Altered expression of αEß7 integrin on intra-epithelial and lamina propria lymphocytes in patients with Crohn's disease

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Abstract

Objective **: To compare the expression of adhesion molecules on intestinal intra-epithelial (IEL) and lamina propria T cells (LPL) from ileum and colon, in patients with Crohn's disease (CD) versus healthy controls, with special reference for the αEß7 integrin.**

Methods **: IEL and LPL were obtained from 18 CD patients and 20 controls by enzymatic extraction, and subsequently characterized by flow cytometry for CD3, CD4, CD8, CD25, LFA-1α (CD11a), CD44, α4 and αEß7 integrin.**

Results **: In LPL of controls, a decreased CD4/CD8 ratio was noted in ileum compared to colon. This regional difference was accompanied by a higher expression of αEß7 integrin in ileum versus colon. In LPL from left hemicolon of CD patients, a decreased CD4/CD8 ratio was noted versus controls. αEß7 expression on T cells of LPL did not discriminate CD from controls. However, an overexpression of this ß7 integrin member was observed on CD25+ T cell subsets from lamina propria of left hemicolon, in CD versus controls.**

Moreover, in IEL, profound alterations in αEß7 integrin were observed in CD, compared to controls. A decreased expression of αEß7 was noted in IEL of ileum of CD patients. This was also apparent in non-inflamed mucosa.

Conclusion **: The observed changes of αEß7 integrin expression in CD patients versus controls are of pathogenic relevance, especially the decreased expression of αEß7 in IEL of non-inflamed CD mucosa. This may be one of the earliest events in the pathogenesis of this disease.** (Acta gastroenterol. belg., **1998**, 61, **288-294**).

Key words : αEß7 integrin, adhesion molecules, Crohn's disease.

Introduction

The aetiology and pathogenesis of inflammatory bowel disease (IBD) is still unknown, although genetic factors, functional or structural abnormalities of the gut, infectious agents, and alterations of the immunologic system have been suggested to play a role in the pathogenesis (1-5). IBD is characterized by a chronic inflammation of the intestine and principally covers two distinct forms of intestinal pathology : ulcerative colitis (UC) and Crohn's disease (CD). The inflammatory process in CD involves the deep mucosa and often extends to the small intestine (6). Lamina propria lymphocytes (LPL) and intra-epithelial lymphocytes (IEL) are considered to be pathogenetically more important in IBD than peripheral blood mononuclear cells (4,7-10).

The intestinal epithelium contains many lymphocytes, that differ from those present in lymphoid organs or in the lamina propria by their environment and phenotypical characteristics. IEL lay close to the laterobasal membranes of epithelial cells and to intraluminal antigens and are separated from lamina propria accessory cells by the basement membrane (11). IEL are predominantly CD8+ and are characterized by a high expression of the αEß7 integrin, which acts as the ligand for E-cadherin that is largely expressed on intestinal epithelial cells (11-13). On the other hand, LPL have mixed CD4/CD8 composition with a low to moderate expression of αEß7 (11,13,14).

A number of differences have been described between T-cells from normal and inflamed mucosa in the representation of various T-cell subsets and the expression of activation markers on intestinal T-cells (7,15-17). More recently, adhesion molecules have been pathogenetically implicated in several animal models of inflammatory bowel disease. Administration of monoclonal antibodies directed to the α4 integrin resulted in a dramatical improvement of the acute colitis in the cotton-top tamarin model, an animal model of ulcerative colitis (18). In addition, anti-α4ß7 treatment in the same animal model, using a monoclonal antibody directed against a combinatorial epitope of the α4ß7 complex, has recently been shown to be beneficial in the chronic phase of the disease (19). Moreover, the development of murine IBD, with histologic features of human CD, has been reported in mice lacking the αEß7 ligand, N-cadherin, on the intestinal epithelial surface (20).

The aim of the present study was to investigate whether intestinal mucosa from patients with CD and controls differ in T cell subsets with special reference for the adhesion molecules CD44 (receptor for hyaluronate), α4 integrin (CD49d), αEß7 integrin (CD103) and LFA-1α (CD11a). Moreover, in the lamina propria the expression of these markers was analysed on activated T cells expressing the interleukin-2 receptor (IL2-R) α-subunit (CD25), as activated T cells have been linked with the pathogenesis of CD (21).

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Table I. — **Clinical characteristics of patients with Crohn's disease**

	Case	Age	Sex	Biopsies	Treatment
IEL	1	21	M	IL,RC*,LC	none
IEL	2	36	M	IL*,RC	mesalazine/methylprednisolone
IEL	3	53	F	RC,LC*	sulfasalazine/methylprednisolone
IEL	4	27	F	IL,RC	azathioprine
IEL	5	14	F	IL,RC	none
IEL	6	30	F	IL,RC*	none
IEL+LPL	7	26	F	IL*,LC*	methylprednisolone
IEL+LPL	8	57	F	IL*,RC,LC	mesalazine
LPL	9	23	F	IL,RC*,LC*	sulfasalazine/methylprednisolone
LPL	10	17	M	RC*,LC	none
LPL	11	29	F	LC	none
LPL	12	31	F	LC	azathioprine/methylprednisolone
LPL	13	34	F	IL,RC*,LC*	none
LPL	14	24	F	RC*,LC*	mesalazine/azathioprine/methylprednisolone
LPL	15	32	M	IL,LC*	mesalazine
LPL	16	27	F	IL,LC*	mesalazine/azathioprine
LPL	17	33	M	IL*,RC,LC	none
LPL	18	48	F	RC*	sulfasalazine

IL : ileum ; RC : right hemicolon ; LC : left hemicolon. * macroscopically inflamed area.

Patients and methods

1. *Patient population*

Eighteen patients with CD (5 males and 13 females), with a median age of 31 years (range 14-57) were included in this study, after giving informed consent. The patients' characteristics are summarized in Table I. Biopsies were obtained by ileocolonoscopy from terminal ileum in 12 patients (4 inflamed/8 non-inflamed) ; from right hemicolon in 13 patients (7 inflamed/6 non-inflamed) and from the left hemicolon in 13 patients (7 inflamed/6 non-inflamed). The different regions from which biopsies were taken in CD patients are indicated in Table I.

As a control population, 20 patients (9 males and 11 females), with a macroscopically and microscopically normal ileocolonoscopy and with a median age of 50 years (range 20-74) were included for extraction of IEL (N = 10) and LPL (N = 10). Ileocolonoscopy in those individuals was performed for abdominal pain or previous history of polyps. From each patient 5 biopsies were taken from ileum (IL), right hemicolon (RC) and left hemicolon (LC), for IEL and LPL extraction.

2. *Cell preparation*

The intestinal biopsies obtained by ileocolonoscopy were stored in 10 ml RPMI 1640 medium (Gibco BRL, UK).

2.1. IEL EXTRACTION

The isolation procedure was performed according to the method described by S. Lynch *et al.* (22). The biopsies were collected in 5 ml of ion-free HBSS (Gibco BRL), containing 5% pooled human serum (HS) and 1 mmol/l dithiotreitol (DTT)/ethylenediaminetetraacetic acid (EDTA) (Sigma). The samples were placed on a test tube rotator at 37°C for 45 min. The supernatant containing single cells was aspirated, washed twice with an equal volume of RPMI 1640 medium, containing HEPES buffer (2%), L-glutamine (1%) and HS (10%) for 10 min at 1800 rpm. Finally, the cells were resuspended in RPMI 1640 medium. No further purification of the cell population was performed in order to retain a maximum number of cells. The median cell yield of the obtained population was 2.9×10^5 (range 2×10^5 - 6.8×10^5) out of 5 biopsies.

2.2. LPL EXTRACTION

The isolation was performed as described by A. Balzano *et al.* (23). All biopsies were suspended in HBSS containing 2 ml collagenase A (1 mg/ml) (Boehringer Mannheim, Mannheim, Germany) and 1 ml DNAse solution (1 mg/ml) (BM) and placed on a test tube rotator at 37°C for 3 h. After incubation, the material was centrifuged at 3000 rpm for 15 min. The pellet, washed with 5 ml HBSS (Gibco BRL), was resuspended in 5 ml of the same solution. The resulting crude cell suspension was purified using a Ficoll-Paque 1077 gradient (Pharmacia, Upssala, Sweden), washed with 5 ml HBSS and centrifuged at 1800 rpm for 15 min and the pellet was resuspended in PBS (Gibco BRL). The median cell yield of mononuclear cells was 3×10^5 (range 2×10^5-15×10^5) out of 5 biopsies.

3. *Monoclonal antibodies*

All monoclonal antibodies (Mab) were directly conjugated with either fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin chlorophyll protein (PerCP). Cellular distribution was investigated using anti-CD3 (Leu4, clone SK7), anti-CD4 (Leu3a, clone SK3), anti-CD8 (Leu2a, clone SK1) (Becton Dickinson,

San Jose, CA, USA). CD25 expression was studied using anti-CD25 (IL-2R, clone 2A3, Becton Dickinson). Adhesion molecules were studied as well: CD44 (HCAM-1, clone F10-44-2), LFA-1α (CD11a, clone B-B15, Serotec, Oxford, UK), α4 integrin (CD49d, clone HP2/1) and αEß7 integrin (CD103, HML-1, clone 2G5, Immunotech, Marseille, France).

Isotype-matched immunoglobulins that did not react with human leukocytes were used as controls (Becton Dickinson).

4. *Immunofluorescent labeling*

Aliquots (100 µl) were incubated with the appropriate amount of monoclonal antibodies in the dark for 30 min at 4°C. Cells were washed with 2 ml PBS and centrifuged at 1600 rpm for 10 min. Cells were fixed with 300 µl CellFIX (Becton Dickinson) and placed at 4°C until analysis.

5. *Flow cytometric analysis*

Flow cytometric analysis was performed using FACSort (Becton Dickinson), by three color analysis. Isotype matched controls were used to determine the nonspecific staining and to set the fluorescent markers. The cut-off was set to encompass 99.5% of the observed events using isotype-matched immunoglobulins. The lymphocytes were gated using FSC/SSC and routinely 5000 events were acquired. The accuracy of lymphocyte gating on the FSC/SSC diagram was confirmed by staining with CD45 FITC/CD14 PE (Leucogate®): > 95% of the gated cells were lymphocytes. Acquired data were analysed using Lysis II® and Attractors® software (Becton Dickinson).

6. *Statistical analysis*

Results are expressed as percentage positive cells with median value and range. Nonparametric statistical tests (the Wilcoxon test for paired samples and the Wilcoxon rank sum test) were used to compare groups. $p < 0.05$ was considered as statistically significant.

Results

1. *Intra-epithelial lymphocytes*

The results of IEL are summarized in Table II (controls versus CD).

1.1. Cellular distribution

As indicated in Table II, no differences in the frequency of CD4+ and CD8+ T-cells were noted between controls and CD, in all localisations studied. Approximately two-thirds of the IEL were CD8+, resulting in a CD4/CD8 ratio < 1. In CD, a higher fraction of CD8+ T-cells was observed in ileum versus right hemicolon ($p < 0.05$; Wilcoxon test for paired samples).

In IEL, minor fractions of CD4+CD8+ and CD4-CD8- T cell subsets were noted (Table II).

1.2. Adhesion molecules

The overall expression of CD44 and α4 integrin on T cells was not different between CD and controls (Table II). The expression of αEß7 however, was significantly lower in CD, compared to controls, most prominent in ileal mucosa ($p < 0.05$; Wilcoxon rank sum test; Figure 1; Table II). Interestingly, the altered expression of this ß7 integrin member, was also present on non-inflamed mucosa, underscoring the specificity of the phenomenon (Figure 1). In addition, an increased expression of LFA-1α was noted in right hemicolon of CD patients, compared to controls ($p < 0.05$; Wilcoxon rank sum test, Table II).

2. *Lamina propria lymphocytes*

The results of LPL are summarized in Table III (controls versus CD) and Table IV and V (expression

Table II. — **Cellular distribution of and adhesion molecule expression on intestinal intra-epithelial T cells**

	Ileum		Right Hemicolon		Left Hemicolon	
	Controls N = 10	Crohn N = 7	Controls N = 10	Crohn N = 7	Controls N = 10	Crohn N = 4
CD4+CD8-	16 (7-64)	17 (8-37)	26 (4-77)	30 (15-46)	36 (9-63)	33 (15-66)
CD8+CD4-	67 (31-84)	73 (52-80)ψ	52 (17-75)	51 (37-62)ψ	53 (31-79)	52 (19-75)
CD4/CD8	0.3 (0.1-2.1)	0.2 (0.1-0.7)	0.6 (0.1-4.5)	0.6 (0.3-1.2)	0.7 (0.1-2.1)	0.7 (0.2-3.5)
CD4-CD8-	6 (2-41)	8 (2-13)	14 (2-26)	13 (6-17)	10 (3-26)	7 (3-23)
CD4+CD8+	6 (0-11)	3 (1-4)	1 (0-58)	2 (1-6)	2 (1-4)	2 (1-8)
CD44	96 (87-99)	96 (96-98)	97 (86-99)	97 (96-99)	98 (78-100)	98 (96-99)
α4	32 (6-68)	19 (2-30)	40 (14-60)	23 (9-36)	48 (11-74)	21 (10-64)
LFA-1α	75 (40-95)	82 (71-93)	66 (37-97)*	86 (76-98)*	74 (46-96)	90 (65-98)
αEß7	84 (27-93)*	53 (22-80)*	56 (13-93)	35 (18-78)	60 (27-87)	25 (8-64)
CD3+CD25+	23 (3-68)	18 (5-37)	15 (6-56)*	27 (24-55)*	24 (5-44)	27 (20-33)
αEß7/CD25+	75 (6-94)	44 (31-71)	41 (7-79)	29 (19-61)	33 (10-77)	20 (5-34)

Flow cytometric expression of CD44, α4 integrin, αEß7 and LFA-1α on CD3 positive IEL from ileum, right and left hemicolon of patients with Crohn's disease and controls. Results are expressed as percentage positive cells. Median and range are displayed. * $p < 0.05$ between controls and Crohn's disease. ψ $p < 0.05$ between ileum and right hemicolon.

Table III. — **Cellular distribution of and adhesion molecule expression on intestinal lamina propria T cells**

	Ileum		Right Hemicolon		Left Hemicolon	
	Controls N = 10	Crohn N = 7	Controls N = 10	Crohn N = 7	Controls N = 10	Crohn N = 11
CD4+CD8-	49 (25-69)ψ	37 (30-61)	61 (43-71)	54 (44-75)	64 (56-78)ψ*	57 (17-75)*
CD8+CD4-	39 (18-65)ψ	51 (29-63)	31 (21-48)	35 (19-46)	27 (11-34)ψ	33 (18-61)
CD4/CD8	1.3 (0.4-3.9)ψ	0.7 (0.5-2.1)	2.0 (0.9-3.0)	1.6 (1.1-4.0)	2.4 (1.8-7.2)ψ*	1.8 (0.3-4.2)*
CD4-CD8-	6 (1-9)	4 (2-15)	5 (1-8)	5 (0-20)	3 (1-21)	4 (0-21)
CD4+CD8+	5 (0-12)	3 (1-8)	3 (0-9)	1 (0-8)	3 (1-8)	2 (0-6)
CD44	97 (94-98)	98 (96-100)	98 (96-99)	99 (96-99)	96 (95-99)	99 (82-100)
α4	35 (26-66)	36 (5-69)	39 (18-72)	43 (9-70)	58 (15-78)	26 (10-83)
LFA-1α	93 (87-98)	ND	96 (94-97)	ND	97 (94-98)	ND
αEß7	42 (16-62)ψ	39 (6-67)	23 (16-54)ψ	27 (13-38)	21 (8-29)ψ	25 (7-82)
CD3+CD25+	27 (11-47)	24 (10-52)	30 (9-44)	30 (15-66)	37 (11-63)	23 (3-54)
αEß7/CD25+	26 (9-54)	29 (12-56)	11 (7-38)	14 (11-22)	5 (2-14)*	12 (3-34)*

Flowcytometric expression of CD44, α4 integrin, αEß7 and LFA-1α on CD3 positive LPL from ileum, right and left hemicolon of patients with Crohn's disease and controls. Results are expressed as percentage positive cells. Median and range are displayed. * $p < 0.05$ between controls and Crohn's disease. ψ $p < 0.05$ between ileum and right/left hemicolon. ND : not determined.

Table IV. — **Expression of adhesion molecules on CD25 positive and negative T cells from lamina propria (LPL) of controls**

		Ileum	Right Hemicolon	Left Hemicolon
		N = 10	N = 10	N = 10
CD3+CD25+	CD44+	99 (93-100)	97 (91-99)	97 (93-100)
	α4+	42 (20-76) [1]	39 (19-80)	64 (24-77)
	αEß7+	26 (9-54) [2]	11 (7-38) [2]	5 (2-14) [2]
CD3+CD25-	CD44+	97 (93-98)	98 (96-99)	96 (95-99)
	α4+	33 (15-59) [1]	39 (17-65)	55 (13-78)
	αEß7+	46 (21-74) [2]	28 (19-62) [2]	28 (11-45) [2]

Flow cytometric expression of CD44, α4 integrin, αEß7 on CD25 positive and negative T cell subsets from ileum, right and left hemicolon are shown. Results are expressed as percentage positive cells. Median and range are displayed. Significant findings ($p < 0.05$) between CD25 positive and negative T cell subsets are indicated for α4 and αEß7, respectively by [1] and [2].

Table V. — **Expression of adhesion molecules on CD25 positive and negative T cells from lamina propria (LPL) of patients with Crohn's disease**

		Ileum	Right Hemicolon	Left Hemicolon
		N = 7	N = 7	N = 11
CD3+CD25+	CD44+	100 (98-100) [1]	99 (96-100)	99 (91-100)
	α4+	40 (6-77) [2]	53 (11-73)	36 (7-87)
	αEß7+	29 (12-56)	14 (11-22)	12 (3-34) [3]
CD3+CD25-	CD44+	97 (96-100) [1]	99 (96-100)	99 (80-100)
	α4+	32 (4-62) [2]	40 (9-63)	29 (11-79)
	αEß7+	35 (5-71)	29 (11-52)	34 (9-87) [3]

Flow cytometric expression of CD44, α4 integrin, αEß7 and on CD25 positive and negative T cell subsets from ileum, right and left hemicolon are shown. Results are expressed as percentage positive cells. Median and range are displayed. Significant findings ($p < 0.05$) between CD25 positive and negative T cell subsets are indicated for CD44, α4 and αEß7 respectively by [1], [2] and [3].

of adhesion molecules on CD25+ versus CD25- T cell subsets).

2.1. Cellular distribution

Contrary to IEL, the majority of T cells in the lamina propria are CD4+, resulting in a CD4/CD8 ratio > 1. Moreover, a clear regional difference was observed in LPL of controls : in ileal mucosa, a significantly lower CD4/CD8 ratio was observed compared to left hemicolon ($p < 0.01$, Wilcoxon test for paired samples ; Table III), due to a higher fraction of CD8+ T cells. This regional difference, however, was not observed in CD patients.

In left hemicolon, a decreased CD4/CD8 ratio was observed in CD versus controls ($p < 0.05$, Wilcoxon rank sum test ; Table III).

Similar to the observations in IEL, minor fractions of CD4+CD8+ and CD4-CD8- T cell subsets were observed (Table III).

2.2. Adhesion molecules

The expression of αEß7 in controls was higher in ileum as compared to right hemicolon ($p < 0.05$, Wilcoxon test for paired samples ; Table III) and left hemicolon ($p < 0.01$, Wilcoxon test for paired samples ; Table III). This regional difference parallels the regional differences in CD8+ T cell distribution noted in the lamina propria of controls.

The overall expression on T cells of CD44, α4 and αEß7 integrin was not different in CD versus controls (Table III). However, the αEß7 integrin was overexpressed in CD25+ T cell subsets in left hemicolon of CD, compared to controls ($p < 0.05$, Wilcoxon rank sum test ; Table III). This was also apparent when data from left and right hemicolon were pooled (Figure 1).

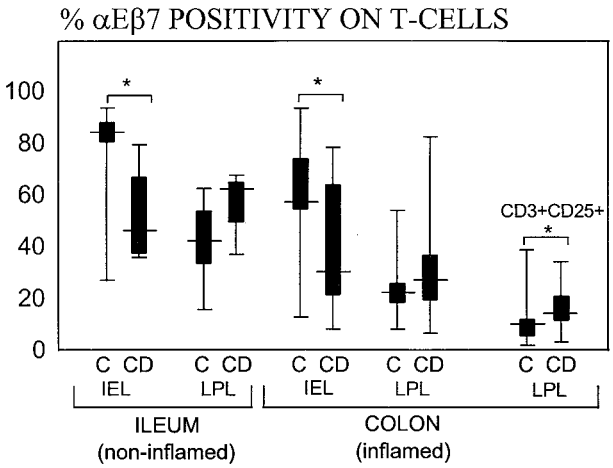

Fig. 1. — Flow cytometric expression of αEß7 integrin on intra-epithelial (IEL) and lamina propria lymphocytes (LPL) from ileum and colon (right and left hemicolon) of patients with Crohn's disease (CD) and controls. Results are expressed as percentage positive T cells. Box plot ; solid line = median, box = interquartile range, error bar = range. * $p < 0.05$ between CD and controls.

In addition to the overall expression of the various tested adhesion molecules on T cells, the distribution in CD25+ versus CD25- T cell subsets was evaluated as well, both in controls and CD (Table IV and V). In LPL of controls, αEß7 integrin expression is preferentially confined to the CD25- T cells, in all the tested localisations ($p < 0.01$; Wilcoxon test for paired samples ; Table IV). In ileum, the α4 integrin was overexpressed in CD25+ versus CD25- subsets ($p < 0.05$, Wilcoxon test for paired samples ; Table IV). In CD patients, the preferential expression of αEß7 on CD25- T cell subsets could only be documented in left hemicolon (Table V). Moreover, in ileum of CD patients, CD44 and α4 integrin expression was overexpressed in CD25+ versus CD25- subsets (Table V).

Discussion

This study focuses on the expression of adhesion molecules on T cells from the intestinal epithelial layer and from the lamina propria of patients with CD and controls. Several previous studies on resected specimens of small and large intestine, using immunohistochemistry as well as flow cytometry, indicated that the CD4/CD8 ratio should be lower than 1 in IEL and higher than 1 in LPL (11,24). In these publications right and left colon were not evaluated separately. The appropiate enrichment of IEL and LPL in the present study, is illustrated by the respective CD4/CD8 ratio's in controls, which are in agreement with earlier reports (11,24). The expression of αEß7 integrin on IEL and LPL from controls is also consistent with previous reports, using a similar enzymatic extraction procedure (11,12,25). It should be noted that the degree of αEß7 expression on IEL and LPL in most of these studies was derived from experiments on human small intestinal tissue (12,13,26) or mixed large and small intestine (14,27). In two studies, the expression of this ß7 integrin family member was evaluated in small and large intestine separately : the number of αEß7 expressing IEL and LPL decreases from the proximal to the distal part of the colon in one report (11), but not in the other (25). However, the results of the latter study are impeded by the small number of specimens analysed (small bowel 3, large bowel 7). In the present report, in controls, a higher level of αEß7 integrin expression was observed on LPL from ileum, compared to colon. A similar regional difference in the CD8+ T cell distribution was noted, with a significantly higher frequency of CD8+ T cells in ileum, compared to left hemicolon. It can not be excluded that contamination of cells from lymphoid follicles may have influenced some results, as previously suggested (28). However, despite a similar extraction procedure used for different regions within one individual, different results were observed in ileum versus colon. Considering the previously reported overexpression of αEß7 on CD8+ versus CD4+ subsets (25), it could be postulated that the regional differences in αEß7 expression reflect the

altered CD4/CD8 ratio. However, in the present study, the expression of αEß7 was only studied on CD3 positive cells.

A first observation in CD, was a decreased CD4/CD8 ratio in the lamina propria from left hemicolon, compared to controls. Similar observations have been previously reported (9), although others failed to demonstrate differences in CD4/CD8 ratio's (22,24,29).

Of particular interest was the expression of the tested adhesion molecules in CD25+ and CD25- T cell subsets. This subdivision was made given the role of IL-2R in T cell activation. Indeed, T cell receptor mediated antigen recognition leads to T cell activation and IL-2 production, which functions as an essential T cell growth and differentiation factor (30,31). The effects of IL-2 are mediated by the IL-2R complex, which is expressed by activated but not by resting T cells. The literature data of CD25 expression in IEL are quite conflicting. Some authors reported a very low to absent expression in IEL whatever the method (immunohistochemistry or flow cytometry) is applied (32,33). Others reported an expression up to 30% (34,35) analysed with flow cytometry. These data might shown that there is an apparent discrepancy between immunohistochemistry and flow cytometry. The use of flow cytometry is more sensitive than immunohistochemistry. This may explain why we and others found an higher expression of CD25 in IEL. In the present study, a preferential expression of αEß7 on CD25- subsets was observed in the lamina propria from controls, and this in all localisations analysed. Moreover, the α4-integrin was overexpressed in CD25+ T cell subsets of ileum. The positive versus negative correlation of CD25 with these markers may reflect: 1) a simultaneous appearance on the cell surface of T cells upon IL-2 mediated activation (positive correlation) or 2) a non-random T cell activation restricted to well-defined T cell subsets (positive and negative correlation).

The most prominent observation of the present study was the altered expression of αEß7 in IEL and LPL in CD versus controls. In the epithelial layer, a decreased expression was observed in patients with CD, despite similar CD4/CD8 ratio's. This was most prominent in ileum. Interestingly, this observation was present in both histologically inflamed as well as non-inflamed tissue, underscoring the specificity of the phenomenon. An overexpression of αEß7 in the lamina propria from left hemicolon of CD patients, was only present within the CD25+ T cell subset. This extends the increased expression of the αEß7 integrin on IL-2 expanded T cell lines from inflamed mucosa of CD-patients versus controls, made by our group (unpublished observations), to the in situ activated T cell subsets.

How to explain the differences between CD and controls ? Given the role of these integrins in diverse aspects of lymphocyte development, trafficking and immune responses, several possibilities are available such as different T cell activation pathways, discriminative cytokine patterns, or distinctive homing patterns. The lowered expression of αEß7 in CD cannot be attributed to a T cell activation event as the expression of this integrin is upregulated in case of T cell activation by various mitogens (25), making alternative explanations more likely. One might postulate that aberrant homing is the result of migration of αEß7+ intra-epithelial T-cells to the lamina propria, although this has not been described previously. Alternatively, the epithelial layer in CD could be enriched with αEß7 low expressors from the lamina propria. In the latter scenario, chemotactic signals released from the intestinal layer under specific circumstances might play a crucial role (36,37). Especially, the observation that αEß7 integrin expression is decreased in IEL, in the absence of histological inflammation, supports the view that this may be one of the earliest events in the pathogenesis of CD.

Aberrant homing may not only play a pathogenic role at the mucosal site in CD patients, but may also be involved in the pathogenesis of extra-intestinal manifestations of CD, such as peripheral arthritis (38). In fact, there are data supporting this concept of T cell migration (39,40). Therefore, if one could define pathogenic T cell subsets in CD based on a specific pattern of adhesion molecule expression, these may form targets for specific immunotherapy. The promising results of anti-adhesion therapy by anti-ICAM-1 antisense therapy may serve as an illustration (41).

In conclusion, in the present study a decreased expression of the αEß7 integrin on IEL from CD compared to controls was noted, in inflamed as well as in non-inflamed intestinal tissue. In addition, an overexpression of this ß7 integrin family member was noted on CD25+ T cells from colon of CD versus controls.

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References

1. SALMI M., GRANFORS K., MACDERMOTT R., JALKANEN S. Aberrant binding of lamina propria lymphocytes to vascular endothelium in inflammatory bowel diseases. *Gastroenterology*, 1994, **106** : 596-605.
2. PODOLSKY D.K. Inflammatory bowel disease (2). *N. Engl. J. Med.*, 1991, **325** : 1008-1016.
3. PODOLSKY D.K. Inflammatory bowel disease (1). *N. Engl. J. Med.*, 1991, **325** : 928-937.
4. MACDERMOTT R.P., STENSON W.F. Alterations of the immune system in ulcerative colitis and Crohn's disease. *Adv. Immunol.*, 1988, **42** : 285-328.
5. STROBER W., JAMES S.P. The immunologic basis of inflammatory bowel disease. *J. Clin. Immunol.*, 1986, **6** : 415-432.
6. SIMPSON S.J., MIZOGUCHI E., ALLEN D., BHAN A.K., TERHORST C. Evidence that CD4+, but not CD8+ T cells are responsible

for murine interleukin-2-deficient colitis. *Eur. J. Immunol.*, 1995, **25** : 2618-2625.
7. NIESSNER M., YOLK B.A. Phenotypic and immunoregulatory analysis of intestinal T-cells in patients with inflammatory bowel disease : evalution of an in vitro model. *Eur. J. Clin. Invest.*, 1995, **25** : 155-164.
8. BRANDTZAEG P., HALSTENSEN T.S., KETT K., KRAJCI P., KVALE D., ROGNUM T.O., SCOTT H., SOLLID L.M. Immunobiology and immunopathology of human gut mucosa : humoral immunity and intraepithelial lymphocytes. *Gastroenterology*, 1989, **97** : 1562-1584.
9. SENJU M., HULSTAERT F., LOWDER J., JEWELL D.P. Flow cytometric analysis of peripheral blood lymphocytes in ulcerative colitis and Crohn's disease. *Gut*, 1991, **32** : 779-783.
10. SELBY W.S., JANOSSY G., BOFILL M., JEWEL D.P. Intestinal lymphocyte subpopulations in inflammatory bowel disease : an analysis by immunohistological and cell isolation techniques. *Gut*, 1984, **25** : 32-40.
11. CERF-BENSUSSAN N., JARRY A., BROUSSE N., LISOWSKA GROSPIERRE B., GUY-GRAND D., GRISCELLI C. A monoclonal antibody (HML-1) definfing a novel membrane molecule present on human intestinal lymphocytes. *Eur. J. Immunol.*, 1987, **17** : 1279-1285.
12. JARRY A., CERF-BENSUSSAN N., BROUSSE N., SELZ F., GUY-GRAND D. Subsets of CD3+ (T cell receptor alpha/beta or gamma/delta) and CD3- lymphocytes isolated from normal human gut epithelium display phenotypical features different from their counterparts in peripheral blood. *Eur. J. Immunol.*, 1990, **20** : 1097-1103.
13. KRUSCHWITZ M., FRITZSCHE G., SCHWARTING R., MICKLEM K., MASON D.Y., FALINI B., STEIN H. Ber-ACT8 : new monoclonal antibody to the mucosa lymphocyte antigen. *J. Clin. Pathol.*, 1991, **44** : 636-645.
14. SCHIEFERDECKER H.L., ULLRICH R., HIRSELAND H., ZEITZ M. T cell differentation antigens on lymphocytes in the human intestinal lamina propria. *J. Immunol.*, 1992, **149** : 2816-2822.
15. KAULFERSCH W., FIOCCHI C., WALDMANN T.A. Polyclonal nature of the intestinal mucosal lymphocyte populations in inflammatory bowel disease. A molecular genetic evaluation of the immunoglobulin and T-cell antigen receptors. *Gastroenterology*, 1998, **95** : 364-370.
16. HARVEY J., JONES D.B., WRIGHT D.H. Leucocyte common antigen expression on T cells in normal and inflamed human gut. *Immunology*, 1989, **68** : 13-17.
17. SCHREIBER S., MACDERMOTT R.P., RAEDLER A., PINNAU R., BERTOVICH M.J., NASH G.S. Increased activation of isolated intestinal lamina propria mononuclear cells in inflammatory bowel disease. *Gastroenterology*, 1991, **101** : 1020-1030.
18. PODOLSKY D.K., LOBB R., KING N., BENJAMIN C.D., PEPINSKY B., SEGHAL P., DEBEAUMONT M. Attenuation of colitis in the cotton-top tamarin by anti-alpha 4 integrin monoclonal antibody. *J. Clin. Invest.*, 1993, **92** : 372-380.
19. HESTERBERG P.E., WINSORHINES D., BRISKIN M.J., SOLER-FERRAN D., MERRIL C., MACKAY C.R., NEWMAN W., RINGLER D.J., WINSOR HINES D., SOLER FERRAN D. Rapid resolution of chronic colitis in the cotton top tamarin with an antibody to a gut homing integrin alpha 4 beta 7. *Gastroenterology*, 1996, **111** : 1373-1380.
20. HERMISTON M.L., GORDON J.I. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science*, 1995, **270** : 1203-1207.
21. MACDONALD T.T., SPENCER J. Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J. Exp. Med.*, 1988, **167** : 1341-1349.
22. LYNCH S., KELLEHER D., FEIGHERY C., WEIR D.G., O'FARRELLY C. Flow cytometric analysis of intra-epithelial lymphocytes from human small intestinal biopsies reveals populations of CD4+CD8+ and CD8aa+ cells. *Eur. J. Gastr. Hepat.*, 1993, **5** : 907-912.
23. BALZANO A., BOVE A., GRANDE G., BEVILACQUA N. Flow cytometric analysis of lymphocytes isolated from endoscopic biopsies in ulcerative colitis. *Eur. J. Gastr. Hept.*, 1994, **6** : 203-208.
24. HIRATA I., BERREBI G., AUSTIN L.L., KEREN D.F., DOBBINS W.O.I. Immunohistological characterization of intraepithelial and lamina propria lymphocytes in control ileum and colon and in inflammatory bowel disease. *Dig. Dis. Sci.*, 1986, **31** : 593-603.
25. SCHIEFERDECKER H.L., ULLRICH R., WEISS BRECKWOLDT A.N., SCHWARTING R., STEIN RIECKEN E.O., ZEITZ M. The HML-1 antigen of intestinal lymphocytes is an activation antigen. *J. Immunol.*, 1990, **144** : 2541-2549.
26. ROBERTS A.I., O'CONNELL S.M., EBERT E.C. Intestinal intraepithelial lymphocytes bind to colon cancer cells by HML-1 and CD11a. *Cancer Res.*, 1993, **53** : 1608-1611.
27. RUTHLEIN J., HEINZE G., AUER I.O. Anti CD-2 and anti CD-3 induced T cell cytotoxicity of human intraepithelial and lamina propria lymphocytes. *Gut*, 1992, **33** : 1626-1632.
28. TREJDOSIEWICZ L.K. Intestinal intraepithelial lymphocytes and lymphoepithelial interactions in the human gastrointestinal mucosa. *Immunology Letters*, 1992, **32** : 13-19.
29. JAMES S.P., FIOCCHI C., GRAEFF A.S., STROBER W. Immunoregulatory function of lamina propria T cells in Crohn's disease. *Gastroenterology*, 1985, **88** : 1143-1150.
30. SMITH K.A. Interleukin-2. *Annu. Rev. Immunol.*, 1984, **2** : 319-333.
31. ROBB R.J. Interleukin-2 : the molecule and its function. *Immunol. Today*, 1984, **5** : 319-333.
32. ABUZAKOUK M., KELLEHER D., FEIGHERY C., O'FARRELLY C. Increased HLA-DR and decreased CD3 on human intestinal intraepithelial lymphocytes : evidence of activation ? *Gut*, 1996, **39** : 396-400.
33. JARRY A., CERF-BENSUSSAN N., BROUSSE N., GUY-GRAND D., MUZEAU F., POTET F. Same peculiar subsets of HML1+ lymphocytes present within normal intestinal epithelium is associated with tumoral epithelium of gastrointestinal carcinomas. *Gut*, 1988, **29** : 1632-1638.
34. ULLRICH R., SCHIEFERDECKER H.L., ZIEGLER K., RIECKEN E.O., ZEITZ M. γδ T cells in the human intestine express surface markers of activation and are preferentially located in the epithelium. *Cellular Immunology*, 1990, **128** : 619-627.
35. SNIJDERS F., MEENAN J., VAN DEN BLINK B., VAN DEVENTER S.J.H., TEN KATE F.J.W. Duodenal intraepithelial and lamina propria T lymphocytes in human immunodeficiency virus-infected patients with and without diarrhoea. *Scan. J. Gastroenterol.*, 1996, **31** : 1176-1181.
36. ECKMANN L., KAGNOFF M.F., FIERER J. Intestinal epithelial cells as watchdogs for the natural immune system. *Trends Microbiol.*, 1995, **3** : 118-120.
37. JUNG H.C., ECKMANN L., YANG S.K., PANJA A., FIERER I., MORZYCKA WROBLEWSKA E., KAGNOFF M.F. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response ot bacterial invasion. *J. Clin. Invest.*, 1995, **95** : 55-65.
38. ELEWAUT D., GRANFORS K., DE KEYSER F., HOFFMAN I., VEYS E.M. Extra-intestinal manifestations of enteropathies : why are the joints involved ? *Rheumatology in Europe*, 1997, **26** : 18-23.
39. SALMI M., ANDREW D.P., BUTCHER E.C., JALKANEN S. Dual binding capacity of mucosal immunoblasts to mucosal and synovial endothelium in humans : dissection of the molecular mechanisms. *J. Exp. Med.*, 1995, **181** : 137-149.
40. SALMI M., GRANFORS K., LEIRSALO REPO M., HAMALAINEN M., MACDERMOTT R., LEINO R., HAVIA T., JALKANEN S. Selective endothelial binding of interleukin-2-dependent human T-cell lines derived from different tissues. *Proc. Natl. Acad. Sci. USA*, 1992, **89** : 11436-11440.
41. YACYSHYN B., WOLOSCHUK B., YACYSHYN M.B., MARTINI D., DOAN K., TAMI J., BENNET F., KISNER D., SHANAHAN W. Efficacy and safety of ISIS 2302 (ICAM-1 antisense oligonucleotide) treatment of steroid-dependent Crohn's disease. *Gastroenterology*, 1997, **112** : A1123.