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Infliximab restores the dysfunctional matrix remodeling protein and growth factor gene expression in patients with inflammatory bowel disease

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DISCLOSURES

Paul Rutgeerts, Séverine Vermeire, Marc Ferrante, Gert Van Assche and Gert De Hertogh report following conflicts of interest: grant support, lecture fees and consulting fees from Centocor and Schering-Plough.

ABSTRACT

Background: Matrix metalloproteinases (MMPs), MMP-inhibitors (TIMPs), ADAM(TS)s and growth factors are involved in inflammation and tissue damage and repair, all occurring in inflammatory bowel disease (IBD). We studied the impact of anti-inflammatory therapy with infliximab on mucosal expression of these tissue remodeling genes in IBD patients.

Methods: Mucosal gene expression of 23 MMPs, 4 TIMPs, 50 ADAM(TS)s and 158 growth factors was investigated in 61 IBD patients before and after first infliximab

therapy and in 12 controls, with microarrays and quantitative RT-PCR. Protein localization, mucosal gelatinase levels and net gelatinolytic activity were investigated by immunohistochemistry, zymography analysis and gelatin degradation assay, respectively.

Results: In active IBD patients before infliximab *vs.* controls, gene expression of many MMPs, TIMPs, ADAM(TS)s and growth factors was upregulated, whereas colonic expression of *MMP28* and *TGFA*, and ileal expression of *ADAMDEC1* and *AGT* were downregulated. After controlling inflammation with infliximab, most gene dysregulations observed at baseline were restored in responders. Increased ratio of *MMP1*/*TIMP1* expression at baseline in active IBD was restored in responders with colonic mucosal healing. Immunohistochemistry for MMP1, MMP3, TIMP1 and REG1A showed higher protein levels in active IBD patients *vs.* inactive patients or controls. With zymography analysis and gelatin degradation assay higher gelatinase levels and net gelatinolytic activity were measured before infliximab and levels normalized after infliximab.

Conclusions: Our data suggest that suppression of inflammation results in arrest of epithelial damage and subsequent mucosal healing. Therefore, the therapeutic potential of agents targeting MMPs or growth factors as primary therapy seems rather complex.

Key Words: inflammatory bowel disease, infliximab, mucosal expression, tissue remodeling genes

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic disabling inflammatory bowel diseases (IBD) with increasing prevalence throughout the whole world. IBD occurs mostly in young people and often leads to a greatly decreased quality of life, with (bloody) diarrhea and abdominal pain as major gastrointestinal symptoms. Despite extensive efforts, the exact pathogenesis of IBD remains unknown. However, it is believed that the chronic intestinal inflammation in IBD is the result of an inappropriate and ongoing activation of the mucosal immune system towards the (normal) luminal microbiota in genetically susceptible individuals. This activation of the mucosal immune system is most likely facilitated by defects in both the intestinal epithelial barrier function ("leaky mucosal barrier") and the mucosal immune system ("loss of immune tolerance") (1).

Chronic intestinal inflammation leads to tissue damage and subsequent tissue repair, and all these events are accompanied by an increased turnover of the extracellular matrix (ECM). Disturbance in the balance between the synthesis and breakdown of ECM components is involved in the pathological findings of IBD, leading to progressive tissue destruction (e.g. ulcers and fistulas) or excessive deposition of collagens (major component of ECM) resulting in fibrosis (2). Matrix metalloproteinases (MMPs) and their inhibitors and growth factors are important players in this tissue remodeling process. MMPs are a family with over 20 members of Zn^{2+}-dependent endopeptidases that degrade most components of the ECM in inflammatory diseases. Their proteolytic activity is tightly controlled by endogenous inhibitors, including the tissue inhibitors of metalloproteinases (TIMPs) (3). Previous selective studies in IBD have shown that the balance between specific MMPs and TIMPs and their expression are dysregulated in IBD (4-6), but a general picture remains so far elusive. The MMPs share sequential and structural motifs with ADAMs

(a disintegrin and metalloproteinase) and ADAMTSs (ADAMs with thrombospondin type 1 motif), which have both adhesive and proteolytic activities. Growth factors are involved in the modulation of intestinal inflammation and repair during IBD, having a critical role in cellular proliferation, differentiation and angiogenesis (7). They also promote wound healing by stimulating ECM synthesis, in part by modulating the balance between MMPs and TIMPs (8). Finally, an intrinsic network of interactions between inflammatory cytokines and growth factors with the balances of proteinases and inhibitors exists in acute and chronic inflammatory diseases, in which tumor necrosis factor (TNF) is a key regulator (9). These interactions not only include the induction of growth factors and proteases by upstream agonists, such as TNF, but also the activation, potentiation and degradation of growth factors and proteinases by extracellular proteases (3;10). Infliximab (Remicade; Centocor, Inc., Malvern, PA, USA), a chimeric monoclonal antibody to tumor necrosis factor-alpha (TNF-α), has become the mainstay of therapy in refractory IBD (11). Infliximab dramatically improves the quality of life in IBD patients. Besides inducing and maintaining remission in refractory IBD patients, treatment with infliximab leads to new treatment goals such as intestinal mucosal healing and a reduction in hospitalizations and surgeries on the long-term. The intestinal mucosa of IBD patients is composed of different and changing cell types. The interactions between the immune cell populations and the non-immune cell types, including epithelial, mesenchymal, and microvascular endothelial cells, are important in the pathogenesis of IBD. Gene expression microarray profiling of the intestinal mucosa will represent an average of these different cell types, and gene expression by some cell populations (e.g. epithelial cells) may be decreased to the total mRNA pool, reflecting mucosal trafficking of inflammatory cell types in IBD(12).

The study of gene expression in patients with IBD who achieved mucosal healing under infliximab in comparison with the gene expression before treatment allows us to obtain insights into the importance of the different mediators of inflammation, tissue degradation and tissue repair. Therefore, we investigated the intestinal mucosal gene expression of MMPs, TIMPs, ADAM(TS)s and growth factors in patients with active IBD, as well as the impact of anti-inflammatory therapy with infliximab on the mucosal expression of these genes, with the use of gene expression microarray technology. Validation of specific microarray data for selected genes was performed by quantitative real-time reverse-transcription PCR (qRT-PCR). In addition, immunohistochemistry was performed to evaluate protein levels and localization of MMP1, MMP3, TIMP1 and REG1A in active, inactive and control colonic and ileal mucosa. Zymography analysis was performed to investigate gelatinase levels before and after treatment with infliximab. Finally, a gelatin degradation assay was used to evaluate net gelatinolytic activity in the mucosal biopsies.

MATERIALS and METHODS

MMPs, TIMPs, ADAM(TS)s and growth factors

The gene expression of 23 MMPs, 4 TIMPs and 50 ADAM(TS)s and the expression of 158 genes encoding peptides/proteins with growth factor activity (see **Supplementary table 1** for a detailed list of the genes) were investigated in intestinal mucosal biopsies obtained from normal controls and from IBD patients before and after their first infliximab treatment, using gene expression microarray technology.

Patients and biopsy specimens

This was a prospective observational cohort study (ClinicalTrials.gov number, NCT00639821). Sixty-one patients with active IBD, including 24 UC, 19 Crohn's colitis (CDc) and 18 Crohn's ileitis (CDi), refractory to corticosteroids and/or immunosuppression were studied. In **table 1** the baseline characteristics of the patients and controls are shown.

A control group with normal mucosa of 12 individuals [6 colon and 6 ileum] who underwent endoscopy for screening for polyps was also included. The patients underwent endoscopy with biopsies from diseased bowel (colon for UC and CDc and ileum for CDi) within a week prior to the first intravenous infliximab infusion of 5 mg/kg body weight. The patients underwent a second endoscopy with biopsies 4 weeks after the first infliximab infusion in case of a single infusion and at 6 weeks if they received a loading dose of infliximab at weeks 0, 2 and 6. The biopsies were taken at sites of active inflammation but at a distance of ulcerations. In the case of healing at control endoscopy, the biopsies were obtained in the areas where lesions were present before therapy. The endoscopist was not blinded to treatment. Half of the biopsies were immediately snap-frozen in liquid nitrogen and stored at -80°C until RNA isolation, except for the biopsies from 1 CDc patient after infliximab treatment because of poor technical quality. The rest of the biopsies were fixed in Carnoy's fixative for up to 5 hours and then dehydrated, cleared and paraffin-embedded. Haematoxylin-eosin stained slides from the paraffin blocks of each patient were used to score the chronic intestinal inflammation, using a previously reported histological scoring system for UC (12) and for CD (13).

The response to infliximab therapy was assessed at the time of the second endoscopy, based on endoscopic and histologic findings. In the colon, the response was defined as a complete endoscopic mucosal healing (absence of ulcers) with a

decrease of at least 3 points on the histological score for CDc (13) and as a decrease to a Mayo endoscopic subscore of 0 or 1 with a decrease to grade 0 or 1 on the histological score for UC (12;14). Patients who did not achieve this healing were considered non-responders although some of them showed endoscopic and/or histologic improvement. Of the 43 IBDc patients, we scored 20 responders (8 UC and 12 CDc) and 23 non-responders (16 UC and 7 CDc). When the same response criteria of CDc were used for CDi, there was only one patient who showed in the ileum a complete endoscopic and histologic healing. Therefore, we used response criteria with lower stringency than complete healing in CDi. Patients with a clear improvement of the ulcerations and a decrease on the histological score (13) were defined as responders. Of the 18 CDi patients, 8 were (partial) responders and 10 were non-responders.

The characteristics of age, sex and smoking were compared between patients and controls. These comparisons were performed with the Mann-Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables, using SPSS software (Chicago, IL) (**Table 1**). A p-value ≤ 0.01 was considered significant.

Whole-genome gene expression analysis

As previously described (15), total RNA was extracted from the biopsy specimens and used to analyze the gene expression via Affymetrix Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA, USA), which are comprised of 54 675 probe sets covering the whole genome. The microarray data have been submitted in MIAME (Minimum Information About a Microarray Experiment) format to the Gene Expression Omnibus database (series accession number GSE16879).

Bioconductor tools (16) in R (version 2.7.2, http://www.r-project.org/) were used to analyze the Affymetrix raw data (.cel files). The robust multichip average method was performed on the Affymetrix raw data to obtain a log2 expression value for each probe set (17). Probe set annotations were obtained through the Affymetrix NetAffx website (http://www.affymetrix.com/analysis/index.affx) or the UCSC Genome Browser website (http://genome.ucsc.edu/) or the NCBI website (http://www.ncbi.nlm.nih.gov/). For comparative analysis, linear models for microarray data (LIMMA) (18) were performed for all probe sets present on the microarray to identify probe sets that are different between the groups, based on moderated *t*-statistics. For multiple testing correction, the false discovery rate (FDR) was estimated from p-values derived from the moderated *t*-statistics using the method of Benjamini and Hochberg (19).

qRT-PCR analysis

To confirm the microarray data, qRT-PCR was performed for *MMP1*, *MMP28*, *TIMP1, ADAM9, TFF1, TGFB1* and β-actin (as endogenous reference gene). cDNA was synthesized from 0.5 µg of total RNA from the same samples as for microarray analysis, using the RevertAid H Minus First Strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany), following the manufacturer's protocol. Primers and dual-labelled probes were designed with OligoAnalyzer software (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/default.aspx) and synthesized by Sigma-Aldrich (Bornem, Belgium) (**Supplementary table 2**). Multiplex real-time PCR was performed in a final reaction volume of 25 µl on a Rotor-Gene 3000 instrument (Corbett Research, Mortlake, Australia), using QuantiTect Multiplex PCR NoROX Kit (Qiagen, Venlo, NL), according to the manufacturer's

instructions. Cycle threshold values were determined by Rotor-Gene 6.0.16 software. All samples were amplified in duplicate reactions. The relative expression of target mRNA levels were calculated as a ratio relative to β-actin reference mRNA (20). The results were analyzed with SPSS software, using Mann-Whitney *U*-test for unpaired samples and Wilcoxon signed-rank test for paired samples. A p-value ≤ 0.01 was considered significant.

Protein expression by immunohistochemistry

To localize MMP1, MMP3, TIMP1 and REG1A in the intestinal mucosa, immunohistochemistry was performed on 5 µm-thick sections that were cut from paraffin blocks of formalin-fixed endoscopic biopsies from IBD patients and control individuals. After drying, deparaffinization and rehydration, epitope retrieval was performed at low pH for MMP1 and TIMP1, and at high pH for MMP3 and REG1A (Dako PT Link machine, Dako Belgium NV, Heverlee, Belgium). Sections were then washed 3 times 5 min (Envision Flex wash buffer, Dako) and Envision Flex Peroxidase-Blocking Reagent (Dako) was applied for 10 min at room temperature. After a second wash step, sections were incubated with an anti-human MMP1 rabbit polyclonal antibody (Bio-Rad AbD Serotec GmbH, Düsseldorf, Germany; dilution 1/100), or with an anti-human MMP3 rabbit polyclonal antibody (Sigma-Aldrich, dilution 1/200), or with anti-human TIMP1 mouse monoclonal antibody (clone 102D1; Millipore, Overijse, Belgium; dilution 1/75), or with anti-human REG1A rabbit polyclonal antibody (Sigma-Aldrich, dilution 1/300) for 30 min at room temperature. Following a third wash step, bound primary antibody was visualized by incubating the slides for 30 min with Envision Flex/HRP (Dako) and application of the Envision DAB+ Chromogen (Dako) for 10 min at room temperature. After rinsing, the slides

were counterstained with haematoxylin, dehydrated, cleared and mounted. All the stains were evaluated by an experienced pathologist (GDH). Aside previous uses of the indicated antibodies, immunohistochemistry controls included omission of primary antibody and always yielded the expected negative signals.

Zymography analysis

Snap-frozen mucosal biopsies from a subset of active CDi patients (n=3) and IBDc patients (n=3) before and after treatment with infliximab, as well as control samples (3 ileum, 2 colon) were investigated with gelatin zymography analysis as described previously (21). Briefly, the weight of the biopsies was determined and 500 µl of lysis buffer was added (50 mM Tris, 0.5 M NaCl, 10 mM $CaCl_2$, 0.5% Triton-X 100, complete EDTA-free protease inhibitors (1 tablet/10 ml (Hoffmann-La Roche, Basel, Switzerland) (pH 7.5)). The tissue was then homogenized with the Precellys 24 system (Bertin Technologies, Montigny-le-Bretonneux, France) and centrifuged 15 min at 20800 g at 4°C. The supernatants, of which the volume was normalized to the starting weight of the biopsies, was then pre-purified using gelatin-Sepharose beads (GE healthcare, Buckinghamshire, United Kingdom) and mini-spin columns (Bio-Rad Laboratories, Hercules, CA, USA) (22). The bound gelatinases were eluted from the column with 20 µl Tris/glycine/SDS non-reducing loading buffer (Invitrogen, Carlsbad, CA, USA) and the pre-purified samples were then separated in 7.5% acrylamide gels copolymerized with 1mg/ml porcine gelatin (Sigma-Aldrich, St. Louis, MO, USA). The gels were then washed with 2.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) for 40 min and incubated overnight at 37°C in 50 mM Tris-HCl (pH 7.5) supplemented with 10 mM $CaCl_2$ (Sigma-Aldrich, St. Louis, MO, USA). The gels were stained with 0.25% Coomassie Brilliant Blue-R (Sigma-Aldrich, St. Louis, MO, USA)

and scanned using standard settings. Band densities were analyzed with Image J 1.48 software (NIH Windows version) and the obtained densities of different gelatinase forms were plotted as a ratio *versus* proMMP2 levels.

Gelatin degradation assay

The gelatin degradation assay was performed on snap-frozen mucosal biopsies from a subset of active CDi patients (n=3) and IBDc patients (n=3) before and after treatment with infliximab, as well as control samples (3 ileum, 3 colon). First, proteins were extracted from the biopsy samples in 500 µl assay buffer (150 mM NaCl, 50 mM Tris, 5 mM $CaCl_2$, 0.05% Tween20). Next, the net gelatinolytic activity was measured with the use of a previously described gelatin degradation assay (23). Briefly, the samples were added (duplicates) to a 96-well plate (chimney, black, clear bottom, Greiner Bio-one, Frickenhausen, Germany) and DQ™-gelatin (Invitrogen, Carlsbad, CA, USA) was added to a final concentration of 2.5 µg/ml. Immediately thereafter, the plate was placed in a fluorescence reader (FL600 Microplate fluorescence reader, Biotek, Highland Park, IL, USA) and fluorescence was measured every 10 min for 4h at 37°C.

ETHICAL CONSIDERATIONS

The study was carried out at the University Hospital Gasthuisberg in Leuven (ClinicalTrials.gov number, NCT00639821). Written informed consent was obtained from all individuals and the study was approved by the University Hospital Ethics Committee.

RESULTS

Gene expression of tissue remodeling genes in IBD intestinal mucosa before and after first infliximab therapy

The intestinal mucosal gene expression microarray profiles were compared between normal controls, patients pre- and post-infliximab therapy in UC, CDc, IBD colitis (IBDc; UC and CDc together) and CDi.

In the current microarray study, we studied the intestinal mucosal gene expression of 23 MMPs, 4 TIMPs, 50 ADAM(TS)s and 158 growth factors. On the Affymetrix Human Genome U133 Plus 2.0 Array, the 23 MMPs were represented by 45 probe sets, the 4 TIMPs by 9 probe sets, the 50 ADAM(TS)s by 112 probe sets and the growth factors by 318 probe sets (**Supplementary table 1**). We only focussed on those probe sets that were expressed in the gut. Therefore, the probe sets with low overall intensity were excluded and only the probe sets with an intensity more than log2(50) in at least 10% of the samples (n=133) were included. This filter criterion leaves 22 probe sets representing 13 MMPs, 8 probe sets representing 3 TIMPs, 18 probe sets representing 14 ADAM(TS)s and 100 probe sets representing 69 growth factors for further analysis (**Supplementary table 1**). The results for these filtered probe sets were selected from all performed comparative analyses (**Supplementary table 3**). In the comparative analyses, the filtered probe sets with > 2-fold change and FDR < 0.05 were considered biologically significant. Additionally, we found that the characteristic age was significantly different between IBD patients and controls (**Table 1**). To investigate if age has an impact on the expression of the tissue remodeling genes in IBD and controls, LIMMA analysis between IBD patients (before therapy) and controls was performed with age as confounding factor. The results of the comparative analysis with age as confounder were similar as for the analysis without age as confounding factor. So there was no impact of the age on the

expression of the tissue remodeling genes between IBD patients and controls (**Supplementary table 4**). Moreover, no evidence was found for age-related differential gene expression in active IBD patients and controls (results not shown).

Expression of MMPs, TIMPs and ADAM(TS)s

First, we studied whether differences existed in gene expression of MMPs, TIMPs and ADAM(TS)s in active UC as compared with active CDc. We observed no significant gene expression differences at baseline (= pre-infliximab therapy) in inflamed colon between UC and CDc (**Supplementary table 3**). Second, we investigated the differential expression in MMP, TIMP and ADAM(TS) genes at baseline between active CDc and active CDi. Only *MMP12* expression was more than 2-fold significantly downregulated at baseline in active CDi as compared to active CDc (**Supplementary table 3**).

Third, we investigated the differential gene expression of MMPs, TIMPs and ADAM(TS)s in inflamed mucosa of IBD patients and the effect of infliximab therapy on the expression of these genes (**Table 2** and **3**). In IBDc, the gene expression levels of *MMP1-3*, *MMP7*, *MMP9-10*, *MMP12*, *MMP19*, *TIMP1-2*, *ADAM9*, *ADAM19*, *ADAM28*, *ADAMTS1* and *ADAMTS9* were more than 2-fold significantly increased, whereas only *MMP28* gene expression was more than 2-fold significantly decreased at baseline in inflamed colon as compared to control colons. Most of the MMP and TIMP genes upregulated at baseline in active IBDc decreased more than 2-fold significantly after infliximab therapy in IBDc responders when compared to baseline, and *MMP28* expression was more than 2-fold significantly increased (**Table 2**). In contrast, no significant changes of these genes were observed in IBDc non-responders after therapy when compared to their baseline samples (**Supplementary**

table 3). These findings validate reciprocally the microarray data versus the endoscopic/histological findings. None of the MMP, TIMP and ADAM(TS) genes remained dysregulated after infliximab therapy in IBDc responders as compared to control colons, whereas most of the dysregulated genes at baseline remained dysregulated after therapy in IBDc non-responders when compared to control colons (**Table 2**).

Furthermore, we observed that in active IBDc before therapy versus control colons the fold change of the significant *MMP1* (85.57x)*, MMP3* (67.93x), *MMP10* (16.98x) and *MMP12* (28.72x) was much higher than the fold change of the significant TIMPs [*TIMP1* (11.51x) *and TIMP2* (2.16x) (**Table 2**). In IBDc responders after therapy versus control colons, the fold change for both MMPs and TIMPs was around 1 (**Table 2**).

In CDi, the gene expression levels of *MMP1*, *MMP3*, *MMP7*, *MMP10* and *TIMP1* were more than 2-fold significantly upregulated, whereas only *ADAMDEC1* gene expression was more than 2-fold significantly downregulated at baseline in inflamed ileum as compared to control ileums. After infliximab therapy, no genes remained dysregulated in CDi responders as compared to control ileums, whereas the expression levels of *MMP1*, *MMP3* and *TIMP1* remained more than 2-fold significantly upregulated in CDi non-responders when compared to control ileums (**Table 3**).

Moreover, the ratio between the fold change of *MMP1* and *TIMP1, MMP3* and *TIMP1*, and *MMP10* and *TIMP1* in active CDi before therapy *vs.* control ileums was also increased (**Table 3**) as in IBDc before therapy.

Next, we studied the correlation of the expression of the MMP, TIMP and ADAM(TS) genes that were dysregulated at baseline in active disease with the gene expression

of granulocyte markers (*S100A8*, *S100A9* and *S100A12*), an inflammatory marker (interleukin 8 (*IL8*)) and an epithelial marker (villin 1 (*VIL1*)). The correlations were analyzed with the Spearman's Rank Correlation test using the microarray log2 mRNA expression values, and a p-value ≤ 0.01 was considered significant (**Supplementary table 5**). The colon (n=91) and ileum (n=42) samples were analysed separately. A highly positive significant correlation was found for the mRNA levels for all upregulated MMPs, TIMPs and ADAM(TS)s with the mRNA levels of *S100A8*, *S100A9*, *S100A12* and *IL8* for both colon and ileum samples. A negative significant correlation was seen between the colonic mRNA levels of the downregulated *MMP28* and *VIL1*, and between the ileal mRNA levels of the downregulated *ADAMDEC1* and *VIL1*.

Expression of growth factor genes

Two rationales incited us to study growth factor gene expression levels in intestinal biopsies. First, many balances between MMPs and inhibitors are regulated by growth factors, and ADAM(TS)s have a prominent role in growth and development. Second, IBD results in epithelial tissue regenerative processes and healing processes involve growth regulating cytokines.

First, we studied the differential gene expression of growth factors at baseline in inflamed colon between active UC and active CDc, and we found no significant differences in gene expression of growth factors at baseline between active UC and active CDc (**Supplementary table 3**). Second, the differential gene expression of growth factors was studied at baseline between active CDc and active CDi. At baseline, the gene expression levels of *FGF9*, *MACC1*, *MST1* and *PDGFC* were more than 2-fold significantly higher, and *AREG*, *BMP2*, *CTGF* and *LEFTY1* gene

expression levels were more than 2-fold significantly lower in active CDi when compared to active CDc (**Table 4**).

Third, the gene expression of growth factors was studied in inflamed mucosa of active IBD patients and the impact of infliximab therapy on the expression of these genes was evaluated (**Tables 5** and **6**). In IBDc, the colonic gene expression of *AGT*, *ANGPTL2*, *AREG*, *CECR1*, *CTGF*, *FGF7/KGFLP1/KGFLP2*, *GMFG*, *HBEGF*, *HDGFRP3*, *INHBA*, *JAG1*, *MANF*, *REG1A*, *TFF1-2*, *TGFB1* and *TYMP* was more than 2-fold significantly upregulated, whereas only *TGFA* gene expression was more than 2-fold significantly downregulated before infliximab therapy in inflamed colon as compared to control colons. The expression of many growth factor genes which were upregulated at baseline in inflamed IBD colon were no longer significantly upregulated after therapy in IBDc responders when compared to control colons. As an exception, we observed that the colonic expression levels of *AREG* and *JAG1* remained more than 2-fold significantly higher in IBDc responders after infliximab therapy as compared to control levels in the colon (**Table 5**). In contrast, most of the growth factor dysregulations observed at baseline in inflamed IBDc colon remained dysregulated after infliximab therapy in IBDc non-responders (**Table 5**). Of notice, the gene expression level of *REG1A* was more than 70 times higher in IBDc when compared to control colons and after infliximab treatment the expression was restored in IBDc responders but not in IBDc non-responders.

In CDi, only *TFF1* mRNA expression was more than 2-fold significantly increased at baseline in inflamed ileum when compared to control ileums. In contrast with inflamed CDc colon, *AGT* mRNA expression levels were more than 2-fold significantly decreased at baseline in inflamed CDi ileum versus control ileums. After infliximab therapy, only *AGT* expression remained downregulated in CDi responders, and *TFF1*

and *AGT* expression remained significantly dysregulated in CDi non-responders as compared to control ileums (**Table 6**).

Finally, all upregulated growth factor genes at baseline in active IBD correlated positively with the granulocyte markers (*S100A12*, *S100A8* and *S100A9*) and the inflammatory marker *IL8*, except for the colonic mRNA levels of *AREG*, *HBEGF*, *JAG1* and the ileal *AGT* mRNA levels. A strong positive significant correlation was found between the colonic mRNA levels of the downregulated *TGFA* and the epithelial marker *VIL1*, and no correlation was seen between the ileal mRNA levels of the downregulated *AGT* and *VIL1* (**Supplementary table 5**).

Validation of selected microarray data by qRT-PCR

We were able to confirm with the use of qRT-PCR the gene expression microarray results for *MMP1*, *MMP28*, *TIMP1*, *ADAM9*, *TFF1, TGFB1*, the ratio *MMP1/TIMP1* (**Figures 1** and **2,** and **Supplementary table 6**) between controls and IBD patients before and after treatment.

As compared to control colons, the gene expression levels of *MMP1*, *TIMP1*, *ADAM9* and *TGFB1* were all significantly increased, and only *MMP28* was significantly decreased in the inflamed colon of active IBDc patients. None of these genes remained significantly dysregulated after therapy in IBDc responders *vs.* control colons, whereas in IBDc non-responders the gene expression of *MMP1*, *TIMP1*, *ADAM9* and *MMP28* remained significantly dysregulated after therapy when compared to control colons. Moreover, the ratio of *MMP1/TIMP1* gene expression was significantly increased in active IBDc versus control colons, and this imbalance was restored after therapy in IBDc responders when compared to control colons.

As compared to control ileums, the gene expression levels of *MMP1*, *TIMP1*, *ADAM9*, *TFF1* and the ratio *MMP1/TIMP1* were all significantly increased in the inflamed ileum of CDi patients. After infliximab therapy, none of these genes remained significantly dysregulated in CDi responders *vs.* control ileums, except for *MMP1* gene expression which remained significantly upregulated after therapy in CDi responders *vs.* control ileums. This increased *MMP1* gene expression after therapy in CDi responders (7.21 times increase) was also observed by microarray analysis but no significance was reached (FDR=0.13) (**Table 3** and **Supplementary table 3**).

Protein expression of MMP1, MMP3, REG1A and TIMP1 in ileal and colonic biopsies

Immunohistochemistry was performed to localize MMP1, MMP3, REG1A and TIMP1 in the intestinal mucosa of controls and IBD patients with active and inactive disease. In normal mucosa MMP1 was expressed in the cytoplasm of primitive cells at the base of the crypts (**Figure 3A**), whereas active IBD mucosa showed an increased expression of MMP1 in immature and surface epithelium cells according to the regeneration of the epithelial layer (**Figure 3A**). Moreover, MMP1 expression was found in endothelial cells of active IBD patients nearby active inflammation areas. In normal mucosa, MMP3 was detected in mononuclear inflammatory cells (**Figure 3B**). In active IBD mucosa, there was an increased expression of MMP3 according to the increased amount of mononuclear inflammatory cells (**Figure 3B**). REG1A was expressed mainly in the Paneth cells at the base of the crypts in normal mucosa (**Figure 3C**), whereas an enhanced expression of REG1A was seen in immature and surface epithelium cells of active IBD patients (**Figure 3C**). TIMP1 expression was seen in enteroendocrine cells at the base of the crypts, but no clear differences could

be found in TIMP1 expression levels between control, inactive or active mucosa (**Figure 3D**).

Gelatinase levels and net gelatinolytic activities before and after treatment with infliximab

Zymography analysis was performed to determine gelatinase (MMP9 and MMP2) levels in mucosal biopsies taken from 3 IBDc and 3 CDi responder patients before and after infliximab, and 5 controls (3 ileum, 2 colon). In **figure 4 and supplementary figure 1**, a clear trend for higher gelatinase levels was seen before infliximab therapy when compared to levels after therapy or control levels. Interestingly, no NGAL-MMP9 or activated MMP9 levels were measured in control tissues. In addition, a gelatin degradation assay was performed to determine the net gelatinolytic activity present in the mucosal biopsies. Overall, a trend was seen for elevated gelatinolytic activity levels before infliximab treatment and a decrease to control activity levels after treatment (**Figure 5**). Moreover, two patients (1 CDi and 1 IBDc responder) had markedly high activity levels before infliximab.

DISCUSSION

In IBD a disturbed and high intestinal turnover is observed during the sequence of inflammation, tissue destruction and repair, resulting in tissue morphological changes (e.g. ulcers, fibrosis) (2;4;7;24;25). MMPs, ADAM(TS)s, TIMPs and growth factors play a major role in this tissue remodeling process. Achievement of mucosal healing in IBD responders to infliximab treatment allowed us to identify, during the evolution of the healing process, the changes that occur with healing in mediators of mucosal damage and repair in IBD.

In the present gene expression microarray study we investigated the intestinal mucosal expression of MMP, ADAM(TS), TIMP and growth factor genes in active IBD and the influence of thorough downregulation of inflammation by infliximab therapy on the mucosal expression of these genes. With the use of qRT-PCR we confirmed the microarray data of selected genes.

In this study, no significant differences in expression of these genes were found at baseline in inflamed colon between CDc and UC, whereas there was a large difference in gene expression before therapy between active CDc and active CDi, especially for the growth factors. Before infliximab therapy, our microarray study further showed that the gene expression of 8 MMPs (*MMP1*, *2*, *3*, *7*, *9*, *10*, *12* & *19*), 3 TIMPs (*TIMP1*, *2* & *3*), 5 ADAM(TS)s (*ADAM9*, *19* & *28* and *ADAMTS1* & *9*) and many growth factors was significantly upregulated, whereas only the expression of *MMP28* and *TGFA* was significantly downregulated before therapy in inflamed colon in UC and/or CDc as compared to control colons. As earlier described (26), the most upregulated gene in IBDc inflamed colon is the cell proliferation gene *REG1A* (>50-fold). Besides IBD, upregulated *REG1A* is also associated with type 1 diabetes, celiac disease and pancreatic cancer (27-29), and has been proposed to act as a mitogenic and/or an anti-apoptotic factor in the development of UC-associated neoplasia (30). Moreover, the analysis of protein levels and localization with immunohistochemistry demonstrated that REG1A was expressed by Paneth cells in normal conditions, whereas in active disease REG1A levels increased and the expression shifted towards all epithelial cells of the mucosal crypts. These data confirm previous studies, whereby REG1A gene and protein expression levels were studied in inflamed and control colonic mucosa(31;32).

The strong significantly upregulated (> 10-fold) *MMP1*, *3*, *7*, 10 & *12* in active IBD colon were previously listed as one of the top upregulated genes in IBD colon in a large scale microarray study using open-access IBD datasets (26). In CDi before therapy, the expression levels of 4 MMPs (*MMP1*, *3*, *7* & *10*), *TIMP1* and *TFF1* were significantly increased, and *ADAMDEC1* and *AGT* were significantly decreased *vs.* control ileums. Of notice, *AGT* expression levels were decreased in active CDi ileum and no correlation was seen with the epithelial marker *VIL1*, whereas it was increased in active IBDc colon and a positive correlation was found with the inflammatory marker *IL8,* suggesting that AGT may play a causative role in CDi ileum. In earlier studies it was shown that the renin-angiotensin system is involved in colonic inflammation and fibrosis associated with IBD (33), but not much is known about its involvement in ileal IBD. The dysregulated expression of nearly all mediators present at baseline normalized after infliximab therapy in responders, but not in non-responders. The expression of almost all upregulated genes at baseline in active disease correlated strongly with the granulocyte markers *S100A8*, *S100A9* and *S100A12,* and the inflammatory marker *IL8,* whereas the expression of the downregulated genes at baseline in active IBD correlated well with the epithelial marker *VIL1.* Both findings suggest that these dysregulations in active IBD are a consequence of the severe inflammation and epithelial damage in the intestine. Only the expression of 3 growth factors (*AREG*, *JAG1* in colon and *AGT* in ileum) remained significantly dysregulated after infliximab therapy in responders *vs.* controls. Further, the balance between MMPs and TIMPs is crucial, since any imbalance can result in an abnormally increased ECM turnover and remodeling, which can promote disease progression. In our study, *MMP1*, *3*, *10* & *12* expression levels were increased to a much greater extent (17-86x) than those of TIMPs (2-12x)

in active IBDc before therapy *vs.* control colons. This is suggestive for an increased net MMP activity in IBDc which might partly explain the tissue destruction in IBD. This is in accordance with previously published data (34). The imbalance of MMP/TIMP was restored in IBDc responders to infliximab who developed complete mucosal healing. The increased ratios of *MMP1*, *3 and 10 vs.TIMP1* was also seen before therapy in CDi inflamed ileum. Previous studies showed that infliximab treatment of IBD intestinal mucosal implants decreased the ratio between MMP1, 3 & 9 and both TIMP1 & 2 (35). A gelatin degradation assay was performed to determine the net gelatinolytic activity in a subset of IBDc (n=3), CDi (n=3) and control individuals (n=6). These data suggested that higher net gelatinolytic activity was present in mucosal biopsies before infliximab and the activity decreased to control levels after infliximab. Zymography analysis performed in this study showed that gelatinase (MMP2 and MMP9) levels had a trend to decrease after infliximab. These data were expected, since previous studies have shown that MMP2 and MMP9 decreased in mucosal biopsies (36) and serum (37) following infliximab therapy in CD. Interestingly, 1 CDi and 1 IBDc responder patient had particularly high net gelatinolytic activity levels and these patients also had the highest gelatinase levels as measured by zymography analysis (CDiR1 w0 and UCR1 w0, see **supplementary figure 1**). In addition, these high mucosal gelatinolytric activities normalized to control levels after treatment of with infliximab. This highlights the advantage of using both zymography analysis and a gelatin degradation assay. The combination of the tests enables to give information on presence, in a semi-quantitative manner, and activity of the gelatinases in a biological sample (21)). Immunohistochemistry analysis of the highest upregulated genes (MMP1, MMP3, TIMP1 and REG1A) confirmed the microarray data and showed higher protein

expression levels in active disease. These data are in line with previous studies (32;34;38).

Various conclusions emerge from our analyses: (i) in comparison with developmentally regulated ADAM(TS)s, inflammation-associated MMPs are stronger induced in IBD tissue *vs.* controls; (ii) *TIMP1* (major inhibitor of *MMP9*) is co-induced at similar levels in IBDc as its major proteinase; (iii) many growth factors are switched on in active IBD tissue biopsies, suggesting regenerative processes; (iv) infliximab restores many of the dysfunctional expressions which underlines the role of TNF as an important switch for MMP and growth factor expression; and (v) the excellent correlation between clinical (endoscopic and histologic) differences of responders and non-responders and alterations in gene expression profiling suggests that both cellular and molecular signatures can be practically used in future studies.

Furthermore, our group showed in a previous microarray study that prior to infliximab therapy the gene expression levels of *MMP1*, *2*, *3, 9*, *10, 13* & *19* were lower in UC and/or CDc responders when compared to UC/CDc non-responders to infliximab (15;39), suggesting that these MMP genes are predictive for non-response of infliximab in IBDc.

Our data suggest that the critical step in mucosal healing in IBD is control of inflammation, blockade of migration of leukocytes and elimination of the inflammatory cells from the tissue. Consequently, the MMPs and TIMPs decrease or normalize and the imbalance of MMP/TIMP is restored in IBD responders to infliximab. Growth factors are upregulated with active inflammation. With mucosal healing, we observe a decrease of most growth factor expression to normal levels, suggesting that there is no need for an excess of these mediators to maintain healing. Our studies, therefore, indicate that the biological targets in IBD are mainly the key inflammatory mediators

and the inflammatory cells. Targeting MMPs, TIMPS, ADAM(TS)s and growth factors may be beneficial in specific patients not responding to anti-TNF therapy but it is unlikely to achieve the same dramatic effects as the approach of targeting upstream inflammatory molecules or cells.

FIGURE LEGENDS

Figure 1: qRT-PCR analysis of *MMP1* (**A**), *MMP28* (**B**), *TIMP1* (**C**), *ADAM9* (**D**), *TFF1* (**E**) and *TGFB1* (**F**) in intestinal mucosa from IBD patients before and after first infliximab treatment and controls. A line between 2 points represents the change in expression before and after treatment for one patient. The x-axis labels of subfigures 1A, 1B, 1C and 1D are similar as shown in subfigure 1E and 1F. CDi: Crohn's ileitis, IBDc: colonic inflammatory bowel disease, NR: infliximab non-responders, R: infliximab responders, *significant p-value ≤ 0.01.

Figure 2: The ratio *MMP1*/*TIMP1* in intestinal mucosa from IBD patients before and after first infliximab treatment and controls, using the qRT-PCR expression data of *MMP1* and *TIMP1*. A line between 2 points represents the change in expression before and after treatment for one patient. CDi: Crohn's ileitis, IBDc: colonic inflammatory bowel disease, NR: infliximab non-responders, R: infliximab responders, *significant p-value ≤ 0.01.

Figure 3: Mucosal tissues from active CDi patients, IBDc patients and controls were stained by immunohistochemistry to localize MMP1 (**A**), MMP3 (**B**), REG1A (**C**) and TIMP1 (**D**). Arrow heads in the lower panel **D** indicate TIMP1 expression in

enteroendocrine cells. Images were taken at 4X and 20X magnification, scale bars are shown in the right lower corner.

Figure 4: Gelatin zymography analysis on mucosal biopsies from active CDi and IBDc responders before and after treatment with infliximab, and controls. Levels of MMP9 and MMP2 forms are represented as a ratio *vs*. proMMP2 levels. Mean values with standard deviation of the mean (SEM) are shown. IBDcR: IBD colitis responder to infliximab, CDiR: CD ileitis responder to infliximab.

Figure 5: Gelatin degradation assay on mucosal biopsies from active CDi and IBDc responders before and after treatment with infliximab, and controls. Gelatinolytic activity is represented by fluorescence units over time. Mean values with standard deviation of the mean (SEM) are shown. IBDcR: IBD colitis responder to infliximab, CDiR: CD ileitis responder to infliximab.

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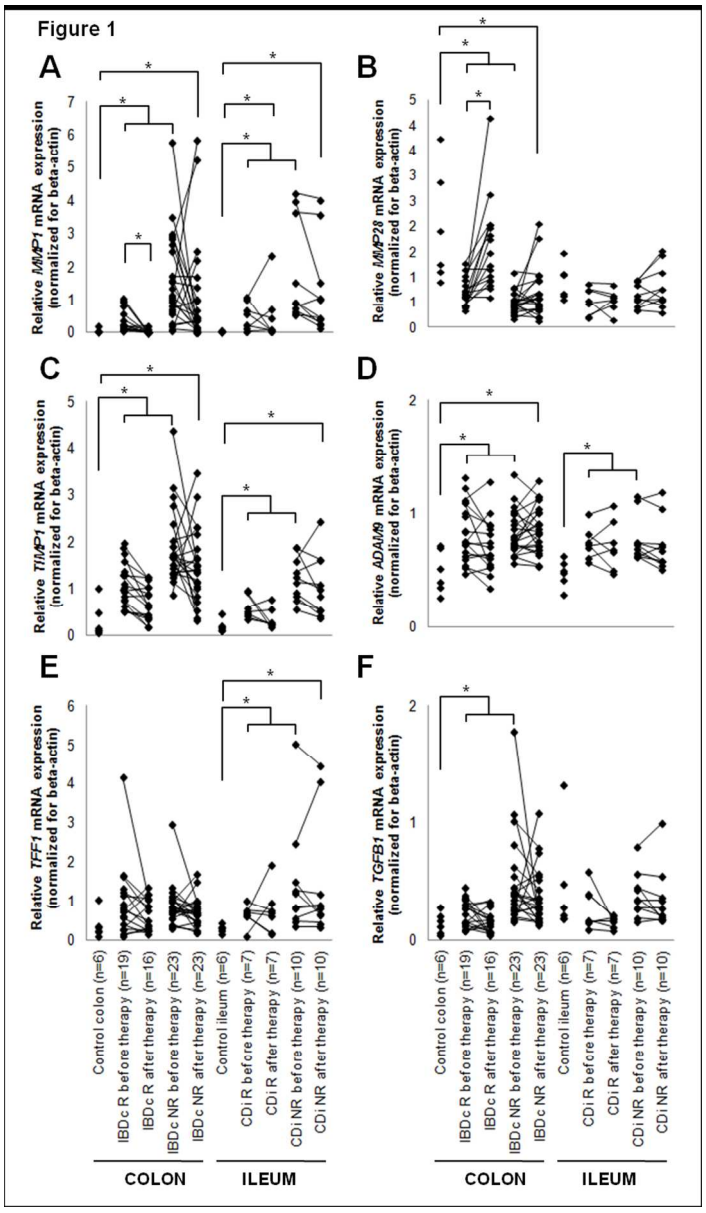

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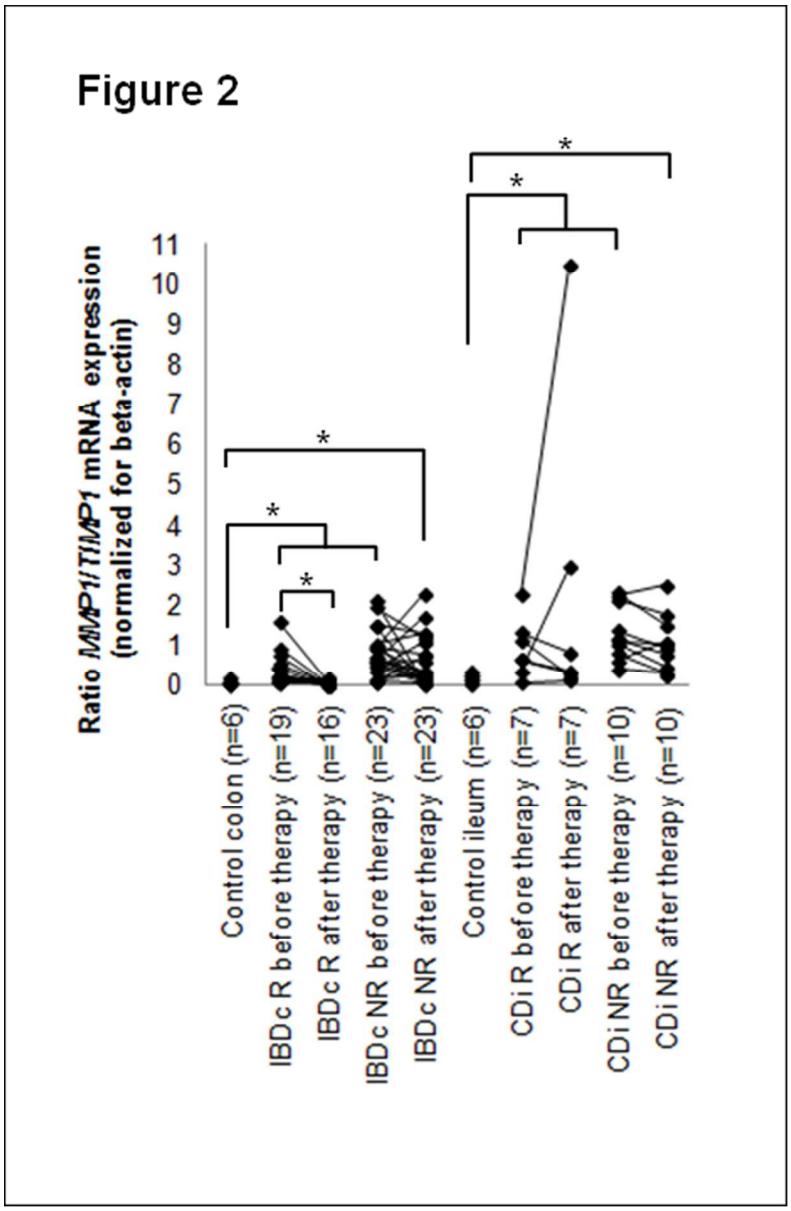

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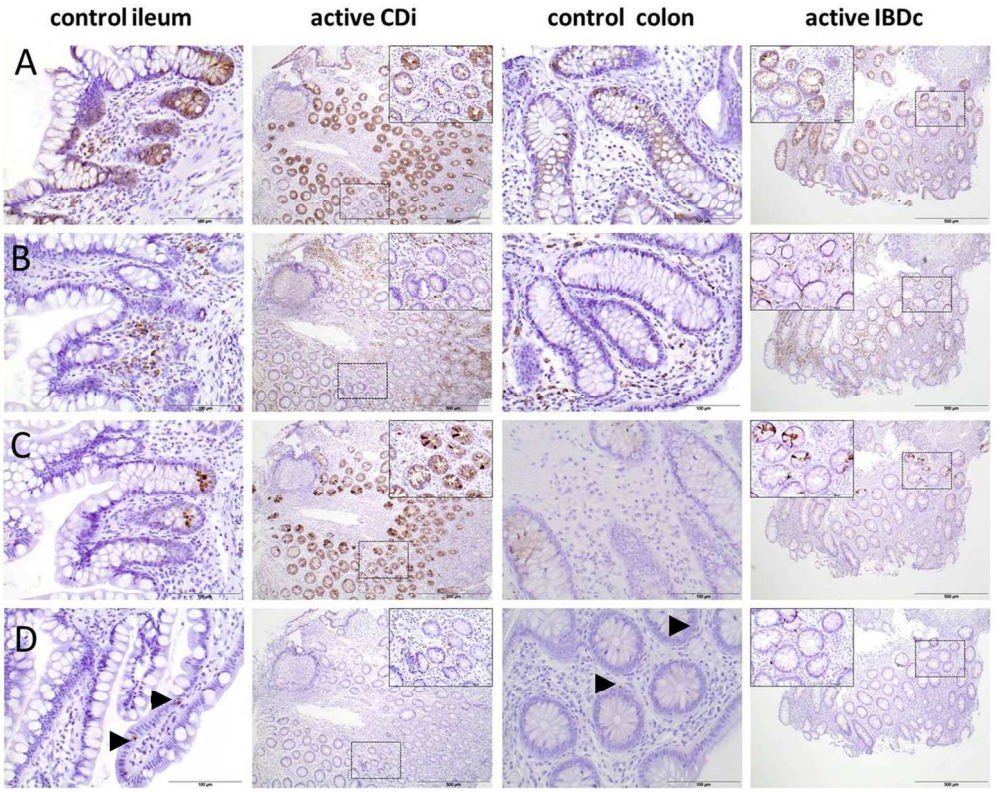

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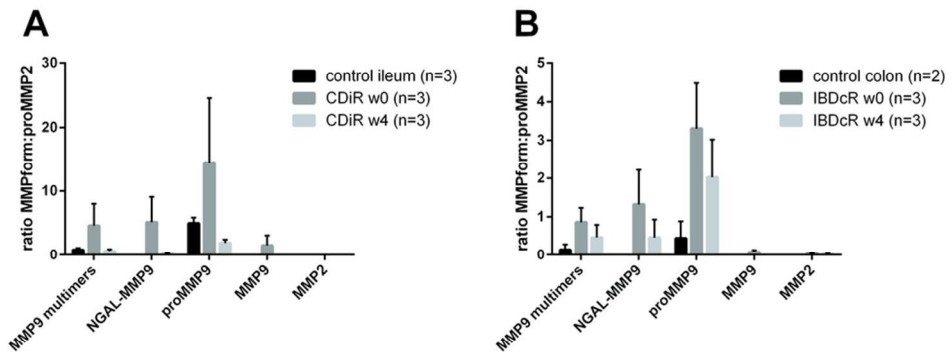

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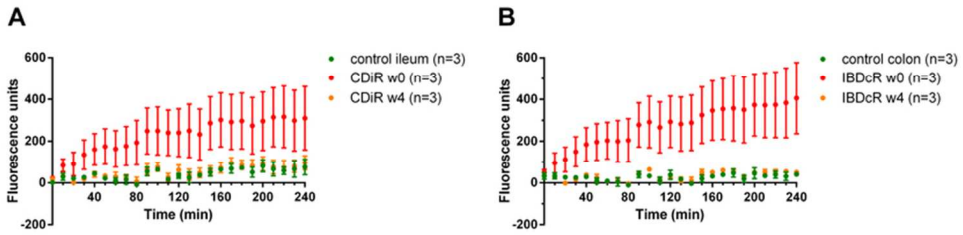

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Table 1: Characteristics of IBD patients at first infliximab infusion and of control individuals.

Baseline characteristics	IBD (n:61)			Controls (n:12)		P-value$_{IBD\ vs.\ controls}$
	UC (n:24)	CDc (n:19)	CDi (n:18)	Controls colon (n:6)	Controls ileum (n:6)	
Male/Female (%)	14/10 (58.3/41.7)	11/8 (57.9/42.1)	9/9 (50/50)	3/3 (50/50)	3/3 (50/50)	0.759
Median (IQR)* age (years)	41.4 (31.9-50.9)	31.8 (23.7-47.5)	46.4 (34-55.3)	73.07 (71.31-76.67)	51.8 (34.11-57.33)	0.002
Median (IQR)* duration of disease prior to first IFX (years)	7.3 (2.7-17.1)	6.4 (3.1-20.9)	22.3 (11.1-28)	NA	NA	NA
Extent of disease						
UC Left-sided colitis (%)	7 (29.2)	NA	NA	NA	NA	NA
Pancolitis (%)	17 (70.8)	NA	NA	NA	NA	NA
CD Ileocolon (%)	NA	5 (26.3)	9 (50)	NA	NA	NA
Ileum (%)	NA	0 (0)	9 (50)	NA	NA	NA
Colon (%)	NA	14 (73.7)	0 (0)	NA	NA	NA
Median (IQR)* C-reactive protein at first IFX (mg/L)	4 (1.8-19.1)	10.2 (4.3-35)	7.4 (2.3-10.9)	NA	NA	NA
Concomitant medication at first IFX (%)						
5-Aminosalicylates	18 (75)	8 (42.1)	5 (27.8)	NA	NA	NA
Corticosteroids	7 (29.2)	4 (21.1)	2 (11.1)	NA	NA	NA
Azathioprine/6-Mercaptopurine	15 (62.5)	14 (73.7)	7 (38.9)	NA	NA	NA
Methotrexate	0 (0)	0 (0)	0 (0)	NA	NA	NA
Corticosteroids + Immunosuppressants	3 (12.5)	2 (10.5)	1 (6)	NA	NA	NA
Active smoking (%)	2 (8.3)	6 (31.6)	6 (33.3)	0 (0)	1 (16.7)	0.438

*: datasets with skewed (non-normal) distributions, IQR: interquartile range, IFX: infliximab, NA: not applicable

Table 2: Fold changes of the probe sets encoding MMP, TIMP and ADAM(TS) genes that were significantly different in the comparative analyses before therapy in IBDc inflamed colon versus control colons, and fold changes of these probe sets from the comparative analyses after infliximab therapy in IBDc. The abbreviations of the individual MMPs, TIMPs and ADAM(TS)s are explained in supplementary table 1.

Probe Set ID	Gene Symbol	IBDc before IFX (n=43) *vs.* control colons (n=6)	IBDc R after IFX (n=19) *vs.* control colons (n=6)	IBDc NR after IFX (n=23) *vs.* control colons (n=6)	IBDc R after IFX (n=19) *vs.* IBDc R before IFX (n=19)	IBDc NR after IFX (n=23) *vs.* IBDc NR before IFX (n=23)
204475_at	*MMP1*	85.57*	1.76	54.66*	0.05*	0.33
201069_at	*MMP2*	4.09*	1.11	4.18*	0.52*	0.62
205828_at	*MMP3*	67.93*	1.27	42.97*	0.04*	0.32
204259_at	*MMP7*	8.00*	1.53	5.36*	0.25*	0.55
203936_s_at	*MMP9*	7.81*	1.34	4.56*	0.27*	0.40
205680_at	*MMP10*	16.98*	1.40	11.50*	0.19*	0.34
204580_at	*MMP12*	28.72*	2.24	21.54*	0.11*	0.54
204575_s_at	*MMP19*	2.08*	1.09	2.42*	0.81*	0.81
219909_at	*MMP28*	0.33*	0.79	0.38*	2.20*	1.21
239273_s_at	*MMP28*	0.42*	0.70	0.41*	1.61*	1.01
201666_at	*TIMP1*	11.51*	2.90	9.95*	0.39*	0.61
224560_at	*TIMP2*	2.16*	1.03	2.00	0.68*	0.71
1555326_a_at	*ADAM9*	2.10*	1.02	1.82	0.51*	0.80
202381_at	*ADAM9*	2.01*	1.33	1.91*	0.69*	0.91
209765_at	*ADAM19*	2.62*	1.21	2.48*	0.61*	0.75
205997_at	*ADAM28*	3.54*	1.25	2.84*	0.50*	0.61
222162_s_at	*ADAMTS1*	3.96*	2.65	6.04*	1.12	0.97
226814_at	*ADAMTS9*	3.17*	1.46	3.36*	0.60*	0.83

*: FDR < 0.05, underline: significant (> 2-fold change and FDR < 0.05), IFX: infliximab, R: responders, NR: non-responders

Table 3: Fold changes of the probe sets encoding MMP, TIMP and ADAM(TS) genes that were significantly different in the comparative analyses before therapy in CDi inflamed ileum versus control ileums, and fold changes of these probe sets from the comparative analyses after infliximab therapy. The abbreviations of the individual MMPs, TIMPs and ADAM(TS)s are explained in supplementary table 1.

Probe Set ID	Gene Symbol	CDi before IFX (n:18) *vs.* control ileums (n:6)	CDi R after IFX (n:8) *vs.* control ileums (n:6)	CDi NR after IFX (n:10) *vs.* control ileums (n:6)	CDi R after IFX (n:8) *vs.* CDi R before IFX (n:8)	CDi NR after IFX (n:10) *vs.* CDi NR before IFX (n:10)
204475_at	*MMP1*	22.04*	7.21	21.12*	0.63	0.57
205828_at	*MMP3*	47.58*	2.82	24.92*	0.15	0.25
204259_at	*MMP7*	3.27*	2.01	2.40	0.86	0.56
205680_at	*MMP10*	6.69*	1.82	3.74	0.40	0.41
201666_at	*TIMP1*	5.71*	1.79	5.57*	0.48	0.70
206134_at	*ADAMDEC1*	0.42*	0.35	0.32	0.79	0.80

*: FDR < 0.05, underline: significant (> 2-fold change and FDR < 0.05), IFX: infliximab, R: responders, NR: non-responders

Table 4: Fold changes of the probe sets encoding growth factor genes that were significant at baseline between active CDi and active CDc. The abbreviations of the individual growth factors are explained in supplementary table 1.

Probe Set ID	Gene Symbol	CDi before IFX (n:18) *vs.* CDc before IFX (n:19)
205239_at	*AREG*	0.23*
205289_at	*BMP2*	0.42*
205290_s_at	*BMP2*	0.42*
209101_at	*CTGF*	0.42*
206404_at	*FGF9*	2.57*
206268_at	*LEFTY1*	0.06*
1559361_at	*MACC1*	2.17*
1566764_at	*MACC1*	3.31*
1566766_a_at	*MACC1*	2.30*
232151_at	*MACC1*	2.50*
205614_x_at	*MST1*	2.41*
216320_x_at	*MST1*	2.69*
218718_at	*PDGFC*	2.03*

*: FDR < 0.05, underline: significant (> 2-fold change and FDR < 0.05), IFX: infliximab

Table 5: The fold changes of the probe sets encoding growth factor genes that were significantly different in the comparative analyses before therapy in IBDc inflamed colon versus control colons, and the fold changes of these probe sets from the comparative analyses after infliximab therapy in IBDc. The abbreviations of the individual growth factors are explained in supplementary table 1.

Probe Set ID	Gene Symbol	IBDc before IFX (n=43) *vs.* control colons (n=6)	IBDc R after IFX (n=19) *vs.* control colons (n=6)	IBDc NR after IFX (n=23) *vs.* control colons (n=6)	IBDc R after IFX (n=19) *vs.* IBDc R before IFX (n=19)	IBDc NR after IFX (n=23) *vs.* IBDc NR before IFX (n=23)
202834_at	*AGT*	2.67*	1.13	2.24*	0.55*	0.69
213001_at	*ANGPTL2*	2.90*	1.12	2.91*	0.66*	0.65
205239_at	*AREG*	3.52*	3.84*	4.67*	1.08	1.28
219505_at	*CECR1*	3.37*	1.33	3.11*	0.56*	0.69
209101_at	*CTGF*	2.70*	1.77	3.24*	0.87	0.93
1554741_s_at	*FGF7/ KGFLP1/ KGFLP2*	3.94*	1.60	4.78*	0.78	0.71
204220_at	*GMFG*	3.35*	1.14	3.57*	0.48*	0.79
203821_at	*HBEGF*	2.34*	3.00	3.56*	1.28	1.43
209524_at	*HDGFRP3*	2.15*	1.44	2.45*	0.98	0.82
210511_s_at	*INHBA*	3.19*	0.77	3.62	0.51*	0.59
231183_s_at	*JAG1*	2.71*	2.30*	2.61*	0.87	0.96
202655_at	*MANF*	2.33*	1.49	2.38*	0.67*	0.97
209752_at	*REG1A*	71.30*	3.36	31.27*	0.05*	0.42
205009_at	*TFF1*	2.94*	1.60	2.52*	0.61*	0.76
214476_at	*TFF2*	2.62*	1.30	1.96	0.58*	0.64
205016_at	*TGFA*	0.45*	0.57	0.42*	1.31	0.91
203085_s_at	*TGFB1*	2.42*	1.10	2.48*	0.72*	0.72
204858_s_at	*TYMP*	2.35*	1.12	2.16*	0.56*	0.79

*: FDR < 0.05, underline: significant (> 2-fold change and FDR < 0.05), IFX: infliximab, R: responders, NR: non-responders

Table 6: The fold changes of the probe sets encoding growth factor genes that were significantly different in the comparative analyses before therapy in CDi inflamed ileum *versus* control ileums, and the fold changes of these probe sets from the comparative analyses after infliximab therapy. The abbreviations of the individual growth factors are explained in supplementary table 1.

Probe Set ID	Gene Symbol	CDi before IFX (n:18) *vs.* control ileums (n:6)	CDi R after IFX (n:8) *vs.* control ileums (n:6)	CDi NR after IFX (n:10) *vs.* control ileums (n:6)	CDi R after IFX (n:8) *vs.* CDi R before IFX (n:8)	CDi NR after IFX (n:10) *vs.* CDi NR before IFX (n:10)
202834_at	*AGT*	0.33*	0.28*	0.28*	0.92	0.80
205009_at	*TFF1*	2.94*	2.03	3.17*	0.95	0.83

*: FDR < 0.05, underline: significant (> 2-fold change and FDR < 0.05), IFX: infliximab, R: responders, NR: non-responders