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

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 B₄ (LTB₄), thromboxane B₂ (TXB₂), and prostaglandin E₂ (PGE₂). 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, LTB₄, PGE₂, and TXB₂.

Results—Soluble FcγRIIIb concentrations were increased in both IBD groups. Significant correlations were seen between sFcγRIIIb and LTB₄ values with histology scores. Mean eicosanoid lavage fluid concentrations in control patients were 14.1 pg/ml for LTB₄, 5.6 pg/ml for PGE₂, and 397 pg/ml for TXB₂. Concentrations of all eicosanoids in IBD patients were significantly increased: LTB₄ in UC: mean 73.2 pg/ml, in CD: 96.4 pg/ml (both $p < 0.01$ v controls). PGE₂ in UC: 20.2 pg/ml, in CD: 43.4 pg/ml ($p < 0.01$). TXB₂ in UC: 719.3 pg/ml, in CD: 180.6 pg/ml (both $p < 0.05$). **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 LTB₄ 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 B₄ (LTB₄), a product of activated neutrophils.²⁻⁴ Amelioration of IBD related mucosal inflammation is associated with declining concentrations of all eicosanoids including LTB₄, prostaglandin E₂ (PGE₂; down regulation of mucosal inflammation), and thromboxane B₂ (TXB₂; 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 α₁ 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.

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

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 pre-defined 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 C₂ (LTB₄ and TXB₂) and C₁₈ (PGE₂) columns (Amersham, England). LTB₄, PGE₂, and TXB₂ 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 LTB₄, PGE₂, nor TXB₂.

Soluble FcγRIII was determined by a radioimmunoassay as previously described.²⁵ The capture antibody for this assay was CLBFCRgrn1 (Central Laboratory of the Red Cross (CLB), Amsterdam), detection being with a ¹²⁵I 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 B₄ (pg/ml) (B); prostaglandin E₂ (pg/ml) (C), and thromboxane B₂ (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 LTB₄ 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 PGE₂ 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 TXB₂ 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 TXB₂ 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 ($r = 0.61$, $p = 0.06$). LTB₄ 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 ($r = 0.92$, $p < 0.01$), LTB₄ ($r = 0.9$, $p < 0.01$), and TXB₂ ($r = 0.72$, $p < 0.05$). Moreover, LTB₄ 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 NA2/NA2 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 LTB₄ 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: $r = 0.89$). LTB₄ concentrations correlated with histology scores (UC: $r = 0.63$, CD: $r = 0.89$). These findings show that sFcγRIII and LTB₄ 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 LTB₄ and TXB₂ as well as the cytoprotective PGE₂ were significantly increased in CD and UC. These results are comparable to previous studies that have found increased concentrations of LTB₄ and PGE₂ 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 LTB₄ 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 LTB₄ within the bowel mucosa, these measurements probably reflect mucosal neutrophil infiltration.

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