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Danger-Associated Molecular Patterns and Inflammatory Bowel Disease: Is There a Connection?

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Key Words
Danger-associated molecular patterns · Inflammasome · Pattern-recognition receptors · Triggering receptor expressed on myeloid cells

Abstract
The innate immune system is of critical importance for maintaining the local tissue homeostasis in the intestinal mucosa. It must recognize and rapidly respond to microbial antigens and danger signals to provide a first line of host defense. This is primarily accomplished through an array of pattern recognition receptors that are located in distinct (sub)cellular compartments and bind pathogen-associated and danger-associated molecular patterns (PAMPs and DAMPs, respectively). The impact of PAMPs, in particular NOD2/CARD15, in the pathogenesis of Crohn's disease is widely established. The involvement of DAMPs in the pathogenesis of inflammatory bowel disease (IBD), however, is much less recognized. DAMPs (also known as alarmins) represent non-pathogen-derived molecules, such as intracellular proteins released from damaged and stressed cells. Although the ligand(s) for the triggering receptor expressed on myeloid cells (TREM)-1 have not yet been fully identified, circumstantial evidence indicates that DAMPs are the inducers of the TREM-1-mediated, excessive induction of proinflammatory effects, also seen in patients with active IBD. Blocking the interactions between TREM-1 with its ligand(s) by the administration of a TREM-1-derived antagonistic peptide even attenuates the progression of established colonic inflammation. Hence, DAMPs can contribute to, and exacerbate, colonic inflammation in mouse models of IBD, in particular when they trigger innate immune cells of the intestinal lamina propria. DAMPs and PAMPs, however, may also be required for maintaining intestinal epithelial barrier functions as demonstrated by the enhanced susceptibility for colitis development in mice deficient for the NLRP6 or NLRC4 sensors in inflammasomes in intestinal epithelial cells.

The appropriate regulation of the local immune system is crucial for maintaining the local tissue homeostasis in the intestinal mucosa. Despite the absence of specific antigen receptors on their surface, the effector cells of the innate immune system recognize and rapidly respond to microbial antigens and danger signals, such as cellular proteins released from damaged or stressed cells. This remarkable task is primarily accomplished through an array of pattern recognition receptors (PRR), which

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are located in distinct (sub)cellular compartments (e.g. the cell surface and cytosolic), and which bind pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs, respectively) (Box 1).

Box 1

DAMPs. Danger-associated molecular patterns (also: damage-associated molecular patterns or 'alarmins'). These are mostly endogenous stress proteins that are expressed and/or released upon cell damage. Examples: HMGB1, calreticulin, monosodium urate crystals and secreted ATP.

PAMPs. Pathogen-associated molecular patterns (syn. MAMPs; microbe-associated molecular patterns). They mediate the initiation and perpetuation of an immune response against infectious microbes and pathogens. Examples: bacterial lipopolysaccharide (LPS), bacterial flagellin [recognized by Toll-like receptor 5 (TLR5)], lipoteichoic acid from Gram-positive bacteria (binds to TLR2), peptidoglycan and nucleic-acid variants normally associated with viruses, such as double-stranded RNA (dsRNA), recognized by TLR3 or unmethylated CpG motifs, recognized by TLR9.

PRR. Pattern recognition receptors. This is a group of receptors expressing hematopoietic and nonhematopoietic cells, which bind PAMPs and DAMPs. Examples: TLRs, nucleotide-binding oligomerization domain protein-like receptors (NLR, including NOD1, NOD2 and NLRP3/NALP3), RIG-1-like receptors (RLR), C-type lectin receptors (CLR) and triggering receptors expressed on myeloid cells (TREMs).

The impact of PAMPs, in particular that of NOD2/CARD15 (which represents an intracellular receptor for bacterial cell wall-derived muramyl dipeptides) in the pathogenesis of Crohn's disease in subgroups of patients has been widely established. The involvement of DAMPs in the pathogenesis of inflammatory bowel disease (IBD), however, is much less established so far.

Pathogen-Associated Molecular Patterns

PAMPs are molecules or motifs associated with groups of pathogens or microbes. PAMPs are recognized by PRR on hematopoietic cells (e.g. cells of the innate immune system) and nonhematopoietic cells (e.g. epithelial cells) and often exert crucial physiological functions in microbes and are thus often highly conserved. Since the molecular motifs of the PAMPS are not restricted to pathogens, the term microbe-associated molecular pattern (MAMPs) has been introduced and is now interchangeably used with the acronym PAMPs.

Damage (or Danger)-Associated Molecular Patterns

Several years ago, it was recognized that cellular stress and cell death are recognized at the cell level through the recognition via specific PRR of (mostly) intracellular proteins, which are released from stressed or dying cells [1]. Due to the fact that some pathogens induce necrotic cell death, they may not only trigger PRR via their PAMPs but also by DAMPs released from dying cells [2]. The protein high mobility group box 1 (HMGB1) represents a prototypic DAMP: it is normally associated with nucleosomes in the nucleus of viable cells where it acts also as a transcriptional regulator [3]. Inflammatory triggers such as endotoxin or TNF induce the release of HMGB1 into the extracellular space in immune cells (e.g. dendritic cells or macrophages). When cells are undergoing apoptosis, HMGB1 is sequestered in the nucleus and is not released. During necrosis, however, it is also released into the extracellular space. Extracellular HMGB1 binds to the receptor for advanced glycation end-products (RAGE), or upon association with bacterial products, also to receptors for PAMPs such as TLR2 and TLR4 [3]. HMGB1 reportedly may also activate – directly or indirectly – the triggering receptor expressed on myeloid cells (TREM-1), and thus mediate the amplification of the inflammatory properties of myeloid cells [4]. Conversely, in addition to its main ligand HMGB1, the receptor RAGE also binds different, structurally related ligands, including members of the S100 protein family [e.g. calgranulin A (MRP8), B (MRP14) and C] or amyloid-like proteins such as serum amyloid A (SAA), and can therefore be considered a PRR, which preferentially binds endogenous stress proteins or damage proteins. RAGE is expressed at high levels in the lung, but also on vascular endothelial cells, neutrophils, monocytes/macrophages, lymphocytes and neurons [3]. The short cytoplasmic tail of RAGE is a docking site for extracellular-signal-regulated kinase (ERK) 1/2, which eventually leads to cell activation, which is particularly in myeloid cells associated with increased production of proinflammatory mediators. The release of HMGB1 (and subsequent triggering of the inflammatory machinery) from necrotic cells, but not from apoptotic cells, illustrates one of the fundamental differences between apoptosis (and autophagy) and necrosis with regard to their consequences on the inflammatory process.

Inflammasomes are large multiprotein complexes in the cytoplasm, which contain one of several nucleotide-binding oligomerization domain protein-like receptor (NLR) proteins, such as NLRP1, NLRP3/NALP3, NLRC4, NLRP6 or NLRP12. These proteins can be regarded as

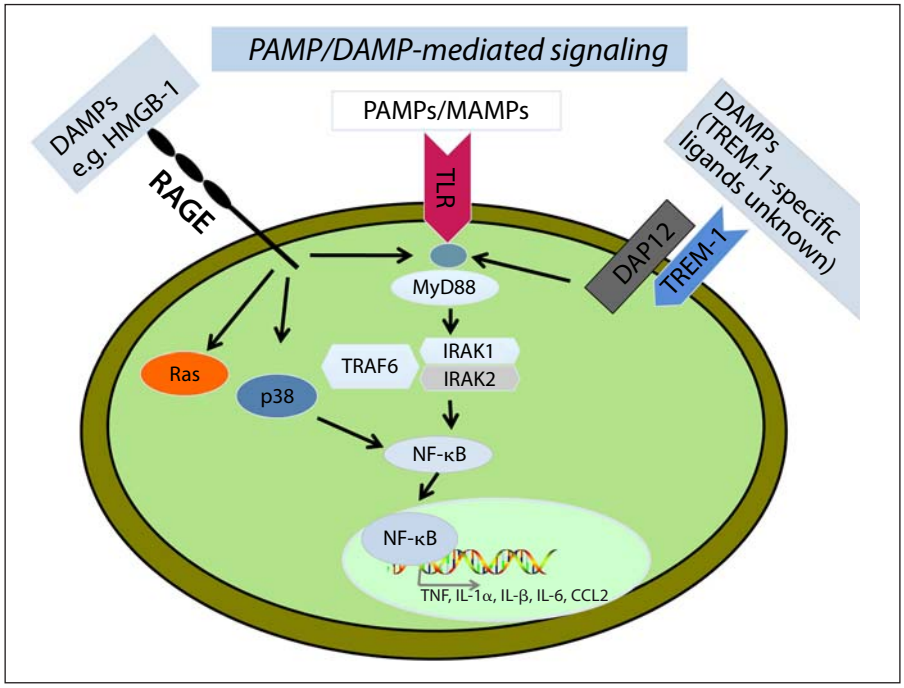

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Fig. 1. DAMP- and PAMP-mediated signaling pathways converge on NF-κB activation and translocation. DAMP-induced signaling (e.g. via RAGE or TREM-1) converges with PAMP-mediated signaling (e.g. via TLR) on NF-κB activation and translocation, which promotes the expression of genes involved in inflammation, but also in tissue repair, regeneration and angiogenesis.

sensors of endogenous or exogenous stress-related molecules and DAMPs [5]. Upon interaction of the NLR with the triggering DAMP, which can be extracellular ATP, cholesterin crystals, monosodium uric acid crystals, alum, silica or titanium oxide, they assemble with additional molecules to form a multiprotein complex that mediates caspase-1 cleavage. Activated caspase 1, in turn, processes the proforms of interleukin (IL)-1 family members such as IL-1β and IL-18 into their bioactive form. Inflammasome activation is crucial for host defense to pathogens. Recent studies have also suggested that excessive activation of the inflammasomes in the pathogenesis may also contribute to several chronic inflammatory diseases such as IBD, rheumatoid arthritis and atherosclerosis. The situation, however, may be further complicated, as secretion of IL-1 family members, in particular IL-18, may also critically regulate the barrier functions of the intestinal epithelium (see below).

Pattern-Recognition Receptors

PRR include several families of innate receptors expressed by hematopoietic and nonhematopoietic cells which are involved in the recognition of PAMPs (MAMPs), including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain protein-like receptors (NLRs) such as NOD1 and NOD2 (which recognize intracellular bacterial muramyldipeptides) and the inflammasome-associated NLRP/NLRC/NALP sensor proteins, C-type lectin receptors and TREMs [6]. Engagement of the PRR often results eventually in NF-κB activation and its nuclear translocation and, thus, enhanced transcription of the genes encoding proinflammatory mediators including TNF, IL-6, CCL2 and IL-1α and IL-1β (fig. 1).

As prototypic PRR, TLRs signal through different adaptor molecules [MyD88, TIRAP (Mal), TRIF and TRAM] to mediate distinct PRR signaling pathways and effects. MyD88, which can be used as an adaptor molecule by all TLRs, with the exception of TLR3, activates the transcription factor NF-κB and mitogen-activated protein kinases (MAPKs) to induce the transcription of genes encoding inflammatory cytokines [7, 8].

Triggering Receptors Expressed on Myeloid Cells

TREMs are noncovalently associated with the ITAM motif containing the DAP12 adaptor molecule. The most prominent members are TREM-1 and TREM-2. Despite their association with the same adaptor molecule, TREM-1 and TREM-2 appear to exert distinct functions: while TREM-1 appears to mediate an amplification of inflam-

matory reactions, TREM-2 seems to downmodulate macrophage functions [9]. Despite numerous efforts by several groups (including ours), the precise nature of the TREM-1 ligand(s) has not yet been determined. Reports about platelet-derived TREM-1 ligand(s) exist [10]; however, bacteria-derived ligands have also been postulated, a notion which might be in agreement with some of the putative ligand(s) reported for TREM-2 [11].

The TREM-1 receptor displays only a short cytoplasmic tail. For signaling, it associates with the DAP12 molecule. After TREM cross-linking – in the absence of known ligands for TREM-1, mimicked by agonistic antibodies, the ITAM motif of DAP12 is phosphorylated and becomes a docking site for protein tyrosine kinases of the SYK family. This eventually leads to the activation of phosphatidylinositol 3-kinase (PI3K) and ERK pathways. These signaling pathways induce Ca^{2+} mobilization and activate transcription factors, such as the nuclear factor of activated T cells (NFAT), activator protein 1 (AP1) and NF-κB to promote the transcription of genes encoding proinflammatory cytokines, chemokines and cell-surface molecules. The TREM-1-triggering of PI3K and ERK pathways also promotes cell survival by inactivating proapoptotic factors, thereby further promoting the survival of myeloid effector cells in the presence of the TREM-1 ligand(s). Furthermore, the TREM signaling cascade synergizes with the IL-1R-TLR signalling pathways, in particular for the activation of NF-κB.

Most macrophages in humans and mice are found in the intestinal lamina propria where they are strategically located in the subepithelial region. They rapidly scavenge bacteria and other luminal antigens that breach through the single-layered epithelium, which forms the barrier between the massive loads of (mostly) commensal bacteria in the gut lumen and the intestinal lamina propria. As an adaptation to this antigen-rich environment, resident intestinal macrophages generally do not express several PRR (including CD14 and TLR4) on their surface, and as a consequence, do not respond to LPS. Intriguingly, we found that intestinal macrophages also lack expression of TREM-1 in the normal intestinal mucosa of humans and mice, whereas macrophages in the spleen and lymph nodes in humans and mice express TREM-1 [12]. In mouse models of colitis and in patients with IBD, however, TREM-1 expression in the intestine is upregulated and correlates with disease activity. Cross-linking TREM-1 by an agonistic specific antibody on intestinal macrophages from IBD patients indeed enhances the secretion of relevant proinflammatory mediators such as IL-6, TNF, MCP1/CCL2 and IL-8. In experimental mouse models of colitis, blocking TREM-1 by the administration of an antagonistic peptide (LP17) substantially attenuates clinical course and histopathological alterations of experimental colitis. This effect is also seen when the antagonistic peptide is administered only after the first appearance of the clinical signs of colitis (fig. 2).

These data in a mouse model of colitis demonstrate that interfering with TREM-1 engagement leads to the simultaneous reduction of production and secretion of a variety of proinflammatory mediators [13]. Therefore, TREM-1 may represent an attractive target for the treatment also of chronic inflammatory disorders, but the consequences of TREM-1 blockade on the course of infectious diseases needs to be considered as well.

TREM-1 is also cleaved from the cell surface of myeloid cells by a protease, and increased levels of secreted TREM-1 (sTREM-1) in the bronchoalveolar lavage fluid in patients receiving mechanical ventilation in intensive care units have been reported to represent a reliable marker for the early detection of bacterial or fungal pneumonia [14]. Hence, we reasoned that in patients with active IBD, increased levels of sTREM-1 might be present when compared to normal controls or IBD patients in remission.

In patients with IBD, endoscopically defined active disease is in fact associated with increased intestinal TREM-1 mRNA expression [13, 15]. Hence, 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, sTREM-1 levels were significantly increased in patients with CD or UC, irrespective of the presence of endoscopically quiescent or active disease. While median serum sTREM-1 levels were almost identical in CD patients with quiescent (1,079 pg/ml) versus active (1,124 pg/ml) disease (fig. 3a), median serum sTREM-1 was slightly elevated in UC patients with active (1,441 pg/ml) versus quiescent disease (950 pg/ml). Patients with IBD displayed a considerable degree of heterogeneity with respect to serum sTREM-1 expression levels (fig. 3a, b). A substantial overlap in expression levels between patients with quiescent and active disease was also observed for CRP, particularly in UC patients (fig. 3c, d) [15]. These data demonstrate that, in contrast to a previous study [16], measuring serum sTREM-1 levels is not likely to represent a reliable surrogate marker for disease activity in patients with IBD. The fact, however, that in some patients with endoscopically defined inactive disease, serum levels of sTREM-1 are several-fold higher than in normal (non-IBD) patients is intriguing. Since in these patients no sub-

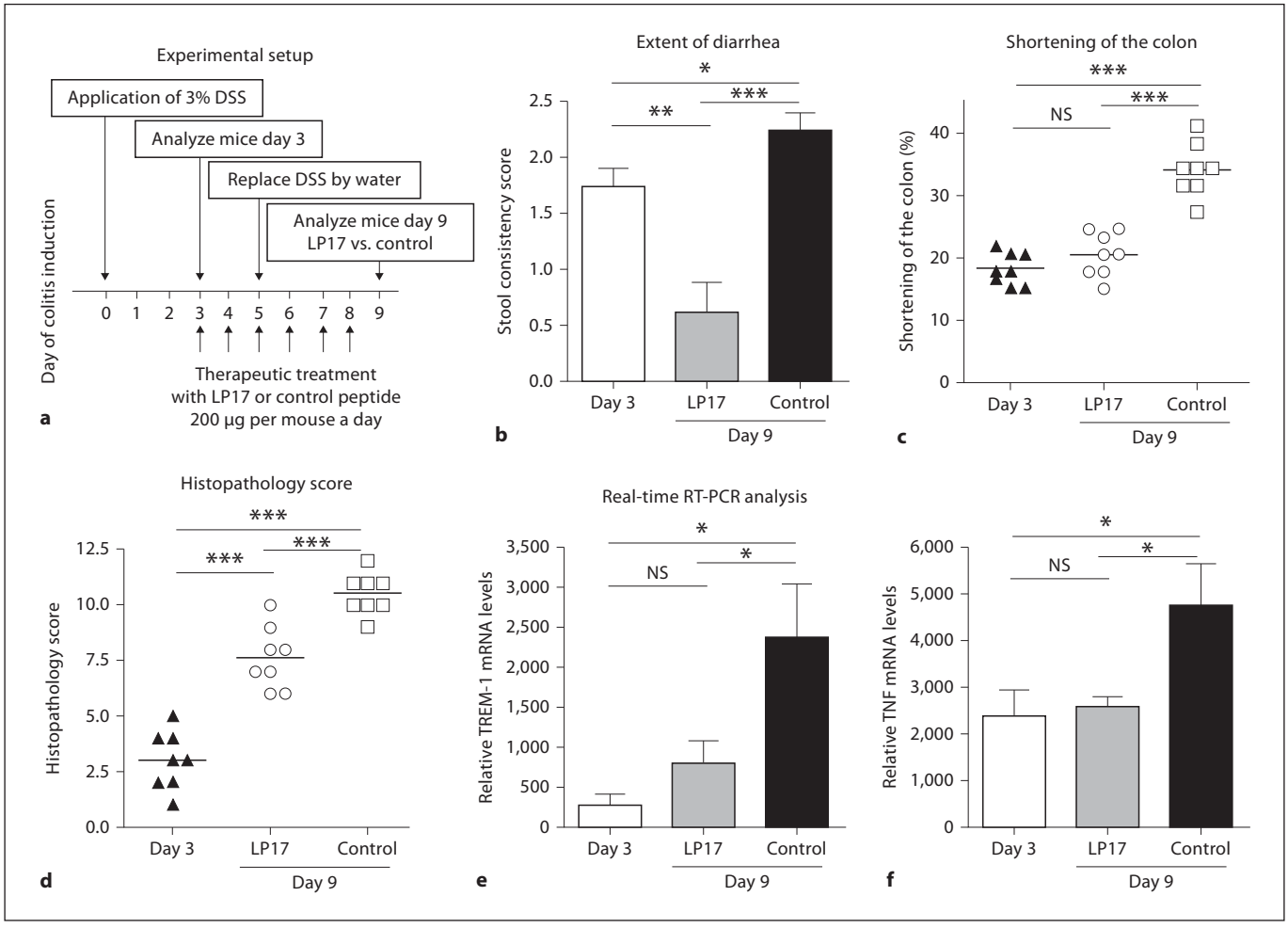

Fig. 2. Blocking TREM-1 attenuates the disease process in established experimental colitis. **a** Colitis was induced by oral administration of dextran sodium sulfate (DSS) in C57BL/6 mice. When experimental mice developed colitis with persistent diarrhea and fecal samples had tested positive for the presence of occult blood (day 3), the first group of mice was analyzed (day 3; n = 8), and the remaining experimental mice were treated daily with either the antagonistic TREM-1 peptide LP17 (n = 8) or with control peptide (n = 8) for an additional 5 days. Treatment with the antagonistic peptide LP17 resulted in a significant reduction of diarrhea score (**b**), no further colon length reduction (**c**), significantly attenuated the histopathological alterations over the next five days (**d**) and attenuated the colonic expression of TREM-1 (**e**) and TNF mRNA (**f**) in comparison to control peptide-treated colitis mice. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. NS = Not significant [13].

sequent imminent flares of the disease were reported, in some patients elevated circulating levels of sTREM-1 may contribute to the attenuation of inflammatory reactions by blocking putative TREM-1 ligands and, thus, maintain remission in patients with IBD.

While our results strongly indicate a disease-exacerbating function of TREM-1-mediated activation of myeloid cells in IBD (and possibly also in other chronic inflammatory disorders), it is less clear whether these findings can be extrapolated to other PRR-mediated effects induced by DAMPs or PAMPs. Intriguingly, in mice deficient for NLRP6 (resulting in reduced or even absent secretion of IL-1β and IL-18 by intestinal epithelial cells), this activation exacerbates rather than attenuates experimental colitis [17]. These findings demonstrate that IL-1 family members are not only involved in mediating inflammation in the intestinal mucosa, but are also critically involved in maintaining intestinal epithelial barrier functions, mostly due to the proposed functions of IL-18 on intestinal epithelial cells, notably, in maintaining epithelial cell barrier function and regeneration. Furthermore, the absence of IL-18 secretion by intestinal epithe-

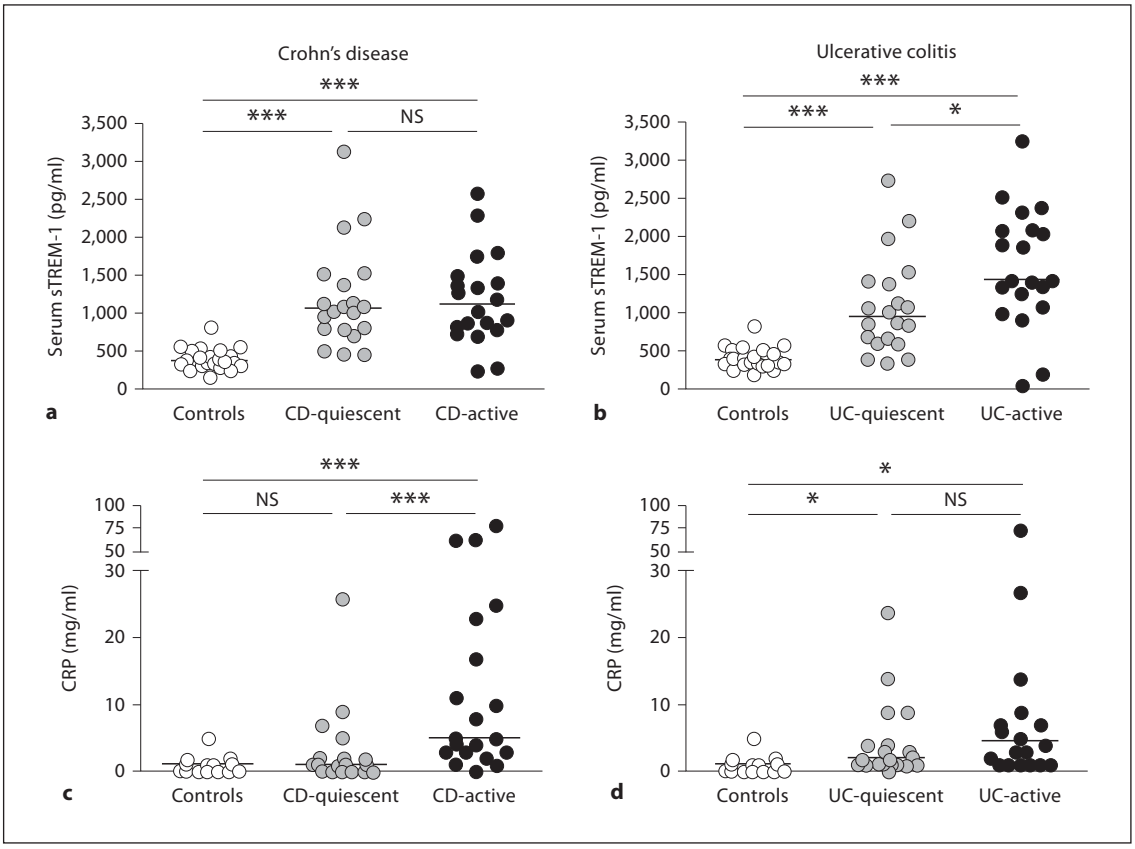

Fig. 3. Serum sTREM-1 levels are increased in patients with active and quiescent IBD. sTREM-1 (**a**, **b**) and CRP (**c**, **d**) levels were determined in the sera of healthy controls (n = 20) and in patients with quiescent (n = 20) and active (n = 20) CD (**a**, **c**) and UC (**b**, **d**), respectively. 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. * $p < 0.05$ and *** $p < 0.001$. NS = Not significant [15].

lial cells in the absence of the NLRP6 inflammasome had profound effects on the composition of the intestinal flora, which, in the absence of IL-18 secretion, shifts towards a highly colitogenic flora. Intriguingly, transfer of this colitogenic flora from NLRP6-deficient mice into wild-type mice leads to similar signs of intestinal inflammation – even in NLRP6-competent mice. A similar situation was previously reported also in T-bet$^{-/-}$ RAG$^{-/-}$ mice, which upon CD4 T cell transfer came down with an exacerbated colitis, a phenotype that could be attributed to the colitogenic microbiota that was established in the absence of T-bet in RAG$^{-/-}$ mice [18].

Taken together, PAMP- and DAMP-derived triggers from stressed or dying cells may contribute to excessive inflammation in patients with active IBD. A shift in the balance between autophagy/apoptosis versus necrotic cell death in favor of the latter may thus contribute to the exacerbation of an ongoing inflammatory response, particularly when nonepithelial cells of the intestinal wall are triggered by DAMPs for enhanced proinflammatory activities. The situation, however, may become even further complicated, as some of the effects mediated by the generally considered inflammatory cytokines of the IL-1 family, in particular IL-18, may also exert critical functions in maintaining the intestinal epithelial barrier, and may thus be anti-inflammatory at the time of induction of early colitis [17, 19], thus dampening the expectations of using IL-18 as a target in IBD [20].

Disclosure Statement

The author declares that no financial or other conflict of interest exists in relation to the content of the article.

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