis, which is mediated by high production of IL-10 [22]. We now demonstrate that human $CD4^+CD45RB^{low}$ T cells produce significantly more IL-10 than $CD4^+CD45RB^{high}$ T cells. It is interesting that in our study, the $CD4^+CD45RB^{low}$ subset from IBD patients produced significantly less IL-10, which has an immune-regulatory role in IBD. The pivotal importance of IL-10 as a regulatory cytokine within the mucosal environment was shown in IL-10−/− mice, which develop severe colitis [23]. IL-10 was identified as a differentiation factor for a subset of T lymphocytes, regulatory T lymphocytes (Tr1 cells). These Tr1 cells have immune-suppressive properties and were found to suppress experimental colitis induced by transfer of pathogenic $CD4^+$/$CD45RB^{high}$ T cells [24, 25]. The source of IL-10 important for the development of regulatory T cells is not defined yet, but special regulatory dendritic cell populations secreting high levels of IL-10 could play a major role [26–28]. Future studies will elucidate their role in IBD.

It is unexpected that $CD4^+CD45RB^{low}$ T cells isolated from UC patients produced low amounts of IL-4 compared with CD patients and controls. However, it was previously reported that IL-5 and not IL-4 plays an important role in UC [29]. Although it was reported that $CD45RB^{high}$ cells produce more IFN-γ [15], we did not observe differences in IFN-γ production between CD45RB subsets obtained from controls. However, the IFN-γ production by the $CD4^+CD45RB^{high}$ subset of IBD patients was decreased. Furthermore, the role of IFN-γ in intestinal inflammation is controversial, as IFN-γ-deficient and IFN-γ receptor-deficient mice are susceptible to experimental 2,4,6,-trinitrobenzene sulphonic acid-induced colitis [30, 31].

In summary, we here report marked differences in the expression of $CD45RB^{high}$ by T lymphocytes present in the inflamed mucosa of patients with CD and UC. The $CD45RB^{high}$ and $CD45RB^{low}$ differentiation could be of clinical importance, as we also observed an important, functional difference between both subgroups, not depending on CD45RA or CD45RO expression. These results indicate that CD45RB could play an important role in the maintenance of the intestinal inflammation by T cell activation.

## REFERENCES


1. Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., Achkar, J. P., Brant, S. R., Bayless, T. M., Kirschner, B. S, Hanauer, S. B., Nunez, G., Cho, J. H. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411,** 603–606.
2. Bouma, G., Strober, W. (2003) The immunological and genetic basis of inflammatory bowel disease. *Nat. Rev. Immunol.* **3,** 521–533.
3. Leach, M. W., Bean, A. G., Mauze, S., Coffman, R. L., Powrie, F. (1996) Inflammatory bowel disease in C.B-17 SCID mice reconstituted with the CD45RBhigh subset of CD4+ T cells. *Am. J. Pathol.* **148,** 1503–1515.
4. Morrissey, P. J., Charrier, K. (1994) Induction of wasting disease in SCID mice by the transfer of normal CD4+/CD45RBhi T cells and the regulation of this autoreactivity by CD4+/CD45RBlo T cells. *Res. Immunol.* **145,** 357–362.
5. Claesson, M. H., Bregenholt, S., Bonhagen, K., Thoma, S., Moller, P., Grusby, M. J., Leithauser, F., Nissen, M. H., Reimann, J. (1999) Colitis-inducing potency of CD4+ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype. *J. Immunol.* **162,** 3702–3710.
6. Powrie, F. (1995) T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity* **3,** 171–174.
7. Tchilian, E. Z., Beverley, P. C. (2002) CD45 in memory and disease. *Arch. Immunol. Ther. Exp. (Warsz.)* **50,** 85–93.
8. Dutton, R. W., Bradley, L. M., Swain, S. L. (1998) T cell memory. *Annu. Rev. Immunol.* **16,** 201–223.
9. Salmon, M., Pilling, D., Borthwick, N. J., Viner, N., Janossy, G., Bacon, P. A., Akbar, A. N. (1994) The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur. J. Immunol.* **24,** 892–899.
10. Mustelin, T., Pessa-Morikawa, T., Autero, M., Gassmann, M., Andersson, L. C., Gahmberg, C. G., Burn, P. (1992) Regulation of the p59fyn protein tyrosine kinase by the CD45 phosphotyrosine phosphatase. *Eur. J. Immunol.* **22,** 1173–1178.
11. Shanafelt, M. C., Yssel, H., Soderberg, C., Steinman, L., Adelman, D. C., Peltz, G., Lahesmaa, R. (1996) CD45 isoforms on human CD4+ T-cell subsets. *J. Allergy Clin. Immunol.* **98,** 433–440.
12. Roman, L. I., Manzano, L., De La Hera, A., Abreu, L., Rossi, I., Alvarez-Mon, M. (1996) Expanded CD4+CD45RO+ phenotype and defective proliferative response in T lymphocytes from patients with Crohn's disease. *Gastroenterology* **110,** 1008–1019.
13. Salmon, M. P. D., Borthwick, N. J., Akbar, A. N. (1997) Inhibition of T-cell apoptosis: a mechanism for persistence in chronic inflammation. *The Immunologist* **5,** 87–92.
14. Res, P. C. M., Couwenberg, F., Vyth-Dreese, F. A., Spits, H. (1999) Expression of pT mRNA in a committed dendritic cell precursor in the human thymus. *Blood* **94,** 2647–2657.
15. Horgan, K. J., Tanaka, Y., Luce, G. E., van Seventer, G. A., Nutman, T. B., Shaw, S. (1994) CD45RB expression defines two interconvertible subsets of human CD4+ T cells with memory function. *Eur. J. Immunol.* **24,** 1240–1243.
16. Salmon, M., Scheel-Toellner, D., Huissoon, A. P., Pilling, D., Shamsadeen, N., Hyde, H., D'Angeac, A. D., Bacon, P. A., Emery, P., Akbar, A. N. (1997) Inhibition of T cell apoptosis in the rheumatoid synovium. *J. Clin. Invest.* **99,** 439–446.
17. Akbar, A. N., Borthwick, N. J., Wickremasinghe, R. G., Panayoitidis, P., Pilling, D., Bofill, M., Krajewski, S., Reed, J. C., Salmon, M. (1996) Interleukin-2 receptor common γ-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-xL) but not pro-apoptotic (bax, bcl-xS) gene expression. *Eur. J. Immunol.* **26,** 294–299.
18. Vella, A. T., Dow, S., Potter, T. A., Kappler, J., Marrack, P. (1998) Cytokine-induced survival of activated T cells in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* **95,** 3810–3815.
19. Akbar, A. N., Borthwick, N., Salmon, M., Gombert, W., Bofill, M., Shamsadeen, N., Pilling, D., Pett, S., Grundy, J. E., Janossy, G. (1993) The significance of low bcl-2 expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T cell memory. *J. Exp. Med.* **178,** 427–438.
20. Boirivant, M., Marini, M., Di Felice, G., Pronio, A. M., Montesani, C., Tersigni, R., Strober, W. (1999) Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology* **116,** 557–565.
21. Ina, K., Itoh, J., Fukushima, K., Kusugami, K., Yamaguchi, T., Kyokane, K., Imada, A., Binion, D. G., Musso, A., West, G. A., Dobrea, G. M., McCormick, T. S., Lapetina, E. G., Levine, A. D., Ottaway, C. A., Fiocchi, C. (1999) Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J. Immunol.* **163,** 1081–1090.
22. Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L., Powrie, F. (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* **190,** 995–1004.
23. Davidson, N. J., Leach, M. W., Fort, M. M., Thompson-Snipes, L., Kuhn, R., Muller, W., Berg, D. J., Rennick, D. M. (1996) T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J. Exp. Med.* **184,** 241–251.
24. Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., Roncarolo, M. G. (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389,** 737–742.
25. Asseman, C., Powrie, F. (1998) Interleukin 10 is a growth factor for a population of regulatory T cells. *Gut* **42,** 157–158.
26. Levings, M. K., Bachetta, R., Schulz, U., Roncarolo, M. G. (2002) The role of IL-10 and TGF-β in the differentiation and effector function of T regulatory cells. *Int. Arch. Allergy Immunol.* **129,** 263–276.
27. Wakkach, A., Fournier, N., Brun, V., Breittmayer, J. P., Cottrez, F., Groux, H. (2003) Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity* **18,** 605–617.
28. Kapsenberg, M. (2003) Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* **3,** 984–993.
29. Fuss, I. J., Neurath, M., Boirivant, M., Klein, J. S., de la Motte, C., Strong, S. A., Fiocchi, C., Strober, W. (1996) Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-γ, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J. Immunol.* **157,** 1261–1270.
30. Dohi, T., Fujihashi, K., Rennert, P. D., Iwatani, K., Kiyono, H., McGhee, J. R. (1999) Hapten-induced colitis is associated with colonic patch hypertrophy and T helper cell 2-type responses. *J. Exp. Med.* **189,** 1169–1180.
31. Camoglio, L., te Velde, A. A., de Boer, A., ten Kate, F. J., Kopf, M., van Deventer, S. J. (2000) Hapten-induced colitis associated with maintained Th1 and inflammatory responses in IFN-γ receptor-deficient mice. *Eur. J. Immunol.* **30,** 1486–1495.