Elevated levels of serum-soluble triggering receptor expressed on myeloid cells-1 in patients with IBD do not correlate with intestinal TREM-1 mRNA expression and endoscopic disease activity

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Abstract

Background & aims: Triggering receptor expressed on myeloid cells-1 (TREM-1) is a potent amplifier of pro-inflammatory responses. We have previously demonstrated a substantial increase in TREM-1-expressing macrophages in the inflamed intestinal mucosa of patients with inflammatory bowel diseases (IBD). TREM-1 is also produced as a soluble receptor (sTREM-1). Here, we aimed to determine whether serum sTREM-1 could be used as a surrogate marker of disease activity in patients with IBD.

Methods: Intestinal biopsies and concurrently collected sera from patients with Crohn’s disease (CD) and Ulcerative colitis (UC) enrolled in the Swiss IBD cohort study were analyzed for intestinal TREM-1 mRNA and serum sTREM-1 expression. TREM-1 mRNA and sTREM-1 were correlated with the endoscopically determined disease activity. Serum sTREM-1 and TREM-1 mRNA expression levels were further determined in sera and colonic tissues collected at various time-points.

Abbreviations: CD, Crohn’s disease; IBD, inflammatory bowel diseases; TLR, Toll-like receptor (s); TREM-1, (soluble) Triggering Receptor-Expressed on Myeloid Cells-1; UC, Ulcerative colitis

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1. Introduction

Inflammatory bowel diseases (IBD) are a heterogeneous group of diseases comprising the two major disease entities Crohn's disease (CD) and ulcerative colitis (UC). While CD and UC differentially affect the gastrointestinal tract and likely differ in their pathogenic mechanisms, both CD and UC are believed to be driven by aberrant innate immune responses to the commensal flora in genetically susceptible individuals. A characteristic feature of both CD and UC is also the fluctuating disease course with episodes of relapse (active disease) and remission (quiescent disease), which complicate an adequate treatment of individual patients. Since endoscopy for accurate assessment of disease activity and treatment efficacy is time-consuming and expensive, considerable efforts have been undertaken to evaluate non-invasive surrogate biomarkers and disease activity indices based on clinical parameters. Among laboratory parameters, CRP, ESR and peripheral blood leukocyte counts are routinely assessed, but representing acute phase reactants, exhibit low specificity for IBD. Clinical disease activity indices such as the Crohn's disease activity index (CDAI) are prone to subjective judgements and have a low sensitivity in detecting subclinical inflammation or discriminating quiescent from active disease.

In contrast, and in keeping with the established role of innate immune cells in driving intestinal inflammation in IBD, presence of proteins derived from degranulating phagocytes in the stool has been shown to closely correlate with endoscopic disease activity. Although these fecal biomarkers, such as calprotectin and lactoferrin, are now considered the gold standard among the currently employed markers, such as the Crohn's disease activity index (CDAI) are prone to subjective judgements and have a low sensitivity in detecting subclinical inflammation or discriminating quiescent from active disease.

As an alternative, triggering receptor expressed on myeloid cells-1 (TREM-1) is a member of the immunoglobulin superfamily predominantly expressed on neutrophils and on subsets of monocytes/macrophages. Binding of the yet unidentified ligands to TREM-1 potently synergizes with distinct Toll-like receptor (TLR) ligands in amplifying oxidative burst and pro-inflammatory cytokine production. The TLR ligands LPS and lipoteichoic acid, but also the NOD-like receptor (NLR) ligand muramyl dipeptide and other stimuli such as the pro-inflammatory cytokines TNFα and GM-CSF can further upregulate surface TREM-1 expression. TREM-1 can also be produced in a soluble form (sTREM-1) which is believed to originate from a protease-dependent shedding of membrane-bound TREM-1.

Elevated serum levels of sTREM-1 are indicative of microbial infections, however, augmented sTREM-1 levels have also been reported for patients with non-infectious inflammatory conditions such as rheumatoid arthritis, acute pancreatitis, chronic obstructive pulmonary disease and cardiac arrest. Thus, sTREM-1 may not only be considered a diagnostic marker for microbial infections, but may also have the potential to serve as a novel surrogate marker to assess disease severity in other inflammatory diseases.

We have recently demonstrated a significant increase in TREM-1 mRNA and protein, relating to an accumulation in TREM-1-expressing macrophages, in the inflamed intestinal mucosa of patients with IBD and mice with experimental colitis while TREM-1 expression was almost undetectable in normal control samples and tissue specimens from unaffected intestinal areas. Importantly, intestinal macrophages from patients with IBD but not from normal controls produced substantially increased levels of pro-inflammatory cytokines upon TREM-1-mediated activation in vitro and blocking TREM-1 in two mouse models of experimental colitis significantly attenuated disease. Based on the observed accumulation of TREM-1-expressing cells in the inflamed intestinal tissue and the seemingly prominent role of TREM-1 in the pathogenesis of IBD, we aimed to determine whether increased intestinal TREM-1 expression is reflected in elevated levels of serum sTREM-1 and whether sTREM-1 in the serum could thus serve as a novel elegant tool to assess disease activity in patients with IBD.

2. Materials and methods

2.1. Patients and samples

Criteria for inclusion of patients into the Swiss IBD cohort study (SIBDCS), the SIBDCS profile and ethical approval have been described previously. Additionally, 40 patients with Crohn's disease (CD) and 40 patients with ulcerative colitis (UC) were selected from the cohort based on the availability of concurrently collected sera and intestinal biopsies. Sera were frozen at −80 °C within 24–48 h post collection.
Intestinal biopsies were directly sampled into RNAlater RNA stabilization reagent (Ambion) and stored at −80 °C.

The 40 patients with CD or UC, respectively, included 20 patients with quiescent disease and 20 additional patients with active disease. Classification of disease activity into quiescent and active was based on macroscopic findings at the time-point of endoscopy. Mucosal healing or slight erythema were considered as quiescent disease whereas overt erythema and edema of the mucosa, aphthous disease or presence of ulcers were considered as active disease. Patients with infectious colitis were excluded from the study. Mean age and gender distribution of patients with CD and UC were as follows: CD: 43±15 years, 42.5% females, and UC: 44.5±13 years, 45% females. The controls were healthy persons recruited from the laboratory staff who had provided written consent for the collection of blood samples.

2.2. Mice

RAG2−/− mice were bred and maintained under SPF conditions in the central animal facility of the Medical School, University of Bern. All animal experiments were approved by the state of Bern committee for animal experimentation (license number: 79/08).

2.3. Determination of sTREM-1 in sera and culture supernatants

Human serum sTREM-1 levels were determined by an in-house optimized ELISA using the anti-human TREM-1 mAb clone 6B1.1 G12 (generous gift from M. Radsak, University of Mainz) at 10 μg/ml for coating and reagents from the Human TREM-1 Duoset (R&D) for detection (according to manufacturers’ instructions). Mouse serum sTREM-1 was measured by ELISA using the Mouse TREM-1 Duoset reagents from R&D.

2.4. RNA isolation

RNA was isolated from one intestinal biopsy per patient using the Qiagen RNeasy Mini kit and following the manufacturers’ instructions. RNA from mouse colonic tissue specimens was isolated with the RNA isolation reagent Tri-reagent (Molecular research center).

2.5. Quantitative RT-PCR for intestinal TREM-1 and S100A9 mRNA

RNA (≤2 μg) from human intestinal biopsies and mouse colonic tissue specimens was DNase treated using a RNase-free DNase I (Ambion) and cDNA was generated with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Expression of TREM-1 and S100A9 mRNA was analyzed on a 7500 Real-time PCR System (Applied Biosystems) using Qiagen Quantitect Primer Assays. The house-keeping gene GAPDH was used for normalization of TREM-1 mRNA expression.

2.6. Flow cytometry

TREM-1 surface expression was determined by staining with the anti-human TREM-1 mAb clone 21C7 (hybridoma supernatant; generous gift from M. Colonna, Washington University School of Medicine) followed by goat-anti-mouse Ig PE-labeled (Fab)2 (BD Biosciences). The mouse IgG1 clone MOPC-21 of irrelevant specificity was used as isotype control. Cells were acquired on a LSRII flow cytometer (BD Biosciences) and analyzed using Flowjo software (Tree Star).

2.7. Leukocyte isolations and in vitro cultures

Leukocytes from healthy controls were isolated from heparinized blood by a 50’ sedimentation of erythrocytes over 12% (w/v) Optiprep (Axis-Shiled, Oslo, Norway) and 16 g/l Methocel (Fluka, Buchs, Switzerland) in 130 mM NaCl. The leukocyte-rich plasma was subsequently collected and washed with IMDM 10% FCS (Sigma). Leukocytes were cultured at 5×10^6 cells/well in 96-well flat bottom plates in the presence of 100 ng/ml LPS (Sigma) for 24 h. Cell-free supernatants were subsequently collected for analysis of stREM-1.

2.8. CD4 CD45RBhi T cell adoptive transfer model of colitis

Colitis was induced in RAG2−/− mice by adoptive transfer of 2×10^5 CD4+ CD25− CD45RBhi FACS-sorted T cells as described previously. Mice were sacrificed at the indicated time-points or when they exhibited clinical signs of colitis (diarrhea, weight loss, signs of abdominal pain). Sera were obtained by tail vein incision with a scalpel or by heart-puncture of the euthanized mice. Two pieces of the middle part of the colon were removed for fixation in 4% paraformaldehyde in PBS (and subsequent paraffin embedding) or for storage in RNAlater (for subsequent isolation of RNA), respectively.

2.9. Clinical and histopathological assessments and determination of disease activity in colitic mice

Clinical scoring was based on the following parameters: % of initial weight loss (0–4%=0, 5–7%=1, 8–10%=2, 11–15%=3), extent of diarrhea (normal stool=0, soft stool=1, visible diarrhea=2, absence of any stool=3) and macroscopic appearance (i.e. thickening and shortening) of colons (normal appearance=0, mild-moderate-severe inflammation=1–3). Clinical scores for mice sacrificed at 10–12 days post disease induction ranged from 0 to 8 (mean: 3.9±2.5). To assess the presence of histopathological alterations, a scoring system ranging from 0 (no alterations) to 15 (most severe signs of colitis) was established, including the following parameters: cellular infiltration (0–3), loss of goblet cells (0–3), crypt abscesses (0–3), epithelial erosions (0–1), hyperemia (0–2), thickness of the colonic mucosa (0–3). Histological scoring was performed by a pathologist (N.S.-S.) blinded to sample identity. Histopathological scores ranged from 1 to 8 (mean: 3.9±2.6). For determination of disease activity in mice, clinical and histopathological scores were combined into a total score ranging from 1 to 15 (mean: 7.7±4).
2.10. Statistical testing

Statistical analyses were carried out with a statistical package program (GraphPad Prism software version 4.0). The Mann Whitney U test was employed for analysis of data presented in Figs. 2, 4 and 5. Correlations were studied using the Spearman rank correlation test. The ability of sTREM-1 and CRP to discriminate patients with either quiescent or active IBD from healthy controls and to discriminate patients with active IBD from patients with quiescent disease was assessed by receiver operator characteristic analyses (ROC) and calculation of the area under the ROC (AUC) with a 95% confidence interval.

3. Results

3.1. TREM-1 mRNA expression is increased in intestinal biopsies of IBD patients with active but not quiescent disease

We first investigated whether intestinal TREM-1 mRNA expression differed in CD and UC patients with endoscopically determined active compared to quiescent disease. In keeping with our previous findings, TREM-1 mRNA was undetectable or expressed at very low levels in intestinal biopsies obtained from patients with quiescent disease (Fig. 1A and B). In contrast, elevated TREM-1 mRNA levels could be detected in biopsies taken from inflamed intestinal sites of patients with active CD or UC (Fig. 1A and B) while TREM-1 mRNA was not observed in matched non-inflamed biopsies from these patients (data not shown). TREM-1 mRNA expression in inflamed intestinal biopsies was reflected by the expression of mRNA for S100A9 (MRP14) (Fig. 1C–D), consistent with an increased presence of activated mononuclear phagocytes at inflamed intestinal sites.

3.2. Serum sTREM-1 levels are augmented in IBD patients with active and quiescent disease

Since endoscopically active disease in fact associated with increased intestinal TREM-1 expression, we subsequently assessed sTREM-1 levels in sera which were collected on the day of endoscopy from patients with quiescent and active disease. Compared to healthy controls, who exhibited median basal serum sTREM-1 levels of 384 pg/ml, sTREM-1 levels were significantly increased in patients with CD or UC irrespective of the presence of endoscopically quiescent or active disease (Fig. 2). While median serum sTREM levels were almost identical in CD patients with quiescent (1079 pg/
ml) compared to active (1124 pg/ml) disease (Fig. 2A), median serum sTREM-1 was distinctly elevated in UC patients with active (1441 pg/ml) compared to quiescent disease (950 pg/ml) (Fig. 2B). Patients with IBD displayed a considerable degree of heterogeneity with respect to serum sTREM-1 expression levels (Fig. 2A and B). A substantial overlap in expression levels between patients with quiescent and active disease was also observed for CRP, particularly in UC patients (Fig. 2C and D). No significant correlation was found for serum sTREM-1 and CRP levels, even in CD or UC patients with active disease (Supporting information Fig. 1).

3.3. Intestinal TREM-1 mRNA expression and serum sTREM-1 levels do not correlate

In contrast to intestinal TREM-1 mRNA, which was barely detectable in patients with quiescent CD or UC, IBD patients with endoscopically quiescent disease exhibited significantly elevated serum sTREM-1 levels compared to normal controls (Figs. 1 and 2). Hence, in IBD patients with endoscopically quiescent disease, intestinal TREM-1 mRNA and serum sTREM-1 did not correlate (Fig. 3A and B). Since TREM-1 mRNA in inflamed biopsies of patients with active disease and serum TREM-1 were expressed in a heterogeneous manner, ranging from low to high expression levels across the different patients, we considered the possibility that at least in IBD patients with endoscopically active disease, increased intestinal TREM-1 mRNA expression could be reflected in higher serum sTREM-1 levels. However, also in IBD patients with active disease, no significant correlation was found for intestinal TREM-1 mRNA and serum sTREM-1 expression (Fig. 3C and D).

3.4. Differential expression of TREM-1 mRNA and serum sTREM-1 in patients with ileal versus colonic CD

Considering that in patients with endoscopically active disease biopsies were analyzed from inflamed intestinal sites only, the heterogeneous expression pattern of mRNA for TREM-1 and S100A9 across the different patients’ samples was striking. While these differences could be attributable to a distinct infiltration by myeloid cells, at least in patients with CD they could also relate to the ileal versus colonic site of disease, as reported previously for the release of calprotectin from intestinal tissue samples. Among the biopsies analyzed in the present study, half of the samples were derived from colonic versus ileal sites of inflammation in patients with quiescent and active CD, respectively. Highest expression levels of TREM-1 and S100A9 mRNA were indeed detected in biopsies from inflamed colonic, but not ileal sites (Fig. 4A and B). Intriguingly, peak expression levels of sTREM-1 were preferentially detected in patients with ileal compared to colonic CD (Fig. 4C). These data further suggested that serum sTREM-1 may not be directly associated with TREM-1 expression in the inflamed intestine.

Figure 2  Serum sTREM-1 levels are increased in patients with active and quiescent IBD. sTREM-1 (A, B) and CRP (C, D) levels were determined in the sera of healthy controls (n=20) and in patients with quiescent (n=20) and active (n=20) CD (A, C) and UC (B, D), respectively. Each symbol represents a value obtained from an individual healthy control or from a patient with either quiescent or active disease. Lines indicate median values. * p<0.05, *** p<0.001.
Healthy control individuals differ with respect to serum sTREM-1 levels and surface TREM-1 expression

Heterogeneity in serum sTREM-1 expression levels was not only observed for patients with IBD but also for healthy controls, albeit to a lesser extent (Fig. 2). We thus speculated that serum sTREM-1 was not only subject to modulation by inflammatory responses but could also be influenced by inherent factors. Indeed, when six healthy controls were systematically investigated with respect to their peripheral blood neutrophil counts, median surface expression of TREM-1 and basal serum sTREM-1, those individuals with the lowest serum sTREM-1 also exhibited reduced neutrophil counts and, compared to healthy controls with intermediate serum sTREM-1, a distinctly lower TREM-1 surface expression on their peripheral neutrophils (Table 1). Moreover, after stimulation of leukocytes from these individuals with saturating concentrations of LPS in vitro, shedding of sTREM-1 into the supernatant was still limited (Table 1). Notably, at least in some of the healthy controls analyzed, low or high expression of surface TREM-1 and serum sTREM-1 appeared to be a stable trait over time (data not shown). Hence, intrinsic variations in surface TREM-1 and serum sTREM-1 may contribute to the high degree of variability in serum sTREM-1 expression levels also in patients with IBD.

In mice with experimental colitis, intestinal TREM-1 mRNA expression is increased at later stages of disease while a rise in sTREM-1 levels is observed early post disease induction

In order to systematically follow the course and association of intestinal TREM-1 mRNA and serum sTREM-1 expression during intestinal inflammation, we decided to employ an experimental mouse model of colitis. Adoptive transfer of CD4 CD45RBHI T cells into lymphopenic RAG2−/− recipients leads to onset of weight loss, diarrhea and clinical signs of colitis, which depending on the microbial colonization of the recipients, may develop within 10–12 days post the colitogenic CD4 T cell transfer (Fig. 5A). In keeping with our previous results and analogous to the situation in human individuals, TREM-1 mRNA expression was very low to undetectable in the colons of healthy control mice (Fig. 5B). In mice analyzed at 5 or 7 days post colitis induction, increased TREM-1 mRNA expression could still not be detected (Fig. 5B), consistent with the absence of macroscopic and histopathological signs of colitis at these early time-points (Fig. 5A and data not shown). However, at 10–12 days post disease induction, distinctly augmented TREM-1 mRNA expression was observed in the colonic tissue samples from the majority of mice (Fig. 5B). TREM-1 expression correlated with the expression of mRNA for S100A9 (data not shown).
Thus, in mice with experimental colitis, S100A9 and TREM-1 expression in the intestinal mucosa represented a late rather than early event in the disease course. From control mice or mice sacrificed at the different time-points post colitis induction (Fig. 5C, filled circles) sera were also obtained and analyzed for the presence of sTREM-1. Moreover, sTREM-1 was assessed in sera from mice that were bled consecutively at days 5, 7 and 10–12 post CD4 T cell transfer (Fig. 5C, other symbols). Serum sTREM-1 was expressed in a heterogeneous manner in healthy control mice (mean: 102± 42 pg/ml). Nonetheless, a distinct increase in serum sTREM-1 was noted as early as 5 days (mean: 141±42 pg/ml) and 7 days (mean: 169±80 pg/ml) post disease induction (Fig. 5C). Taken together, this kinetics indicated that activation of TREM-1-expressing phagocytes in the inflamed intestinal mucosa and local shedding of TREM-1 did likely not represent the predominant source of the increased serum sTREM-1 observed in experimental colitis.

Colonic TREM-1 mRNA expression and serum sTREM-1 levels in control mice and mice sacrificed at 10–12 days post disease induction were subsequently correlated with disease activity. To this end, mice were grouped into different categories of mild to moderate or severe disease depending on the overall scores for clinical and histopathological signs of colitis (see Materials and methods). While a minority of mice (n=5) only developed a mild form of colitis and almost completely lacked colonic TREM-1 expression similar to the healthy control group, the majority of mice (n=9) had moderate to severe disease, which associated with significantly increased TREM-1 mRNA expression (Fig. 5D). Intriguingly, an inverse relationship was observed for serum sTREM-1 levels, and mice with a mild, but not severe, form of colitis tended to exhibit increased serum sTREM-1 (Fig. 5E).

### 4. Discussion

With the present study we have aimed to assess the potential of serum sTREM-1 to serve as a novel surrogate marker of disease activity in patients with IBD. Our findings demonstrate significantly upregulated levels of serum sTREM-1 in patients with CD or UC compared to normal controls. However, in contrast to intestinal TREM-1 mRNA, which was exclusively expressed in inflamed biopsies from patients with active disease, elevated serum sTREM-1 was also detected in patients with quiescent disease at similar levels as observed in patients with active disease. These results thus
only partially consent with the findings from two previous studies that have reported increased, but largely non-overlapping levels of serum sTREM-1 in IBD patients with mild compared to severe disease.25,26 Intriguingly, in these studies median values measured for sTREM-1 were as low as 0–50 pg/ml in healthy controls and in the case of Park’s et al. investigation only reached 66.5 pg/ml even in patients with IBD,26 illustrating potential differences in the
methodological approach for detection of sTREM-1. Notably, when employing the commercialized specific ELISA kit that was likely utilized by the other two investigations, we consistently failed to detect serum sTREM-1 above background levels, and it was only with the use of an alternative (monoclonal) capture antibody and an in-house optimized ELISA that we were able to successfully measure sTREM-1. While differences in the ELISA protocols may account for some of the disparities in the results obtained with respect to the potential value of sTREM-1 as surrogate marker of disease activity, other parameters such as the size of the patient pool and determination of actual disease activity are likely more relevant. In the present study, we have analyzed equally sized groups of n = 20 healthy controls and CD or UC patients with either quiescent or active disease, respectively. Importantly, since clinical activity indices tend to correlate poorly with the actual endoscopic disease activity, we have taken care to include only patients with endoscopically determined disease activity and to analyze sTREM-1 in serum samples that were actually obtained on the day of endoscopy. This set-up, respectively, the concurrent availability of intestinal biopsies and sera, has further allowed us to directly associate the presence of serum sTREM-1 with intestinal TREM-1 mRNA expression. Based on our previous observations that TREM-1-expressing mononuclear phagocytes are substantially increased in the inflamed intestinal mucosa of patients with IBD and mice with experimental colitis,19,20 we have speculated that local activation-induced shedding of surface TREM-1 may translate into increased serum sTREM-1. Our latest results indeed show an association of intestinal TREM-1 mRNA expression with endoscopically determined active versus quiescent IBD. It remains curious that not all of the inflamed biopsies from patients with active disease exhibited substantially increased TREM-1 mRNA expression. Since expression of TREM-1 mRNA significantly correlated with the expression of mRNA for S100A9 (data not shown), we assume that the heterogeneous expression levels observed for TREM-1 and S100A9 may primarily be attributable to a distinct infiltration by myeloid cells. In addition, at least in patients with active CD, the colonic versus ileal localization of disease also appears to account for higher expression levels of TREM-1 mRNA, similarly to what has previously been reported for the release of S100 proteins from colonic tissue samples.24 Unfortunately, due to the small size of the individual biopsies, a histological assessment of the extent and composition of the inflammatory infiltrate to further investigate into the mechanism behind the heterogeneous expression levels of TREM-1 and S100A9 mRNA was not attainable.

One of the most unanticipated and striking findings of the present study was the lack of correlation between intestinal TREM-1 mRNA expression and serum sTREM-1. Accordingly, while TREM-1 mRNA expression was undetectable in intestinal biopsies from patients with quiescent disease, these patients exhibited significantly increased levels of serum sTREM-1 compared to healthy controls. Moreover, even in patients with active disease, distinctly augmented expression of TREM-1 mRNA did not associate with peak levels of serum sTREM-1. Since in an experimental mouse model of colitis increased serum sTREM-1 levels clearly preceded an upregulated expression of intestinal TREM-1 mRNA expression, we may conclude that, contrary to our initial assumptions, activation of TREM-1-expressing myeloid cells in the inflamed intestinal mucosa and local shedding of TREM-1 can hardly represent the principal source of the increased serum sTREM-1 observed in patients with IBD.

Currently, we can at best speculate about the systemic cellular source of the elevated serum sTREM-1 observed in patients with IBD, as well as the activating stimuli that trigger the release of surface TREM-1. Shedding of TREM-1 is induced by TLR ligands such as LPS,10,11 but based on more recent evidence possibly also by non-microbial factors such as Prostaglandin E2 (PGE2),27 hypoxic conditions18 or potential ligands of NOD family receptors such as monosodium urate crystals.29 In contrast, pro-inflammatory cytokines such as TNFα, IL-1β, or IFNγ appear insufficient in mediating substantial sTREM-1 release.11 We were not able to trigger the release of sTREM-1 by incubation of human PBMC with PGE2 or block an LPS-induced shedding of sTREM-1 with synthetic Cox- inhibitors in vitro (data not shown), arguing against a simple effect of general pro-inflammatory mediators in stimulating sTREM-1 release. We thus hypothesize that even in patients with quiescent IBD and in mice with experimental colitis at the early stages of disease induction, subclinical intestinal inflammation may be sufficient to cause minor intestinal epithelial barrier defects and translocation of bacterial products into the systemic circulation, which may subsequently trigger the release of sTREM-1 distantly from the intestinal mucosa.

The ability of serum sTREM-1 to detect subclinical inflammation in IBD patients with quiescent disease is remarkable (AUC in CD patients compared to controls: 0.95 ± 0.03 and in UC patients: 0.92 ± 0.04) and in this respect appears superior to CRP (AUC in CD patients: 0.60 ± 0.09 and in UC patients: 0.77 ± 0.07). Nonetheless, like CRP, serum sTREM-1 cannot be considered a specific marker of IBD and thus has little value for an initial diagnosis of the disease. Furthermore, although median serum sTREM-1 levels were significantly elevated at least in patients with active compared to quiescent UC, a substantial overlap in expression levels was still observed between the two patient groups. Hence, from the analysis of the confined number of patients included in this study, we deduce that serum sTREM-1 only has limited potential to serve as a novel surrogate marker of disease activity in patients with IBD. This conclusion is corroborated by our findings from an experimental mouse model of colitis, where serum sTREM-1 levels tended to inversely associate, rather than directly correlate with disease activity. We have demonstrated previously that administration of the synthetic peptide LP17, representing the extracellular domain of TREM-1, to mice with established colitis was able to significantly attenuate the disease process.19 Treatment with a recombinant TREM-1 fusion protein or with LP17 peptide even protected mice from LPS-induced endotoxemic lethality,5,30 and in patients with sepsis, elevated serum sTREM-1 at the time-point of admission associated with increased survival.31 Serum sTREM-1 thus not only has to be looked at as a passive marker of infection or disease, but also has to be considered as a decoy receptor with protective functions that could actively modulate the disease process.

The use of serum sTREM-1 as surrogate marker of disease activity may further be complicated by intrinsic parameters that could influence its expression in the absence of overt inflammation. Our preliminary analyses have indicated that healthy control subjects can differ substantially with respect to their basal serum sTREM-1 levels. Moreover, low serum
sTREM-1 in some of these individuals associated with low surface expression of TREM-1 on neutrophils and appeared to be a stable trait over time. We are currently investigating whether these inherent differences in TREM-1 expression could be explained by polymorphisms in the TREM-1 gene, similar to what has been described for SNPs identified in the CRP promoter region and CRP plasma levels.32

As intrinsic variations in TREM-1 expression in patients with IBD may obscure any inflammation-induced changes on a population level, we have performed repeated analyses of serum sTREM-1 levels in eight patients with CD over a time-period of 38 months. Intriguingly, no consistent pattern of upregulated or downmodulated serum sTREM-1 expression could be observed as these patients went through periods of active and quiescent disease, respectively (data not shown). Since in these analyses determination of disease activity was not strictly based on endoscopy but largely relied on clinical assessments, more systematic time-course studies on individual patients will be required to conclusively investigate the value of serum sTREM-1 in diagnosing or predicting relapses or even responses to treatments.

In summary, our current data argues against the use of serum sTREM-1 as a straightforward novel marker of disease activity in patients with IBD. However, considering the upregulated expression of TREM-1 mRNA in the inflamed intestinal mucosa of patients with active, but not quiescent IBD, and in mice with a severe, as opposed to a mild, form of experimental colitis, the potential role of TREM-1 in IBD cannot be overlooked. Thus, future evaluations on the possible value of sTREM-1 as a fecal marker of disease activity or efforts to therapeutically interfere with TREM-1 signalling may still lead to a significant advance in the management of IBD.

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Appendix

Members of the SIBDCS:


References