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

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Abstract

Background—Activated neutrophils cause tissue injury in inflammatory bowel disease (IBD). Upon activation, they shed soluble Fc γ IIIb receptors (sFcyRIIIb). 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 sFcyRIII 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 sFcyRIIIb, LTB4, PGE2, and TXB2.

Results—Soluble FcγRIIIb concentrations were increased in both IBD groups. Significant correlations were seen between sFcyRIIIb 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 39.7 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 vs 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.

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). A significant mediator of neutrophil mucosal recruitment is leukotriene B4 (LTB4), a product of activated neutrophils. 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; vasoconstrctor). Clinically, the effects of eicosanoids are manifested as the occurrence of diarrhoea and abdominal cramps resulting from their effect on smooth muscle contraction, and epithelial secretion.

Fc gamma receptor III (FcyRIII) 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. Soluble forms of FcγRIIib (sFcyRIIib) are released upon neutrophil activation, and the sFcyRIIib concentration has been shown to increase at inflammatory sites. Consequently, luminal release of this molecule may reflect local neutrophil infiltration.

Whole gut lavage has been used to study humoral immunity in IBD 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. The aim of this study was to assess gut lavage concentrations of both sFcyRIIIB 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, 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, with a maximal score of 22 points. Disease activity was assessed using the Powell-Tuck index (PTI) for UC patients, and the Crohn’s Disease Activity Index (CDAI) 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. 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 sFcyRIII 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 FcyRIII was determined by a radioimmunoassay as previously described. The capture antibody for this assay was CLB-FcRgαn1 (Central Laboratory of the Red Cross (CLB), Amsterdam), detection being with a ¹²⁵I labelled pan-FcyRIII 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 FcyRIIIα or FcyRIIIb gene expression, and therefore does not specify its cellular origin. The FcyRIIIb gene encodes for the codominant biallelic NA1/NA2 system. To establish the cellular origin of sFcyRIII in lavage fluid, we determined the FcyRIIIb NA1/NA2 alleles in plasma of eight study patients using an NA1-RIA. Using donor serum samples known to be FcyRIIIb-NA1/NA1 positive, the binding of CLB GRAN1 (anti-FcyRIIIb-NA1) relative to BW209/2 (anti-pan-FcyRIII) was determined and set as 100% relative binding. The NA phenotype of soluble FcyRIIIb in patients was assigned as FcyRIIIb-NA1/NA1 (>80% relative binding of CLB GRAN1), FcyRIIIb-NA1/NA2 (10–80%), or FcyRIIIb-NA2/NA2 (<10%). sFcyRIII values are expressed in arbitrary units.

**Statistical analysis**

Statistics were calculated using 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).

**sFcyRIII**

The Figure (A) shows the sFcyRIII concentrations. In control patients, the sFcyRIII concentration ranged from 1.0 to 6.0 units (mean 3.2 (0.5)). In both study groups, sFcyRIII 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 sFcyRIII, 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-sFcyRIII was detected in considerable amounts (mean 233 arbitrary units). In all NA2NA2 patients, lavage fluid concentrations of NA1-sFcyRIII were negative. Hence, the NA-polymorphism that was predicted from serum was also found in lavage fluid. This finding suggests that the sFcyRIII detected in lavage fluid originated from neutrophils.
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, sFcyRIII correlated well with the endoscopic score (r=0·77, p<0·001), weaker correlations were found between sFcyRIII 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, sFcyRIII 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 sFcyRIII concentration in gut lavage fluid obtained from patients with IBDs reflects the mucosal inflammatory reaction. Both in CD and UC, the lavage sFcyRIII concentration correlated well with the histologically determined degree of mucosal inflammation.

FcyRIII is the low affinity receptor of IgG, which is expressed by neutrophils, natural killer T cells, and macrophages.15-18 Two forms of FcyRIII have been characterised: a transmembrane protein (FcyRIIIa) that is expressed by macrophages and natural killer cells, and FcyRIIIb that is expressed exclusively by neutrophils. FcyRIII binds dimers, trimers, immune complexes, and opsonised particles, thereby activating neutrophils. Upon activation, proteolytic cleavage of FcyRIII from the membrane takes place, and soluble forms of FcyRIII are released.26,27

The assay used to determine the lavage sFcyRIII concentration in this study does not distinguish between FcyRIIIa or FcyRIIIb gene expression. Although it has been reported that most, if not all plasma sFcyRIII is derived from neutrophils,27 we attempted to establish the cellular origin of sFcyRIII in lavage fluid. Firstly, by using the anti-NA1-FcyRIIIb-antibody (CLB GRAN11), the NA allozygous or all CD patients were determined. Because most plasma FcyRIII is derived from neutrophils, and the FcyRIIIb-gene encodes exclusively for FcyRIII on neutrophils,15,18 the sFcyRIIIb-NA1/FcyRIII 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-sFcyRIIIb analysis in lavage fluids showed a significant amount in all NA1 positive patients, and no NA1-sFcyRIIIb 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 FcyRIII in lavage fluid originated from neutrophils.

A significant correlation was seen between sFcyRIII and LTB4 lavage concentrations, a leukotriene with well recognised neutrophil chemoattracting and activating action. sFcyRIII 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 sFcyRIII and LTB4 in lavage fluid in IBD patients reflect the mucosal neutrophil induced inflammation. The score derived from the endoscoplc 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 cytotoxic 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

Box plots of lavage fluid concentrations: soluble FcyRIII 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.
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 sFcyRIII and LTB4 lavage fluid concentration reflects mucosal inflammation. Because the sFcyRIII 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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