Gut 1996; 38: 564–567

# Soluble Fcγ receptor III (CD16) and eicosanoid concentrations in gut lavage fluid from patients with inflammatory bowel disease: reflection of mucosal inflammation

D W Hommes, J Meenan, M de Haas, F J W ten Kate, A E G Kr von dem Borne, G N J Tytgat, S J H van Deventer

Centre for Haemostasis, Thrombosis, Atherosclerosis and Inflammation Research
D W Hommes
S J H van Deventer

Department of Gastroenterology
D W Hommes
J Meenan
G N J Tytgat
S J H van Deventer

Department of Pathology
F J W ten Kate

Department of Haematology
A E G Kr von dem Borne

Academic Medical Centre, University of Amsterdam, the Netherlands

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, Amsterdam, the Netherlands
M de Haas

Correspondence to: Dr D W Hommes, Department of Gastroenterology and Hepatology, Academic Medical Centre, Meibergdreef 9, 1005 AZ Amsterdam, the Netherlands.

Accepted for publication 23 October 1995

## Abstract

***Background*—Activated neutrophils cause tissue injury in inflammatory bowel disease (IBD). Upon activation, they shed soluble Fc gamma IIIb receptors (sFcγRIIIb). The subsequent inflammatory response is modulated by several mediators, including neutrophil derived leukotriene $B_4$ ($LTB_4$), thromboxane $B_2$ ($TXB_2$), and prostaglandin $E_2$ ($PGE_2$). The aim of this study was to determine the value of gut lavage sFcγRIII and eicosanoid measurements for the assessment of mucosal inflammation in IBD.**

***Methods*—A total of 18 patients with active IBD, 10 ulcerative colitis (UC), and eight Crohn's disease (CD), and 12 control patients underwent whole gut lavage. Disease activity, endoscopic appearance, and histopathology were graded. Samples were processed for the determination of sFcγRIIIb, $LTB_4$, $PGE_2$, and $TXB_2$.**

***Results*—Soluble FcγRIIIb concentrations were increased in both IBD groups. Significant correlations were seen between sFcγRIIIb and $LTB_4$ values with histology scores. Mean eicosanoid lavage fluid concentrations in control patients were 14·1 pg/ml for $LTB_4$, 5·6 pg/ml for $PGE_2$, and 397 pg/ml for $TXB_2$. Concentrations of all eicosanoids in IBD patients were significantly increased: $LTB_4$ in UC: mean 73·2 pg/ml, in CD: 96·4 pg/ml (both $p<0{\cdot}01$ *v* controls). $PGE_2$ in UC: 20·2 pg/ml, in CD: 43·4 pg/ml ($p<0{\cdot}01$). $TXB_2$ in UC: 719·3 pg/ml, in CD: 180·6 pg/ml (both $p<0{\cdot}05$).**

***Conclusions*—Whole gut lavage fluid analysis is an effective method to study mucosal eicosanoid production. Soluble FcγRIIIb concentrations in gut lavage fluid closely correlate with histological signs of mucosal inflammation and with lavage $LTB_4$ concentration. These data suggest that lavage FcγRIIIb assessment may be used as a simple assay to estimate mucosal neutrophil infiltration in IBD.**

(*Gut* 1996; **38:** 564–567)

Keywords: inflammatory bowel disease, Fcγ receptor III, eicosanoids, gut lavage fluid.

Neutrophil infiltration of the intestinal mucosa is a feature of active inflammatory bowel disease (IBD).[1] A significant mediator of neutrophil mucosal recruitment is leukotriene $B_4$ ($LTB_4$), a product of activated neutrophils.[2–4] Amelioration of IBD related mucosal inflammation is associated with declining concentrations of all eicosanoids including $LTB_4$, prostaglandin $E_2$ ($PGE_2$: down regulation of mucosal inflammation), and thromboxane $B_2$ ($TXB_2$: vasoconstrictor).[5–8] Clinically, the effects of eicosanoids are manifested as the occurrence of diarrhoea and abdominal cramps resulting from their effect on smooth muscle contraction,[9] and epithelial secretion.[10]

Fc gamma receptor III (FcγRIII) is a low affinity neutrophil surface membrane IgG receptor. FcγRIIIb, expressed exclusively on neutrophils and contributes to the inflammatory response by binding and internalising antibody coated micro-organisms or immune complexes.[11–15] Soluble forms of FcγRIIIb (sFcγRIIIb) are released upon neutrophil activation, and the sFcγRIIIb concentration has been shown to increase at inflammatory sites.[16] Consequently, luminal release of this molecule may reflect local neutrophil infiltration.

Whole gut lavage has been used to study humoral immunity in IBD[17 18] and the assessment of albumin, IgG, and $\alpha_1$ antitrypsin in lavage fluid has been proposed as a new approach to measuring disease activity in IBD.[19 20] The aim of this study was to assess gut lavage concentrations of both sFcγRIIIb and eicosanoids, and their correlations with disease activity, to evaluate their usefulness as markers for mucosal inflammation in patients with active IBD.

## Methods

### *Patients*

Eighteen patients with a previous diagnosis of IBD (ulcerative colitis, UC: Crohn's disease, CD) based on histological, radiological, and endoscopic findings (10 UC and eight CD) underwent whole gut lavage for clinically indicated endoscopy. In three CD patients, the terminal ileum was involved, the remaining CD patients suffered from large bowel disease. Control patients (12) underwent colonoscopy for the investigation of abdominal pain of unknown origin or polyps. The caecum was reached in all cases.

Endoscopic appearance was scored in UC patients using a previously described scoring scale,[21] in which an increasing score (0 to 18) pointed to greater macroscopic inflammation. Four biopsy specimens for histopathology were obtained from macroscopically inflamed areas, and scored in a blinded fashion, using a predefined scoring scale with 14 relevant parameters,[22] with a maximal score of 22 points. Disease activity was assessed using the Powell-Tuck index (PTI)[23] for UC patients, and the Crohn's Disease Activity Index (CDAI)[24] for CD patients. This study was approved by the medical ethical committee of the Academic Medical Centre in Amsterdam.

*Gut lavage procedure*

An isotonic non-absorbable lavage fluid (sodium 125 mmol/l, potassium 10 mmol/l, sulphate 40 mmol/l, chloride 35 mmol/l, bicarbonate 20 mmol/l) was given through a gastric tube at a rate of 1–1·5 litre per hour. When clear fluid passed the rectum (usually within two hours), free of faecal debris, 20 ml was collected from each patient. Within 10 minutes the lavage fluid was processed as described previously.[18–20] In brief, fluid was filtered through a GF/A glass fibre filter (Whatman Scientific, Kent, England) and to the filtrate, soybean trypsin inhibitor, sodium EDTA, phenyl methyl sulphonyl fluoride, sodium azide, and fetal calf serum was added. Samples were then stored at −70°C until assay.

*Eicosanoid and sFcγRIII measurements*

Samples for eicosanoid estimation were extracted through Amprep $C_2$ ($LTB_4$ and $TXB_2$) and $C_{18}$ ($PGE_2$) columns (Amersham, England). $LTB_4$, $PGE_2$, and $TXB_2$ were measured using commercial Biotrak enzyme linked immunosorbent assays (ELISAs) (Amersham). The lower detection limits of these ELISAs were 0·31 pg, 1 pg, and 0·5 pg respectively. The extraction procedure and assays were validated by the use of samples of lavage fluid containing added known quantities of each eicosanoid. Recovery for all eicosanoids was >90%. There was no detectable cross reactivity between $LTB_4$, $PGE_2$, nor $TXB_2$.

Soluble FcγRIII was determined by a radioimmunoassay as previously described.[25] The capture antibody for this assay was CLBFcRgran1 (Central Laboratory of the Red Cross (CLB), Amsterdam), detection being with a $^{125}I$ labelled pan-FcγRIII antibody (BW209/2, a gift of Dr R Kurrle, Behringwerke AG, Marburg). The intra-assay coefficient of variation for this assay was <5%. This assay does not distinguish between FcγRIIIa or FcγRIIIb gene expression, and therefore does not specify its cellular origin. The FcγRIIIb gene encodes for the codominant biallelic NA1NA2 system. To establish the cellular origin of sFcγRIII in lavage fluid, we determined the FcγRIIIb NA1/NA2 allotypes in plasma of eight study patients using an NA1-RIA.[17 25] Using donor serum samples known to be FcγRIIIb-NA1/NA1 positive, the binding of CLB GRAN11 (anti-FcγRIIIb-NA1) relative to BW209/2 (anti-pan-FcγRIII) was determined and set as 100% relative binding. The NA phenotype of soluble FcγRIIIb in patients was assigned as FcγRIIIb-NA1/NA1 (>80% relative binding of CLB GRAN11), FcγRIIIb-NA1/NA2 (10–80%), or FcγRIIIb-NA2/NA2 (<10%). sFcγRIII values are expressed in arbitrary units.

*Statistical analysis*

Statistics were calculated using the SPSS 6.0 for Windows (SPSS Inc, USA). Values are given as means (SEM). Differences between groups were analysed using the Mann-Whitney test, and analysis of variance where appropriate. Correlations were calculated using Spearman correlation coefficients. Alpha was set at 0·05.

## Results

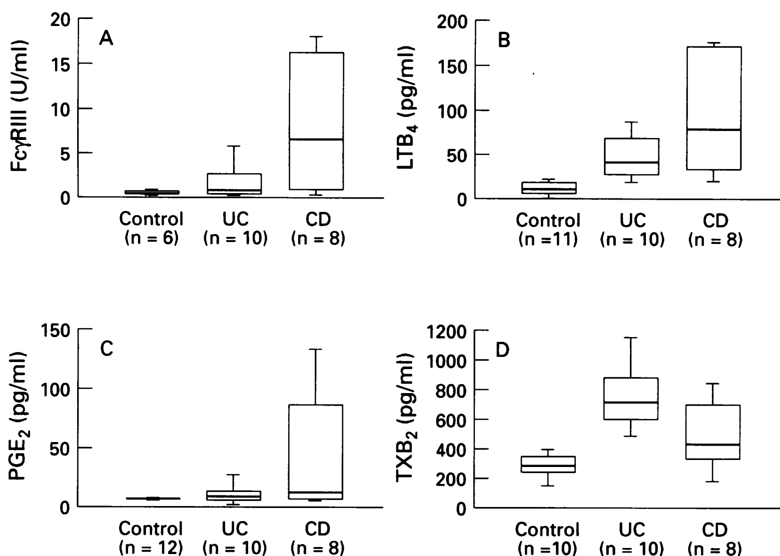
*Patients*

In UC patients, disease activity (PTI) ranged from 2–11 points (mean 5·4 (1·0)), in CD patients disease activity (CDAI) ranged from 21–390 points (mean 174·5 (40)). The distribution of treatment regimens were similar in both disease groups with respect to the use of sulphasalazine/5-ASA, corticosteroids, and immunosuppressive drugs. The lavage procedure was well tolerated in all patients and all samples were processed immediately after colonoscopy. In UC patients, the endoscopic scores varied between 2 and 14 points (mean 5·7 (1·1)) and the histopathology scores in this group ranged from 5 to 15 (8·9 (0·9)). The mean histopathology score in CD patients was 11·4 (1·3) points (range 6–17).

*sFcγRIII*

The Figure (A) shows the sFcγRIII concentrations. In control patients the sFcγRIII concentration ranged from 1·0 to 6·0 units (mean 3·2 (0·5)). In both study groups, sFcγRIII values were increased: in UC patients a mean value was seen of 35 (20) units ($p=0·05$ *v* control), in CD patients the mean value was 113 (47) units ($p=0·05$ *v* control).

To determine the cellular origin of sFcγRIII, the NA-polymorphism was determined in the serum samples of eight patients with active CD: five patients were found to be homozygous NA2NA2, one patient homozygous NA1NA1, and two patients were heterozygous NA1NA2. In the lavage fluid of the three NA1 positive patients, NA1-sFcγRIII was detected in considerable amounts (mean 233 arbitrary units). In all NA2NA2 patients, lavage fluid concentrations of NA1-sFcγRIII were negative. Hence, the NA-polymorphism that was predicted from serum was also found in lavage fluid. This finding suggests that the sFcγRIII detected in lavage fluid originated from neutrophils.




*Box plots of lavage fluid concentrations: soluble FcγRIII receptor (arbitrary units) (A); leukotriene $B_4$ (pg/ml) (B); prostaglandin $E_2$ (pg/ml) (C), and thromboxane $B_2$ (pg/ml) (D) in patients with UC and CD versus controls. Boxes and heavy lines represent 25–75% interquartile range and median respectively. Light lines represent range of results.*

*Eicosanoids*

The mean $LTB_4$ value measured 14·2 (3·1) pg/ml in controls, whereas this was 73·2 (29·3) pg/ml in UC patients ($p<0·01$ *v* control) and 96·4 (22·7) pg/ml in CD patients ($p<0·01$ *v* control) (Fig (B). Figure (C) shows $PGE_2$ values. In UC patients, a mean of 20·2 (10·5) pg/ml versus 5·6 (0·23) pg/ml in control patients was measured. In CD patients these values increased even further (mean of 43·4 (17·9) pg/ml). The difference between CD patients and controls was significant ($p<0·01$). Lavage $TXB_2$ concentrations in control patients ranged from 155·4 to 397 pg/ml (mean 287·3 (22·2) pg/ml) (Fig 1D), in UC patients these concentrations were significantly higher (mean 719·3 (76·7) pg/ml, $p<0·01$). In CD patients the mean $TXB_2$ value was 549·7 (120·8) pg/ml, which also differed significantly from controls ($p<0·05$).

*Correlations*

In UC patients, sFcγRIII correlated well with the endoscopic score ($r=0·77$, $p<0·01$), weaker correlations were found between sFcγRIII and histology scores ($r=0·60$, $p=0·08$) and disease activity ($r=0·61$, $p=0·06$). $LTB_4$ concentrations correlated with the histology score in UC patients ($r=0·63$, $p<0·05$).

In CD patients, sFcγRIII values correlated strongly with histology scores ($r=0·92$, $p<0·01$), $LTB_4$ ($r=0·9$, $p<0·01$), and $TXB_2$ ($r=0·72$, $p<0·05$). Moreover, $LTB_4$ values correlated strongly with histology scores in CD patients ($r=0·89$, $p<0·01$).

## Discussion

The results of this study show that the sFcγRIII concentration in gut lavage fluid obtained from patients with IBDs reflects the mucosal inflammatory reaction. Both in CD and UC, the lavage sFcγRIII concentration correlated well with the histologically determined degree of mucosal inflammation.

FcγRIII is the low affinity receptor of IgG, which is expressed by neutrophils, natural killer T cells, and macrophages.[11–14] Two forms of FcγRIII have been characterised: a transmembrane protein (FcγRIIIa) that is expressed by macrophages and natural killer cells, and FcγRIIIb that is expressed exclusively by neutrophils. FcγRIII binds dimers, trimers, immune complexes, and opsonised particles, thereby activating neutrophils. Upon activation, proteolytic cleavage of FcγRIII from the membrane takes place, and soluble forms of FcγRIII are released.[26 27]

The assay used to determine the lavage sFcγRIII concentration in this study does not distinguish between FcγRIIIa or FcγRIIIb gene expression. Although it has been reported that most, if not all plasma sFcγRIII is derived from neutrophils,[27] we attempted to establish the cellular origin of sFcγRIII in lavage fluid. Firstly, by using the anti-NA1-FcγRIIIb-antibody (CLB GRAN11), the NA allotypes in all CD patients were determined. Because most plasma FcγRIII is derived from neutrophils, and the FcγRIIIb-gene encodes exclusively for FcγRIII on neutrophils,[15 28] the sFcγRIIIb-NA1/FcγRIII ratio in plasma permits prediction of the NA1/NA2 phenotype. Of the eight tested CD patients, five were typed to be NA2/NA2 homozygous, one NA1/NA1 homozygous, and two NA1/NA2 heterozygous. NA1-sFcγRIIIb analysis in lavage fluids showed a significant amount in all NA1 positive patients, and no NA1-sFcγRIIIb in NA2NA2 positive patients. Hence the NA polymorphism that was predicted from plasma was also found in lavage fluid. This finding strongly suggests that the FcγRIII in lavage fluid originated from neutrophils.

A significant correlation was seen between sFcγRIII and $LTB_4$ lavage concentrations, a leukotriene with well recognised neutrophil chemoattracting and activating action. sFcγRIII values correlated well with the histology scores (UC: $r=0·60$, CD: $r=0·89$). $LTB_4$ concentrations correlated with histology scores (UC: $r=0·63$, CD: $r=0·89$). These findings show that sFcγRIII and $LTB_4$ in lavage fluid in IBD patients reflect the mucosal neutrophil induced inflammation. The score derived from the endoscopic scoring system used reflects the greatest degree of inflammation seen rather than assesses the geographical extent of this process. Consequently, comparatively small areas of mucosal inflammation may have a significant influence on the concentrations of luminal inflammatory mediators detected.

The concentrations of both the pro-inflammatory $LTB_4$ and $TXB_2$ as well as the cytoprotective $PGE_2$ were significantly increased in CD and UC. These results are comparable to previous studies that have found increased concentrations of $LTB_4$ and $PGE_2$ in rectal dialysis of UC patients,[29] and in the mucus of ulcerative proctocolitis.[30] Measurement of eicosanoids in lavage fluid does not require insertion of a rectal dialysis bag, and therefore is more feasible. In addition, lavage fluid

eicosanoid concentration may better reflect the amount of eicosanoids produced by the total area of inflamed bowel, especially in CD, which may be patchy. Hence this method could be used to monitor mucosal inflammation in clinical intervention studies in IBD. Our results emphasise the difficulties of attempting to correlate mucosal inflammatory mediator production with clinical parameters of IBD activity.

In conclusion, we have shown that the sFcγRIII and $LTB_4$ lavage fluid concentration reflects mucosal inflammation. Because the sFcγRIII in lavage fluid was shown to originate from neutrophils, and neutrophils are known to be the major source of $LTB_4$ within the bowel mucosa, these measurements probably reflect mucosal neutrophil infiltration.


1 Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; **320:** 365–76.
2 Ford-Hutchinson AW. Leukotriene $B_4$ in inflammation. *Crit Rev Immunol* 1990; **10:** 1–12.
3 Sharon P, Stenson WF. Enhanced synthesis of leukotriene $B_4$ by colonic mucosa in inflammatory bowel disease. *Gastroenterology* 1984; **86:** 453–60.
4 Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. In vivo profiles of eicosanoids in ulcerative colitis, Crohn's colitis and Clostridium difficile colitis. *Gastroenterology* 1988; **95:** 11–7.
5 Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. Effects of topical 5-aminosalicylic acid and prednisolone on prostaglandin $E_2$ and leukotriene $B_4$ levels determined by equilibrium in vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* 1986; **91:** 837–44.
6 Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. *Immunol Today* 1991; **12:** 349–52.
7 Hawkey CJ, Karmeli F, Rachmilewitz D. Imbalance of prostacyclin and thromboxane synthesis in Crohn's disease. *Gut* 1983; **24:** 881–5.
8 Whittle BJR, Kauffman GL, Moncada S. Vasoconstriction with thromboxane $A_2$ induces ulceration of the gastric mucosa. *Nature* 1981; **292:** 472–4.
9 Percy WH, Burton MB, Fallick F, Burakoff R. A comparison in vitro of human and rabbit distal colonic muscle responses to inflammatory mediators. *Gastroenterology* 1990; **99:** 1324–32.
10 Donowitz M. Arachidonic acid metabolites and their role in inflammatory bowel disease. *Gastroenterology* 1985; **88:** 580–7.
11 Fleit HB, Wright SD, Unkeless JC. Human neutrophil Fcγ receptor distribution and structure. *Proc Natl Acad Sci USA* 1982; **79:** 3275–9.
12 Perussia B, Trinchieri G. Antibody 3G8, specific for the human neutrophil Fc receptor, reacts with natural killer cells. *J Immunol* 1984; **132:** 1410–5.
13 Clarkson SB, Kimberly RB, Valinsky JE, Witmer MD, Bussell JB, Nachman RL, Unkeless JC. Blockade of clearance of immune complexes by anti-FcR monoclonal antibody. *J Exp Med* 1986; **164:** 474–89.
14 Clarkson SB, Ory PA. CD16: developmentally regulated IgG Fc receptors on cultured human monocytes. *J Exp Med* 1988; **167:** 408–20.
15 Huizinga TW, Kleijer M, Tetteroo PA, Roos D, von dem Borne AEGKr. Biallelic neutrophil NA-antigen system is associated with a polymorphism on the phospho-inositol-linked Fc gamma receptor III (CD16). *Blood* 1990; **75:** 213–20.
16 Fleit HB, Kobasiuk CB, Doly C, Furie R, Levy PC, Webster RO. A soluble form of FcγRIII is present in human serum and other body fluids and is elevated at sites of inflammation. *Blood* 1992; **79:** 2721–8.
17 O'Mahony S, Barton JR, Crichton S, Ferguson A. Appraisal of gut lavage in the study of intestinal humoral immunity. *Gut* 1990; **31:** 1341–4.
18 O'Mahony S, Choudari CP, Barton JR, Walker S, Ferguson A. Gut lavage fluid proteins as markers of activity of inflammatory bowel disease. *Scand J Gastroenterol* 1991; **26:** 940–4.
19 Choudari CP, O'Mahony S, Brydon G, Mwantembe O, Ferguson A. Gut lavage protein concentrations: objective measures of disease activity in inflammatory bowel disease. *Gastroenterology* 1993; **104:** 1064–71.
20 Brydon WG, Choudari CP, Ferguson A. Relative specificity for active inflammatory bowel disease of plasma-derived proteins in gut lavage fluid. *Eur J Gastroenterol Hepatol* 1993; **5:** 269–73.
21 Mulder CJJ, Tytgat GNJ, Wiltink EHH, Houthoff HJ. Comparison of 5-aminosalicylic acid (3 g) and prednisolone phosphate sodium enemas (30 mg) in the treatment of distal ulcerative colitis: a prospective, randomized double blind trial. *Scand J Gastroenterol* 1988; **23:** 1005–8.
22 Seldenrijk CA, Morson BC, Meuwissen SGM, Schipper NW, Lindeman J, Meijer CJLM. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease; diagnostic implications. *Gut* 1991; **32:** 1514–20.
23 Powell-Tuck J, Bown RL, Lennard-Jones JE. A comparison of oral prednisolone given as a single or multiple daily doses for active proctocolitis. *Scand J Gastroenterol* 1978; **13:** 833–7.
24 Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index: National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; **70:** 439–44.
25 Huizinga TWJ, de Haas M, van Oers MHJ, Kleijer M, Vile H, van der Wouw PA, *et al.* The plasma concentration of soluble Fc-gamma RIII is related to production of neutrophils. *Br J Haematol* 1994; **87:** 459–63.
26 Huizinga TWJ, van der Schoot CE, Jost C, Klaassen R, Kleijer M, von dem Borne AEG, *et al.* The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature* 1988; **333:** 667.
27 Huizinga TW, de Haas M, Kleijer M, Nuijens JH, Roos D, von dem Borne AEGKr. Soluble Fc gamma receptor III in human plasma originates from release by neutrophils. *J Clin Invest* 1990; **86:** 416–23.
28 De Haas M, Kleijer M, Minchinton RM, Roos D, von dem Borne AEGKr. Soluble FcγRIIIa is present in plasma and is derived from natural killer cells. *J Immunol* 1994; **152:** 900–7.
29 Laursen LS, Naesdal J, Bukhave K, Lauritsen K, Rask-Madsen J. Selective 5-lipoxygenase inhibition in ulcerative colitis. *Lancet* 1990; **335:** 683–5.
30 Zijlstra FJ, Wilson JH. 15-HETE is the main eicosanoid present in mucus of ulcerative proctocolitis. *Prostaglandins Leukot Essent Fatty Acids* 1991; **43:** 55–9.