Poor diagnostic value of colonic CD44v6 expression and serum concentrations of its soluble form in the differentiation of ulcerative colitis from Crohn's disease

W Reinisch, K-H Heider, G Oberhuber, C Dejaco, M Müllner, G R Adolf, C Gasché

Abstract

Background—Increased expression of CD44v6 on colonic crypt epithelial cells in ulcerative colitis has been suggested as a diagnostic tool to distinguish ulcerative colitis from colonic Crohn's disease.

Aims—To investigate colonic CD44v6 expression and serum concentrations of soluble CD44v6 (sCD44v6) in patients with ulcerative colitis and Crohn's disease.

Methods—Colonic biopsy samples were obtained from 16 patients with ulcerative colitis, 13 with ileocolonic Crohn's disease, and 10 undergoing polypectomy. Serum samples were obtained from 15 patients with active ulcerative colitis, 20 with active Crohn's disease, and 20 healthy donors. Colonic CD44v6 expression was evaluated immunohistochemically by monoclonal antibody 2F10 and the higher affinity monoclonal antibody VFF18. Serum sCD44v6 concentrations were measured by ELISA.

Results—2F10 stained colonic epithelium of inflamed ulcerative colitis and Crohn's disease samples in 80% and 40% of cases, respectively, and VFF18 in 95% and 87%, respectively. Both monoclonal antibodies displayed a sensitivity and specificity of 60% and 87% to differentiate ulcerative colitis from colonic Crohn's disease. Serum concentrations of sCD44v6 were lower in patients with ulcerative colitis (median 153 ng/ml; interquartile range (IQR) 122–211) compared with Crohn's disease (219; IQR 180–243) and healthy donors (221; IQR 197–241 ($p=0.002$)). Its sensitivity and specificity to discriminate ulcerative colitis from Crohn's disease was 75% and 71%, respectively.

Conclusion—Colonic CD44v6 and serum sCD44v6 concentrations do not facilitate reliable differential diagnosis between ulcerative colitis and Crohn's disease.

(Gut 1998;43:375–382)

Keywords: CD44 variant 6; differential diagnosis; immunohistochemistry; soluble CD44v6

precise diagnosis is required as both diseases differ in their natural course and complications,¹ the opposing effect of cigarette smoking on disease severity,^{5,6} and the response to treatment. In particular, the high risk for recurrence of Crohn's disease within the ileal reservoir after ileal pouch-anal anastomosis supports the necessity of a correct differential diagnosis.⁷

The investigation of several immunological parameters, such as adhesion molecules, immunoglobulins, cytokines, and their receptors in serum and intestinal lamina propria of patients with IBD could not identify a sensitive and specific test to distinguish between ulcerative colitis and Crohn's disease.^{8–12} An increased prevalence of perinuclear antineutrophil cytoplasmic antibodies (pANCA) has been described in serum of patients with ulcerative colitis and their clinically unaffected family members, suggesting that pANCA is a potential marker of genetic susceptibility to ulcerative colitis.¹³ However, the significant proportion of pANCA negative ulcerative colitis patients and the identification of a small subgroup of Crohn's disease patients with pANCA positivity as a specific clinical phenotype with features of ulcerative colitis limits the clinical utility of this serological marker for a precise differentiation between both diseases.¹⁴ The detection of matrix metalloproteinase I mRNA in inflamed lamina propria of the colon in patients with ulcerative colitis and the increased expression of substance P receptors on enteric neurones of patients with Crohn's disease seem to be novel, promising approaches to distinguish between the two entities of IBD, but the significance of these observations needs to be further substantiated by examination of larger patient populations.^{15,16} As no differentiating single gold standard has been established, discrimination of ulcerative colitis and Crohn's disease continues to be based on a combination of clinical, laboratory, endoscopic, histopathological, radiographic, and sonographic observations.^{2,17,18}

Recently, Rosenberg *et al* reported the immunohistochemical detection of increased expression of CD44 variant 6 (CD44v6) by staining with monoclonal antibody 2F10 on colonic crypt epithelium in ulcerative colitis, but not colonic Crohn's disease and suggested a reliable diagnostic procedure to distinguish these two disorders.¹⁹ CD44 encompasses a polymorphic family of transmembrane glycoproteins which serve as adhesion molecules for

Department of Clinical Pharmacology
W Reinisch

Clinic of Internal Medicine IV,
Department of Gastroenterology and Hepatology
C Dejaco
C Gasché

Clinic of Pathology
G Oberhuber

Department of Emergency Medicine,
University of Vienna
M Müllner

Boehringer Ingelheim Research and Development,
Department of Cell Biology, Vienna, Austria
K-H Heider
G R Adolf

Correspondence to:
Dr W Reinisch, Univ.-Klinik für Klinische Pharmakologie, Neues AKH, Waehringer Guertel 18–20, A-1090 Vienna, Austria.

Accepted for publication
10 March 1998

Crohn's disease and ulcerative colitis are regarded as distinct entities of inflammatory bowel disease (IBD)^{1,2}; however, in approximately 10–20% of cases the definite classification remains impossible by macroscopic and microscopic examination.^{3,4} A

Table 1 Patient characteristics

Variable	Ulcerative colitis	Crohn's disease
No of patients (F/M)	16 (6/10)	13 (8/5)
Median (IQR) age (y)	41 (27–50)	32 (23–38)
Median (IQR) disease duration (y)	7 (5–10)	6 (4–12)
No (%) of patients receiving		
No medication	3 (19)	6 (46)
5-aminosalicylic acid, sulphasalazine	10 (63)	7 (54)
Steroids	4 (25)	2 (15)
Azathioprine	3 (19)	1 (8)
Total number of colonic biopsy samples investigated	24	17
No (%) of UC colonic biopsy samples histologically graded as		
Remission	4 (17)	
Mild	8 (33)	
Moderate	8 (33)	
Severe	4 (17)	
No (%) of CD colonic biopsy samples histologically graded as		
Normal mucosa		2 (12)
Active CD lesions		15 (88)

CD, Crohn's disease; UC, ulcerative colitis.

extracellular matrix components, such as hyaluronan, fibronectin, and fibrin mediating lymphocyte activation and homing, haematopoiesis, cell migration, and tumour metastasis.^{20–21} CD44 exists as a broadly distributed 80–90 kDa standard isoform (CD44s) and multiple, higher molecular weight isoforms with different extracellular regions due to alternative RNA splicing of variant exons (CD44v) and post-translational modifications.^{22–23}

Whereas in normal colon a CD44v6 epitope is not or just weakly detectable at the base of the crypt epithelium, increased expression is found in adenomatous colorectal polyps and most invasive colon carcinomas.^{24–27} The initial description of an upregulation of CD44v6 on colonic crypt epithelial cells in ulcerative colitis in contrast to colonic Crohn's disease has raised particular interest both in clinicians and pathophysiologists. However, results of subsequent investigations from other groups resulted in disagreement and concerns about the specificity of this finding.^{28–30} Immunohistochemical analysis of CD44v6 in IBD has been confined to the application of monoclonal antibody 2F10, which shows only low binding affinity compared with other CD44v6 specific antibodies.³¹ Since data on the distribution of CD44 variants is conflicting, depending on the antibodies used, further examinations of colonic CD44v6 in IBD with different monoclonal antibodies are warranted.³²

In the present study we investigated whether colonic samples of patients with ulcerative colitis can be differentiated from colonic Crohn's disease by means of epithelial CD44v6 expression. The quantitative and qualitative immunohistochemical staining with the monoclonal antibody 2F10 was compared with our higher affinity monoclonal antibody VFF18.³¹ Furthermore, we evaluated the diagnostic performance of serum concentrations of sCD44v6 to discriminate between patients in the clinically active phase of ulcerative colitis and Crohn's disease.

Patients and methods

PATIENT POPULATION

The study was performed according to the Helsinki Declaration and after ethical approval was obtained from the local ethics committee.

The diagnoses of ulcerative colitis and Crohn's disease were based on established clinical, radiological, endoscopic, and histopathological criteria.^{33–34} In all patients a specific disease history of at least three years had to be recorded. Extent of bowel involvement was documented by radiological and/or endoscopic investigations within one year before study entry. Extensive ulcerative colitis was defined as macroscopic disease extending at least to the hepatic flexure.

Twenty four colonic biopsy samples from 16 patients with ulcerative colitis, 17 colonic samples from 13 patients with ileocolonic Crohn's disease, and 10 normal colonic samples from patients undergoing surveillance after polypectomy were obtained by endoscopy (table 1). The serum samples of 15 other consecutive patients with ulcerative colitis (seven women, eight men; median age 32 years (interquartile range (IQR) 27–40); median disease duration four years (IQR 3–8)) and 20 with Crohn's disease (13 women, seven men; median age 25 years (IQR 21–36); median disease duration five years (IQR 3–8)), as well as 20 age and sex matched healthy donors (13 women, seven men; median age 27 years (IQR 20–49)) were studied. Disease activity of ulcerative colitis and Crohn's disease was determined by the clinical activity index (CAI) according to Rachmilewitz and the Crohn's disease activity index (CDAI), respectively.^{35–36} For the serum study protocol only clinically active patients with ulcerative colitis (CAI>4; left sided colitis 7/15, 47%; pancolitis 8/15, 53%) and ileocolonic Crohn's disease (CDAI>200), who had not received steroids or other immunosuppressive drugs within two months were included. Medication with 5-aminosalicylates and antidiarrhoeal drugs was allowed.

Blood samples from patients and healthy donors were obtained by venepuncture. After clotting, sera were centrifuged and stored within one hour in volumes of 0.5 ml at –20°C.

ANTIBODY CHARACTERISATION

The CD44v6 specific monoclonal antibody VFF18 (IgG1) was generated by immunising BALB/c mice with a glutathione S-transferase (GST) fusion protein containing the human variant CD44 portion v3–v10. Epitope mapping of VFF18 was performed by enzyme linked immunosorbent assay (ELISA) with several peptides spanning the exon v6 domain as described by Heider *et al.*³¹ The CD44v6 specific monoclonal antibody 2F10 (IgG1) was purchased from R&D Systems, Abingdon, UK.

Affinity and kinetics of monoclonal antibodies VFF18 and 2F10 were determined by surface plasmon resonance using a BIAcore 2000 system (Pharmacia Biosensor) and competitive binding ELISAs with a GST CD44 fusion protein containing a region encoded by exons v3–v10 as immobilised antigen.³¹

IMMUNOHISTOCHEMISTRY

Formalin fixed and paraffin wax embedded sections of biopsy samples were dewaxed in xylol and rehydrated in a descending alcohol

series. The sections were briefly washed in distilled water and microwave based antigen retrieval was performed. Subsequently, sections were washed in phosphate buffered saline (PBS), preincubated with normal goat serum (10% in PBS), and after three additional washes with PBS incubated with the primary antibody (VFF18, 250 ng/ml; 2F10, 500 ng/ml; isotype matched negative control 500 ng/ml) for one hour. Endogenous peroxidases were then blocked in 0.3% hydrogen peroxide in PBS and sections were incubated with the secondary biotinylated antibody for 30 minutes (anti-mouse IgG, F(ab')₂, Dako, Glostrup, Denmark). Visualisation of the immunocomplex was performed by incubation with a streptavidin-biotin-peroxidase complex (Dako) for another 30 minutes and with 3,3-amino-9-ethylcarbazole substrate (Sigma, Poole, Dorset, UK) for 5–10 minutes. The sections were counterstained in haematoxylin and mounted. As positive control for the staining reaction, normal human skin sections were used, as keratinocytes express CD44v3–v10.³⁷

Immunohistochemistry sections were read by two observers blinded for the diagnosis. Histological severity of ulcerative colitis was evaluated on haematoxylin-eosin stained sections only and graded as non-inflamed, mildly, moderately, or severely inflamed. Samples from patients with Crohn's disease were rated as normal or inflamed. For evaluation of staining results with anti-CD44v6 monoclonal antibodies VFF18 and 2F10, the percentage of positive colonic epithelial cells on each section was expressed in 10% increments.

Staining intensity of CD44 antibodies was graded from 0 to +3, with keratinocyte staining serving as a +3 reference for strong expression; +2 signified moderate expression, +1 weak expression, and 0 equivocal or no expression.

LABORATORY ASSESSMENTS

Serum C reactive protein

Serum C reactive protein was measured by a turbidimetric method (Tina-quant a CRP turbidimetrie BM, Hitachi-747, Boehringer Mannheim GmbH, Mannheim, Germany).

Soluble CD44v6

For determination of serum soluble CD44v6 (sCD44v6), 96 well immunoassay plates (Nunc Immunoplate I) were coated with monoclonal antibody VFF18 (5 µg/ml in 50 mM sodium carbonate, pH 9.6) overnight at 4°C. The plates were washed with PBS/0.05% Tween 20 (washing buffer) and blocked for one hour at room temperature with PBS/0.05% Tween 20/0.5% bovine serum albumin (assay buffer). After additional washes the plates were filled with patient sera (serial dilutions; 100 µl/well) and a solution of peroxidase conjugated monoclonal antibody BU-52 (anti-CD44s) for three hours at room temperature. The plates were washed three times; a solution of streptavidin coupled to horseradish peroxidase (Boehringer) was added (150 µl/well) and incubated for two hours at room temperature. After an additional three washes, tetramethyl benzidine substrate solution was added (Ki-

erkegaard and Perry Laboratories, Gaithersburg, USA; 100 µl/well) for 10–20 minutes at room temperature and the reaction was stopped with 1 M phosphoric acid (100 µl/well). The absorbance was read in a Hewlett-Packard ELISA reader. All samples were run in duplicate; the detection limit was 10 ng/ml.

STATISTICAL ANALYSIS

Continuous data are presented as median and range from the 25th to the 75th quartile (IQR). The comparison between groups of continuous data was performed by means of the Mann-Whitney U test, or if appropriate by means of Kruskal-Wallis analysis of variance. Dichotomous data were compared by means of Fisher's exact test. The association between the staining pattern of monoclonal antibodies and the histological activity of ulcerative colitis was assessed by means of Rank Spearman correlation (r_s). Bonferroni correction was used to control for multiple comparisons. In order to determine the diagnostic value of the monoclonal antibodies, receiver operating characteristic (ROC) curves were constructed.³⁸ Sensitivity and specificity were calculated according to standard formulae. To facilitate the detection of a diagnostic cut off value the Youden Index was determined ((sensitivity + specificity) – 100).³⁹ The closer the index approaches 100, the better is the performance of a test. Agreement between the two methods was assessed according to a method described by Bland and Altman and is presented as the bias (mean difference between the two methods), the upper and lower limit of agreement.⁴⁰

Results

IMMUNOHISTOLOGICAL DETECTION OF CD44v6 EXPRESSION IN ULCERATIVE COLITIS AND CROHN'S DISEASE

Competitive ELISA and surface plasmon resonance measurements revealed that the CD44v6 specific monoclonal antibodies 2F10 and VFF18 used in this study, recognise a centrally located 14 mer (amino acid residues 18–31) encoded by CD44 exon v6. However, VFF18 showed a threefold higher dissociation constant compared with 2F10 indicating a stronger binding affinity to its epitope.³¹ Therefore, 2F10 was used in a higher concentration than VFF18 in our standard immunohistochemistry protocol to give similar staining intensities of keratinocytes in skin tissue sections. In colonic samples only cells with a clear membrane staining were scored positive.

In normal colonic tissue from control patients, staining for CD44v6 by 2F10 and VFF18 was either negative or limited to single cells at the crypt base. In all colonic sections from patients with ulcerative colitis and Crohn's disease, CD44v6 expression was predominantly detected on colonic epithelial cells of the crypts. Only distinct lymphocytic cells of the lamina propria in ulcerative colitis and Crohn's disease stained positive with VFF18. Therefore, the cut off level for epithelial

Table 2 Percentage of CD44v6 positive crypt epithelial cells and intensity of staining with 2F10 and VFF18 in inflamed colonic samples of patients with ulcerative colitis and Crohn's disease

Antibody	Ulcerative colitis (n=20)	Crohn's disease (n=15)	p Value
2F10 (%)	75 (20–80)	10 (0–50)	0.01
2F10 (intensity)	2 (1–2)	1 (0–1)	0.02
VFF18 (%)	90 (80–100)	60 (50–80)	0.03
VFF18 (intensity)	3 (2–3)	2 (1–2)	0.002

Results are expressed as median (IQR).

reactivity of both monoclonal antibodies was set at more than 10% positive colonic epithelial cells.

Staining with 2F10 in IBD samples

Sixteen of 20 ulcerative colitis samples (80%) with mild, moderate, or severe inflammation and six of 15 Crohn's disease samples (40%) with inflamed mucosa displayed epithelial reactivity ($p=0.019$). The percentage of positive colonic epithelial cells per sample and staining intensity were higher in ulcerative colitis than in Crohn's disease (table 2). From non-inflamed ulcerative colitis lesions one of four samples showed epithelial reactivity with 20% positive cells. The non-inflamed mucosa in two samples from patients with Crohn's disease remained negative. The percentage of positive staining was not associated with histological grade of inflammation in patients with ulcerative colitis ($r_s=0.27$, $p=0.20$, $n=24$). Overall, there was a good correlation between the percentage of CD44v6 positive cells and

staining intensity by 2F10 in the IBD samples ($r_s=0.89$, $p<0.001$, $n=41$).

Staining with VFF18 in IBD samples

Epithelial reactivity was assessed in 95% (19/20) of the ulcerative colitis samples and 87% (13/15) of the Crohn's disease samples with histological signs of inflammation ($p=0.56$). The percentage of positive colonic epithelial cells per sample and staining intensity were higher in ulcerative colitis than in Crohn's disease (table 2). Among non-inflamed ulcerative colitis samples a clear staining of 70% and 40% positive cells was counted in two of four sections, respectively, whereas 20% positive cells were detectable in the other two samples. In one of two Crohn's disease samples with non-inflamed mucosa 40% of colonic epithelial cells reacted with VFF18. In ulcerative colitis no association between the extent of histological inflammation and the percentage of positive cells was observed ($r_s=0.27$, $p=0.20$, $n=24$). The percentage of positive cells correlated with staining intensity when calculated for all IBD samples ($r_s=0.82$, $p<0.001$, $n=41$).

COMPARISON BETWEEN MONOCLONAL ANTIBODIES 2F10 AND VFF18

When we compared the staining results of VFF18 with 2F10 in all IBD samples a significantly higher percentage of positive colonic epithelial cells (80 (IQR 50–90) versus 20 (IQR 0–80), $p<0.001$) was displayed by the

Figure 1 Biopsy sample from inflamed colon of a patient with ulcerative colitis stained with anti-CD44v6 monoclonal antibody 2F10 (A) and VFF18 (B). Biopsy sample from inflamed colon of a patient with colonic Crohn's disease stained with anti-CD44v6 monoclonal antibody 2F10 (C) and VFF18 (D). Immunoperoxidase, original magnification $\times 200$.

Table 3 Sensitivity and specificity of intensity of staining by 2F10 and VFF18 in the diagnosis of ulcerative colitis

Score	Samples included	Sensitivity (%)	Specificity (%)
2F10			
3	4	20	100
2	15	60	80
1	29	95	33
0	35	100	0
VFF18			
3	14	65	93
2	29	95	33
1	34	100	7
0	35	100	0

Figure 2 ROC curves of the diagnostic properties for the percentage of positive staining with 2F10 and VFF18 to diagnose ulcerative colitis in inflamed colonic samples of patients with ulcerative colitis and Crohn's disease.

former (fig 1). However, a highly significant association between the percentage of positive cells by 2F10 and VFF18 was obtained ($r=0.66$, $p<0.001$, $n=41$). The overall agreement in 54 samples (healthy donors, ulcerative colitis, Crohn's disease) between the two monoclonal antibodies exhibited a mean bias of 23%; the upper limit of agreement was 80%, and the lower limit of agreement was -33%.

DIAGNOSTIC VALUE OF MONOCLONAL ANTIBODIES 2F10 AND VFF18 IN THE DIFFERENTIATION BETWEEN ULCERATIVE COLITIS AND CROHN'S DISEASE

For the differentiation of ulcerative colitis from Crohn's disease by 2F10, the maximum Youden Index achieved was 47 at a cut off value of 60% positive colonic epithelial cells with a corresponding sensitivity of 60% and specificity of 87%. At a cut off value at 10% the corresponding sensitivity and specificity were 85% and 47%, respectively (fig 2). Table 3 shows the diagnostic properties of 2F10 staining intensities. Data reveal a high specificity at cut off values of 2 and 3, respectively, combined with low sensitivity values.

The diagnostic qualities of monoclonal antibody VFF18 to differentiate ulcerative colitis from Crohn's disease are given by the maximum Youden Index of 47 at a cut off value of 90% positive colonic epithelial cells with a corresponding sensitivity of 60% and specificity of 87% (fig 2). Table 3 shows the diagnostic value of VFF18 staining intensity to differentiate ulcerative colitis from Crohn's disease.

Figure 3 Box-Whisker plots of soluble serum CD44v6 concentrations in age matched healthy donors, patients with Crohn's disease (CD), and patients with ulcerative colitis (UC).

Figure 4 ROC curve of the diagnostic properties of soluble serum CD44v6 to diagnose ulcerative colitis in serum samples of patients with clinically active ulcerative colitis and Crohn's disease.

SERUM SOLUBLE CD44V6 CONCENTRATIONS IN ULCERATIVE COLITIS AND CROHN'S DISEASE

In patients with clinically active ulcerative colitis serum concentrations of sCD44v6 were significantly lower (153 ng/ml (IQR 122-211); $n=15$) compared with patients with active Crohn's disease (219 ng/ml (IQR 180-243); $n=20$) and healthy donors (221 ng/ml (IQR 197-241); $n=20$) ($p=0.002$; fig 3). Figure 4 presents the diagnostic properties of sCD44v6 to exclude ulcerative colitis. The maximum Youden Index was 46 at a cut off value of 187 ng/ml with a corresponding sensitivity of 75% and specificity of 71%.

A significant negative correlation was found between serum sCD44v6 and C reactive protein concentrations ($r_s=-0.88$, $p<0.001$) in patients with active ulcerative colitis. In patients with active Crohn's disease no significant correlation could be calculated ($r_s=0.161$, $p=0.55$).

Discussion

The present study investigated the diagnostic potential of CD44v6 expression on colonic epithelium and of its serum soluble forms to differentiate ulcerative colitis from colonic Crohn's disease. In contrast to the initial report

by Rosenberg *et al*,¹⁹ our results obtained by immunohistochemical application of two different specific monoclonal antibodies do not confirm that colonic CD44v6 is a promising diagnostic parameter in IBD. This discrepancy is due to a notable positive staining for CD44v6 in our samples of colonic Crohn's disease. Likewise, serum concentrations of soluble CD44v6 provide limited diagnostic information to distinguish patients with clinically active ulcerative colitis from those with Crohn's disease.

By immunohistochemical analysis on paraffin wax sections Rosenberg *et al* described elevated crypt epithelial expression of CD44v6 in 23 of 25 colonic biopsy samples from patients with ulcerative colitis compared with three of 18 colonic Crohn's disease samples, whereas normal colon remained negative.¹⁹ For the description of staining results a semiquantitative scoring system was introduced which graded staining of fewer than 25% of crypt epithelial cells per sample as negative, 25–75% as single positive, and more than 75% as double positive. Increased colonic expression of CD44v6 in ulcerative colitis was also reported by Kitano *et al* on frozen sections. However, by simply evaluating the intensity of epithelial staining as negative or positive, positivity was found in only 42% of ulcerative colitis samples and not in Crohn's disease.⁴¹ In contrast, Papadogiannakis *et al* detected a similar upregulation of colonic CD44v6 on paraffin wax ulcerative colitis and Crohn's disease samples as estimated by a four grade scale for staining intensity.²⁹ All these discordant results obtained by application of different scoring systems for epithelial staining were performed using a single CD44v6 specific monoclonal antibody 2F10.

We examined colonic expression of CD44v6 in IBD and normal colon with the two different specific monoclonal antibodies 2F10 and VFF18. Staining results were assessed quantitatively by determining the percentage of positive epithelial cells as well as qualitatively by grading staining intensity on a four degree scale. 2F10 and VFF18 bind an epitope of CD44v6 located on amino acid residues 18–31, but VFF18 shows a higher affinity compared with 2F10, both to bacterially expressed recombinant protein and native CD44v6 on intact cells.³¹ The distribution of CD44v6 by staining with VFF18 on a representative panel of normal tissues has been shown to be very similar to that reported by other specific monoclonal antibodies.^{24 26 42} On normal colon CD44v6 epitopes are absent or restricted to the crypt base by applying different specific monoclonal antibodies.^{24 25 27 43} This is in line with our findings with VFF18 on normal paraffin wax embedded colonic tissue in the present study. Therefore, VFF18 is a suitable monoclonal antibody for immunohistochemical detection of CD44v6 expression in IBD.

Our results confirm a quantitative and qualitative upregulation of CD44v6 on colonic epithelium of inflamed ulcerative colitis lesions detected by 2F10. Staining with VFF18

resulted in an even higher reactivity of ulcerative colitis samples which might be due to the superior binding affinity of this monoclonal antibody. However, a pronounced difference in the pattern of CD44v6 expression between ulcerative colitis and colonic Crohn's disease is not supported by our study. 2F10 positively stained 40% of colonic Crohn's disease samples, a finding which was barely affected when scoring of positivity was alternatively performed according to Rosenberg *et al* (data not shown). Our preliminary experience with other low affinity CD44v6 specific monoclonal antibodies, such as VFF4 and VFF7, verifies the findings with 2F10 on colonic Crohn's disease (data not shown).³¹ By application of the high sensitivity monoclonal antibody VFF18 epithelial expression of CD44v6 can be detected in 87% of colonic Crohn's disease samples. The discrepancy between the enhanced reactivity of 2F10 in our Crohn's disease samples and the lack of remarkable staining in some previous investigations might be explained by the higher number of patients taking steroids in the latter ones. The impact of steroids on the regulation of CD44 isoforms is unknown, therefore, steroid mediated inhibition of CD44v6 cannot be excluded. Evidently, those two patients of our study who were on prednisolone at time of colonic biopsy displayed a negative colonic CD44v6 staining pattern by 2F10.

An absolute differentiating diagnostic marker between ulcerative colitis and Crohn's disease should meet the criteria of high sensitivity and specificity. Due to the high reactivity of 2F10 and VFF18 on colonic Crohn's disease in our series, the quantitative and qualitative determination of epithelial CD44v6 expression poorly distinguishes ulcerative colitis from colonic Crohn's disease. Depending on the cut off levels for positive staining, both monoclonal antibodies display low sensitivity associated with high specificity and vice versa. Quantitative staining with 2F10 and VFF18 optimally achieved a corresponding sensitivity and specificity of 60% and 87%, respectively.

The regulation and function of increased expression of CD44v6 in IBD remains obscure. The induction of CD44v6 on mononuclear haematopoietic cells by activation dependent mechanisms and on epithelial derived cell lines on incubation with inflammatory cytokines and growth factors has been shown.^{24 44–46} However, the distribution of this isoform predominantly on colonic crypt compared with luminal epithelium and the lack of association between staining positivity and histological grade of inflammation in our study argue against an inflammatory driven event. Moreover, the expression of CD44v6 on histopathologically unaffected mucosa in IBD might indicate an early epithelial alteration, similar to morphological irregularities on colonic epithelium revealed by scanning electron microscopy.⁴⁷ In normal colonic epithelium CD44v6 is essentially restricted to the generative cell compartment and most likely serves an adhesive function integrated into the ongoing process of epithelial proliferation and regeneration.^{24 48} Therefore, the enhanced ex-

pression of CD44v6 on colonic crypt cells in IBD might reflect the increased epithelial cell proliferation rates observed in these chronic inflammatory disease states.⁴⁹ Furthermore, CD44v6 induction has been discussed as an early event in colorectal carcinogenesis prior to K-ras and p53 gene mutations and is found on adenomatous colorectal polyps and most invasive colon carcinomas.^{24-27 50} Whether CD44v6 is associated with the increased risk for colorectal cancer in IBD remains an intriguing field of research.⁵¹

Soluble forms of various cell surface structures, such as adhesion molecules or cytokine receptors, have recently been described in blood circulation.^{52 53} Likewise, soluble CD44 isoforms, which are most likely generated by proteolytic cleavage from cell surfaces, are present in the serum of normal individuals and increased concentrations have been detected in patients with malignant lymphoma, and advanced gastric and colon cancer.⁵⁴⁻⁵⁶ In our study serum concentrations of soluble CD44v6 in Crohn's disease patients with clinically active disease were not different to those of healthy controls, whereas patients with active ulcerative colitis displayed significantly lower concentrations which correlated with the degree of systemic inflammation as evaluated by C reactive protein. Nevertheless, the corresponding sensitivity and specificity of 75% and 71%, respectively, of serum sCD44v6 provide only poor diagnostic information to distinguish ulcerative colitis from Crohn's disease. The opposite finding of an increased in situ expression of CD44v6 on colonic epithelium in ulcerative colitis and lower serum concentrations of its soluble form might suggest specific protection of enzymatic cleavage sites on the CD44 molecule or an impaired functionality of the responsible enzymes. The ensuing stabilisation of membrane CD44v6 would contribute to a higher immunohistochemical reactivity of the low affinity monoclonal antibody 2F10 in ulcerative colitis compared with Crohn's disease.

Due to the lack of an absolute differentiating marker the precise diagnosis of ulcerative colitis and Crohn's disease may often not be possible at a given point in time.¹ Therefore, the bias of incorrect allocation of patients to the ulcerative colitis or Crohn's disease group, especially in cases of confined colonic Crohn's disease is inherent in studies investigating the differential diagnostic potential of a new parameter. To minimise this error only patients with a recorded, specific disease history of at least three years and current bowel investigations were enrolled in our protocol. Furthermore, patients with Crohn's disease needed to have a combined ileocolonic involvement for inclusion in the study. Our investigations on colonic CD44v6 expression and serum concentrations of its soluble form were performed on different populations of IBD patients. Whereas the immunohistochemical staining was intended to differentiate affected colonic lesions of ulcerative colitis from Crohn's disease, the study of sCD44v6 aimed to discriminate IBD patients with clinically active disease. Whether the

simultaneous measurement of both parameters would be more helpful than each single analysis for the differential diagnosis between ulcerative colitis and Crohn's disease has yet to be examined.

Our data confirm the different expression of CD44v6 on colonic epithelial cells between ulcerative colitis and Crohn's disease, which is, however, too weak for an accurate distinction between the two diseases. Therefore, immunohistochemical detection of CD44v6 on large bowel sections does not end the clinicians' search for a diagnostic gold standard in IBD.

- Rubin PH, Present DH. Differential diagnosis of chronic ulcerative colitis and Crohn's disease of the colon: one, two or many diseases? In: Kirsner JB, Shorter RG, eds. *Inflammatory bowel disease*. 4th edn. Baltimore: Williams and Wilkins, 1995:355-79.
- Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 1991;325:928-37,1008-16.
- Price AB. Overlap in the spectrum of non-specific inflammatory bowel disease—"colitis indeterminate". *J Clin Pathol* 1978;31:567-77.
- Wells AD, McMillan I, Price AB, et al. Natural history of indeterminate colitis. *Br J Surg* 1991;78:179-81.
- Cosnes J, Carbonell F, Beugerie L, et al. Effects of cigarette smoking on the long-term course of Crohn's disease. *Gastroenterology* 1996;110:424-31.
- Thomas GAO, Rhodes J, Mani V, et al. Transdermal nicotine as maintenance therapy for ulcerative colitis. *N Engl J Med* 1995;332:988-92.
- Hyman NH, Fazio VW, Tuckson WB, et al. Consequences of ileal pouch-anal anastomosis for Crohn's colitis. *Dis Colon Rectum* 1991;34:653-7.
- Pooley N, Ghosh L, Sharon P. Up-regulation of E-selectin and intercellular adhesion molecule-1 differs between Crohn's disease and ulcerative colitis. *Dig Dis Sci* 1995;40:219-25.
- Seldenrijk CA, Meuwissen SGM, Schipper NW, et al. Value of counting colonic mucosal Ig-containing cells in differential diagnosis of chronic inflammatory bowel disease. *J Clin Pathol* 1992;45:241-7.
- Mahida YR, Kurlac L, Gallagher A, et al. High circulating concentrations of interleukin-6 in active Crohn's disease but not ulcerative colitis. *Gut* 1991;32:1531-4.
- Mullin GE, Lazenby AJ, Harris ML, et al. Increased interleukin-2 messenger RNA in the intestinal mucosal lesions of Crohn's disease but not ulcerative colitis. *Gastroenterology* 1992;102:1620-7.
- Choy MY, Walker-Smith JA, Williams CB, et al. Differential expression of CD25 (interleukin-2 receptor) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative colitis. *Gut* 1990;31:1365-70.
- Shanahan F, Duerr RH, Rotter JL, et al. Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. *Gastroenterology* 1992;103:456-61.
- Vasiliauskas EA, Plevy SE, Landers CJ, et al. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996;110:1810-19.
- Matthes H, Ulrich K, Stallmach A, et al. MMP I mRNA expression is a predictive parameter to discriminate between ulcerative colitis and Crohn's disease [abstract]. *Gastroenterology* 1996;110:A959.
- Mantyh CR, Vigna SR, Bollinger RR, et al. Differential expression of substance P receptors in patients with Crohn's disease and ulcerative colitis. *Gastroenterology* 1995;109:850-60.
- Theodossi A, Spiegelhalter DJ, Jass J, et al. Observer variation and discriminatory value of biopsy features in inflammatory bowel disease. *Gut* 1994;35:961-8.
- Hata J, Haruma K, Suenaga K, et al. Ultrasonographic assessment of inflammatory bowel disease. *Am J Gastroenterol* 1992;87:443-7.
- Rosenberg WMC, Prince C, Kaklamanis L, et al. Increased expression of CD44v6 and CD44v3 in ulcerative colitis but not colonic Crohn's disease. *Lancet* 1995;345:1205-9.
- Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv Immunol* 1993;54:271-335.
- Henke CA, Roongta U, Mickelson DJ, et al. CD44-related chondroitin sulfate proteoglycan, a cell surface receptor implicated with tumor cell invasion, mediates endothelial cell migration on fibrin and invasion into a fibrin matrix. *J Clin Invest* 1996;97:2541-52.
- Screation GR, Bell MV, Jackson DG, et al. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc Natl Acad Sci USA* 1992;89:12160-4.
- Jackson DG, Bell JL, Timans J, et al. Proteoglycan forms on the lymphocyte homing receptor CD44 are alternatively spliced variants containing the v3 exon. *J Cell Biol* 1995;128:673-85.
- Mackay CR, Terpe H-J, Stauder R, et al. Expression and modulation of CD44 variant isoforms in humans. *J Cell Biol* 1994;124:71-82.

- 25 Heider K-H, Hofmann M, Hors E, et al. A human homologue of the rat metastasis-associated variant of CD44 is expressed in colorectal carcinomas and adenomatous polyps. *J Cell Biol* 1993;120:227-33.
- 26 Heider K-H, Mulder J-WR, Ostermann E, et al. Splice variants for the surface glycoprotein CD44 associated with metastatic tumour cells are expressed in normal tissues of humans and cynomolgus monkeys. *Eur J Cancer* 1996;31A:2385-91.
- 27 Gotley DC, Fawcett J, Walsh MD, et al. Alternatively spliced variants of the cell adhesion molecule CD44 and tumour progression in colorectal cancer. *Br J Cancer* 1996;74:342-51.
- 28 Ilyas M, Abbasi AM, Talbot IC, et al. CD44v6 and CD44v3 expression in ulcerative colitis and Crohn's disease [letter]. *Lancet* 1995;346:256.
- 29 Papadogiannakis N, Gad A, Chenard M-P, et al. Expression of CD44 variants in differential diagnosis of ulcerative colitis and Crohn's disease [letter]. *Lancet* 1996;347:1413-14.
- 30 Cellier C, Patey N, Leborgne M, et al. In situ expression of CD44 in ulcerative colitis (UC) and colonic Crohn's disease (CD) [abstract]. *Gastroenterology* 1996;110:A880.
- 31 Heider K-H, Sproll M, Susani S, et al. Characterization of a high-affinity monoclonal antibody specific for CD44v6 as candidate for immunotherapy of squamous cell carcinomas. *Cancer Immunol Immunother* 1996;43:245-53.
- 32 Terpe HJ, Stark H, Prehm P, et al. CD44 variant isoforms are preferentially expressed in basal epithelia of non-malignant human fetal and adult tissues. *Histochemistry* 1994;101:79-89.
- 33 Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol* 1989;24(suppl 170):2-6.
- 34 Le Berre N, Heresbach D, Kerbaol M, et al. Histological discrimination of idiopathic inflammatory bowel disease from other types of colitis. *J Clin Pathol* 1995;48:749-53.
- 35 Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989;298:82-6.
- 36 Best WR, Beckett JM, Singleton JW, et al. Development of a Crohn's disease activity index—National Crohn's Disease Study. *Gastroenterology* 1976;70:439-44.
- 37 Hofmann M, Rudy W, Zöller M, et al. CD44 splice variants confer metastatic behaviour in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Res* 1991;51:5292-7.
- 38 Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
- 39 Armitage P, Berry G. Diagnostic tests and screening procedures. In: Armitage P, Berry G, eds. *Statistical methods in medical research*. Oxford: Blackwell Scientific Publications, 1994:522-9.
- 40 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-10.
- 41 Kitano A, Oshitani N, Matsomoto T, et al. CD44 variants in ulcerative colitis and Crohn's disease [letter]. *Lancet* 1996;348:266-7.
- 42 Fox SB, Fawcett J, Jackson DG. Normal human tissues, in addition to some tumors, express multiple different CD44 isoforms. *Cancer Res* 1994;54:4539-46.
- 43 Mulder JWR, Wielenga VJM, Polak MM, et al. Expression of mutant p53 protein and CD44 variant proteins in colorectal tumorigenesis. *Gut* 1995;36:76-80.
- 44 Koopman G, Heider K-H, Horst E, et al. Activated human lymphocytes and aggressive non-Hodgkin lymphomas express a homologue of the rat metastasis-associated variant of CD44. *J Exp Med* 1993;177:897-904.
- 45 Weiss JM, Sleeman J, Renkl AC, et al. An essential role for CD44 variant isoforms in epidermal Langerhans cell and blood dendritic cell function. *J Cell Biol* 1997;137:1137-47.
- 46 Zhang M, Wang MH, Singh RK, et al. Epidermal growth factor induces CD44 gene expression through a novel regulatory element in mouse fibroblasts. *J Biol Chem* 1997;272:14139-46.
- 47 Nagel E, Bartels M, Pichlmayr R. Scanning electron-microscopic lesions in Crohn's disease: relevance for the interpretation of postoperative recurrence. *Gastroenterology* 1995;108:376-82.
- 48 Kaya G, Rodriguez I, Jorcano JL, et al. Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. *Genes Dev* 1997;11:996-1007.
- 49 Ryder SD, Parker N, Ecclestone D, et al. Peanut lectin stimulates proliferation in colonic explants from patients with inflammatory bowel disease and colon polyps. *Gastroenterology* 1994;106:177-24.
- 50 Kim H, Yang XL, Rosada C, et al. CD44 expression in colorectal adenomas is an early event occurring prior to K-ras and p53 gene mutation. *Arch Biochem Biophys* 1994;310:504-7.
- 51 Ekblom A. Cancer in inflammatory bowel disease. *Curr Opin Gastroenterol* 1995;11:316-20.
- 52 Schleiffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. *J Cell Biol* 1992;119:229-38.
- 53 Heaney ML, Golde DW. Soluble cytokine receptors. *Blood* 1996;87:847-57.
- 54 Bazil V, Strominger JL. Metalloprotease and serine protease are involved in cleavage of CD43, CD44, and CD16 from stimulated human granulocytes. *J Immunol* 1994;152:1314-22.
- 55 Ristamäki R, Joensuu H, Salmi M, et al. Serum CD44 in malignant lymphoma: an association with treatment response. *Blood* 1994;84:238-43.
- 56 Guo YJ, Liu G, Wang X, et al. Potential use of soluble CD44 in serum as indicator of tumor burden and metastasis in patients with gastric or colon cancer. *Cancer Res* 1994;54:422-6.