

Review

Clinical review: Role of triggering receptor expressed on myeloid cells-1 during sepsis

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Critical Care 2005, **9**:485-489 (DOI 10.1186/cc3732)**Abstract**

Triggering receptor expressed on myeloid cells (TREM)-1 is a recently identified molecule that is involved in monocytic activation and in the inflammatory response. It belongs to a family related to the natural killer cell receptors and is expressed on neutrophils, mature monocytes and macrophages. The inflammatory response mediated by Toll-like receptor-2 and -4 stimulation is amplified by the engagement of TREM-1. The expression of membrane-bound TREM-1 is greatly increased on monocytes during sepsis. Moreover, infection induces the release of a soluble form of this receptor, which can be measured in biological fluid and may be useful as a diagnostic tool. Modulation of the TREM-1 signalling pathway by the use of small synthetic peptides confers interesting survival advantages during experimental septic shock in mice, even when this treatment is administered late after the onset of sepsis.

Introduction

Sepsis is a complex clinical syndrome that results from a harmful host response to infection. The initial line of defence against invading pathogens is the immediate, innate host immune response, which prevents proliferation of pathogens until the more specialized adaptive response, provided by specific T and B cells, can occur. The innate response involves the coordinated action of effector cells such as phagocytes and natural killer cells, which express numerous membrane-bound receptors. Of these, the Toll-like receptors (TLRs) detect microbial structures such as lipopolysaccharide (LPS), lipoteichoic acid, flagellin and bacterial DNA, all of which are present in various micro-organisms [1-3]. Innate effectors also express members of the immunoglobulin and lectin-like superfamilies, which recognize endogenous structures such as major histocompatibility complex I molecules and CD47 [4]. These receptors contain cytoplasmic immunoreceptor tyrosine-based inhibitory motifs that recruit tyrosine phosphatases, which mediate inhibition.

Thus, in its basal state the innate immune system is subject to constant inhibitory signalling. On detection of an infectious agent, these inhibitory signals are overwhelmed by stimulatory signals triggered by engagement of pathogen receptors.

The triggering receptor expressed on myeloid cells (TREM) family is a member of the immunoglobulin superfamily and includes at least two activating receptors, namely TREM-1 and TREM-2, as well as an inhibitory receptor called TREM-like transcript (TLT)-1 [5,6]. TREM-1 and TREM-2 are transmembrane glycoproteins with a single extracellular immunoglobulin-like domain, a transmembrane region with a charged lysine residue, and a short intracellular region [5]. Engagement of TREMs, after association with the adapter protein DAP12 (which contains an immunoreceptor tyrosine-based activation motif), triggers a signalling pathway involving ζ -chain-associated protein 70 (ZAP70) and spleen tyrosine kinase. This in turn leads to the recruitment and tyrosine phosphorylation of adaptor molecules such as growth factor receptor binding protein 2, and activation of phosphatidylinositol 3-kinase, phospholipase C- γ , extracellular signal regulated kinase-1 and -2, and p38 mitogen-associated protein kinase [7]. Activation of these pathways leads to intracellular calcium mobilization, actin cytoskeleton rearrangement, and activation of transcription factors. TREM-1 has been implicated in mounting the inflammatory response, whereas TREM-2 regulates dendritic cells, osteoclasts and microglia [6,8,9]. An alternative mRNA splice variant of TREM-1 has also been detected, which encodes a putative protein that lacks transmembrane and cytoplasmic domains [10]. The TREM-1 gene cluster also includes a gene that encodes an inhibitory receptor, namely TLT-1, that is found exclusively in platelets and megakaryocytes [11-13]; its expression is upregulated on platelet activation. TLT-1 does

BAL = bronchoalveolar lavage; CDR = complementary determining region; CLP = caecal ligation and puncture; IL = interleukin; LPS = lipopolysaccharide; MFI = mean fluorescence intensity; NF- κ B = nuclear factor- κ B; TNF = tumour necrosis factor; sTREM = soluble TREM; TLR = Toll-like receptor; TLT = TREM-like transcript; TREM = triggering receptor expressed on myeloid cells.

not inhibit other members of the TREM family but it helps to maintain vascular homeostasis and regulate coagulation at sites of injury [12,13]. Murine counterparts of TREM-1 and TREM-2 have also been described, along with a third cDNA that encodes TREM-3 (a pseudogene in humans) [5,14-16].

TREM-1 as an amplifier of the inflammatory response

TREM-1 is expressed by neutrophils, macrophages and mature monocytes [5]. Its expression by effector cells is dramatically increased in skin, biological fluids and tissues infected by Gram-positive and Gram-negative bacteria and fungi [17,18]. In contrast, TREM-1 is not upregulated in samples from patients with noninfectious inflammatory disorders such as psoriasis, ulcerative colitis, or vasculitis caused by immune complexes [18]. In mice engagement of TREM-1 with monoclonal agonist antibodies has been shown to stimulate the production of proinflammatory cytokines and chemokines such as IL-8, monocyte chemoattractant protein-1 and -3, and macrophage inflammatory protein-1 α [5,19], as well as stimulating rapid neutrophil degranulation and oxidative burst [20]. Activation of TREM-1 in the presence of TLR-2 or TLR-4 ligands amplifies the production of proinflammatory cytokines (tumour necrosis factor [TNF]- α , IL-1 β , and granulocyte-macrophage colony-stimulating factor) while inhibiting the release of IL-10 [19]. In addition, activation of these TLRs increases expression of TREM-1 [5,21] by activating a phosphatidylinositol-3-kinase-dependent pathway [5,21].

Thus, TREM-1 and TLRs appear to cooperate to produce an inflammatory response. Expression of TREM-1 may be under the control of nuclear factor- κ B (NF- κ B; activated by the TLRs), with engagement of TREM-1 possibly leading to activation of several transcription complexes that synergize with NF- κ B in order to elicit transcription of proinflammatory genes. The role of TREM-1 as an amplifier of the inflammatory response has been confirmed in a mouse model of septic shock in which blockade of TREM-1 signalling was able to reduce mortality [18]. Moreover, transgenic mice that overexpress DAP12 develop leucocytosis and pulmonary macrophage infiltration, and are highly susceptible to LPS [22].

Expression of TREM-1 in sepsis

Using experimental models of polymicrobial infection induced by caecal ligation and puncture (CLP) in mice, we and others [18,23] investigated whether sepsis alters membrane-bound TREM-1 expression. In sham-operated animals, TREM-1 was present at low levels on the surface of peripheral monocytes and neutrophils, and peritoneal macrophages and neutrophils, as well as splenic macrophages. Sepsis induced a marked (threefold to fivefold) increase in TREM-1 expression on the surface of all cell types, with the most pronounced increase observed on peritoneal macrophages. Conversely, TREM-1 was undetectable on lymphocytes in both groups of

mice. Sepsis also induced the appearance of an approximately 30-kDa protein in peritoneal lavage fluid samples that was specifically recognized by a monoclonal antibody directed against the extracellular domain of TREM-1 in Western blot analysis. The release of this soluble form of TREM-1 (sTREM-1) was markedly increased in peritoneal lavage fluid from septic animals but barely detectable in sham-operated animals.

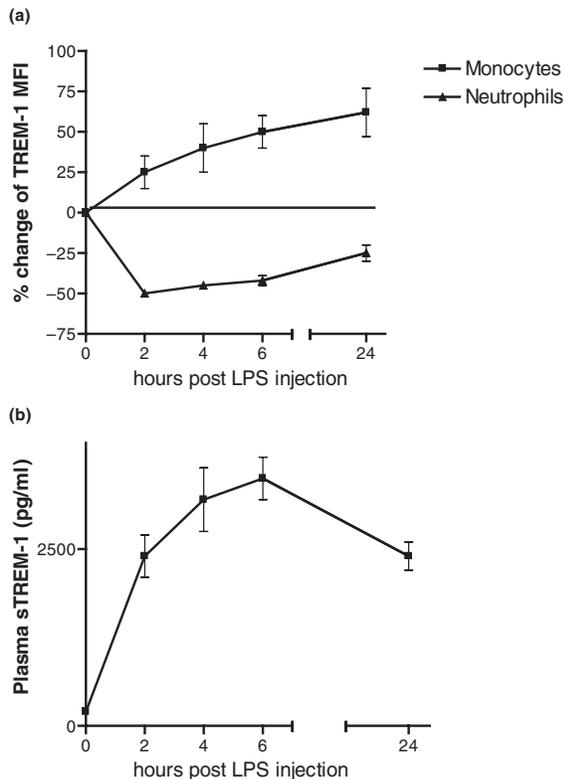
In healthy volunteers challenged with intravenous LPS, granulocyte TREM-1 expression – initially high at baseline – was immediately downregulated on LPS exposure, which occurred together with an increase in sTREM-1 levels (Fig. 1). In contrast, monocytes exhibited a progressive increase in TREM-1 [21]. Interestingly, ligands for the predominantly dendritic cell and B cell expressed TLRs (namely TLR-3, TLR-7 and TLR-9) did not alter TREM-1 expression, and neither did the surrounding concentrations of TNF- α [21]. This pattern of monocytic TREM-1 expression found in healthy volunteers was confirmed in septic shock patients [24].

Taken together, these data demonstrate that expression of membrane-bound TREM-1 on neutrophils and monocytes/macrophages is strongly altered during sepsis, as is the release of its soluble form. Given that both cell surface TREM-1 and sTREM-1 are upregulated during sepsis, this protein may be useful in the diagnosis of infection.

TREM-1 as a diagnostic tool

The specific involvement of TREM-1 solely in cases of infection led us to investigate the diagnostic value of a plasma sTREM-1 assay in distinguishing sepsis from severe systemic noninfectious inflammation among newly admitted critically ill patients with suspected infection [25]. Baseline plasma levels of C-reactive protein, procalcitonin and sTREM-1 were higher among septic patients than in patients with systemic inflammatory response syndrome only. Plasma sTREM-1 levels appeared to be the most helpful parameter in differentiating patients with sepsis from those with systemic inflammatory response syndrome. Median plasma sTREM-1 levels at admission were 0 pg/ml (range 0–144 pg/ml) in noninfected patients and 149 pg/ml (range 30–428 pg/ml) in patients with sepsis ($P < 0.001$). Plasma sTREM-1 levels yielded the highest discriminative value (Table 1).

The diagnostic value of sTREM-1 has also been investigated in the context of a more localized infectious process, namely pneumonia, in a series of 148 consecutive mechanically ventilated patients [26]. sTREM-1 levels were higher in bronchoalveolar lavage (BAL) fluid from patients with community-acquired and ventilator-associated pneumonia than in BAL fluid from patients without pneumonia, but the levels did not differ significantly between patients with community-acquired pneumonia and those with ventilator-associated pneumonia. The presence of elevated levels of sTREM-1 in BAL fluid was the strongest predictor of

Figure 1

TREM-1 expression and release in healthy volunteers administered lipopolysaccharide. **(a)** TREM-1 cell surface expression in healthy volunteers administered 4 ng/kg lipopolysaccharide intravenously. **(b)** Corresponding plasma concentrations of the soluble form of TREM-1. Adapted with permission from Knapp and coworkers [21]. LPS, lipopolysaccharide; TREM, triggering receptor expressed on myeloid cells.

pneumonia (Table 1). Furthermore, Richeldi and coworkers [27] recently studied TREM-1 expression levels in BAL specimens from patients with community-acquired pneumonia, tuberculosis (an intracellular infection that is unable to induce upregulation of TREM-1 *in vitro*) and interstitial lung disease, the latter being used as a model of noninfectious inflammatory lung disease. TREM-1 expression was significantly increased in lung neutrophils and in lung macrophages of patients with pneumonia ($n = 7$; 387.9 ± 61.4 MFI [mean fluorescence intensity] and 660.5 ± 18.3 MFI,

respectively) in comparison with patients with pulmonary tuberculosis ($n = 7$; 59.2 ± 13.1 MFI and 80.6 ± 291.2 MFI) and patients with interstitial lung diseases ($n = 10$; 91.8 ± 23.3 MFI and 123.9 ± 22.8 MFI).

Hence, sTREM-1 appears to represent a reliable marker of infection, particularly in plasma during sepsis and in BAL fluid in cases of pneumonia.

TREM-1 as a follow-up marker

In a recent study [28] we sequentially measured plasma sTREM-1 concentrations in 63 consecutive septic patients. Soluble TREM-1 concentrations were significantly lower at admission in nonsurviving patients than in surviving patients, and an elevated baseline sTREM-1 level was found to be an independent protective factor (an explanation for this intriguing finding is given below). Moreover, sTREM-1 concentrations remained stable or even increased in nonsurvivors whereas they decreased in survivors (Fig. 2). A similar differential pattern was found with regard to cell surface TREM-1 expression [24]. Although monocytic TREM-1 expression did not differ on admission between septic survivors and nonsurvivors, expression in these two groups diverged significantly by day 3, with high and stable level in nonsurvivors, but with levels in surviving patients rapidly declining to those observed in healthy volunteers and nonseptic patients. A progressive decline in plasma sTREM-1 or of its monocytic expression could therefore indicate a favourable clinical evolution during the recovery phase of sepsis.

The main cellular origin of sTREM-1 production is still unclear (monocytes or neutrophils), and in view of the different patterns of expression of TREM-1 between monocytes and neutrophils [21], we require further clarification of the relationship between soluble and membrane-bound forms of TREM-1.

TREM-1 modulation as a therapeutic tool

Bouchon and coworkers [18] demonstrated that blockade of TREM-1 with mTREM-1/IgG₁ (a murine TREM-1 extracellular domain and human IgG₁ Fc fragment fusion protein) protected mice against both LPS-induced shock and microbial sepsis caused by administration of live *Escherichia coli* or by CLP. We therefore designed a synthetic peptide

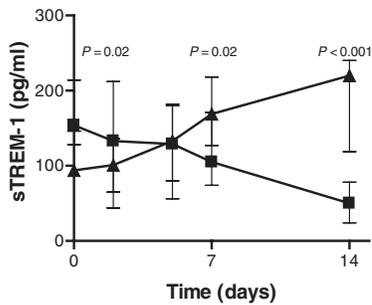
Table 1

Diagnostic accuracy of sTREM-1 determination in sepsis

Setting [ref.]	sTREM-1 threshold (pg/ml)	Sensitivity (% [95% CI])	Specificity (% [95% CI])	Positive likelihood ratio	Area under the ROC curve (95% CI)
Pneumonia [26]	5	98 (95–100)	90 (84–96)	10.4	0.93 (0.92–0.95)
Sepsis [25]	60	96 (92–100)	89 (82–95)	8.6	0.97 (0.94–1.00)

CI, confidence interval; ROC, receiver operating characteristic; sTREM, soluble triggering receptor expressed on myeloid cells.

Figure 2

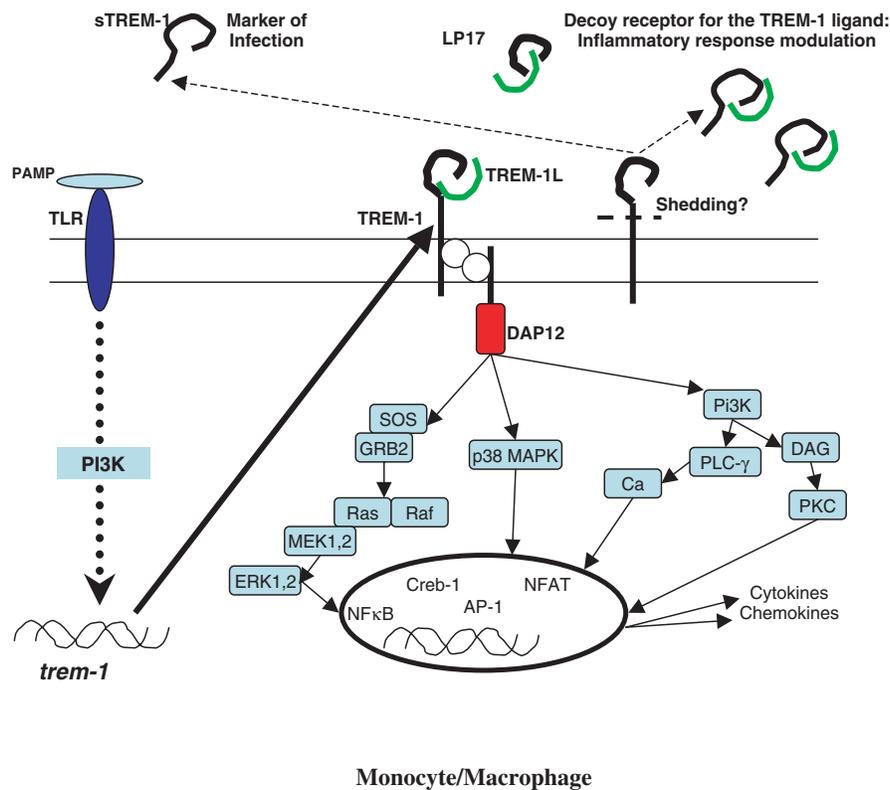


Time course of median plasma levels of sTREM-1 in septic patients. Patients are subgrouped according to whether they survived (squares; $n = 42$) or did not survive (triangles; $n = 21$). Adapted with permission from Gibot and coworkers [29]. sTREM, soluble triggering receptor expressed on myeloid cells.

(LP17) to mimic part of the extracellular domain of TREM-1 and examined its action both *in vitro* and in a mouse model of endotoxaemia [29]. In monocytes cultured with LPS, LP17

reduced the production of TNF- α and IL-1 β in a concentration-dependent manner. In the mouse model, single administration of LP17 60 min before a lethal dose of LPS reduced mortality in a dose-dependent manner. Treatment with LP17 after the onset of endotoxaemia also conferred significant protection against a lethal dose of LPS, reducing cytokine levels by 30% compared with controls. Similar results were also obtained in a CLP model of polymicrobial sepsis. The modulation of TREM-1 signalling reduced but did not abolish NF- κ B activation and cytokine production, and protected septic animals from hyper-responsiveness and death. Although crystallographic analyses [30,31] can predict TREM-1 recognition by using antibody-equivalent complementary determining region (CDR) loops (such as T-cell receptors, CD8 and cytotoxic T-lymphocyte associated antigen-4), its natural ligand has yet to be identified. Nevertheless, LP17 overlaps the CDR-3 and the 'F' β strand of the extracellular domain of TREM-1, with the 'F' β strand containing a tyrosine residue that mediates dimerization. LP17 could therefore compete with the natural ligand of TREM-1, thus acting as a decoy receptor, and/or it could impair TREM-1 dimerization. Along similar lines, this hypothe-

Figure 3



Overview of the role of TREM-1 in sepsis. DAG, diacylglycerol; ERK, extracellular signal regulated kinase; GRB, growth factor receptor binding protein; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PAMP, pathogen-associated molecular pattern; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; SOS, son of sevenless; TLR, Toll-like receptor; TREM, triggering receptor expressed on myeloid cells; TREM-1L, TREM-1 ligand.

sis may also account for the protective effect of elevated sTREM-1 concentrations observed in septic patients [28].

Conclusion

TREM-1 is a recently described cell surface molecule on neutrophils and macrophages that acts as an amplifier of inflammatory responses. During sepsis there is a significant increase in both the expression of membrane-bound TREM-1 and in the release of its soluble form (Fig. 3). Although it remains to be confirmed in larger and more heterogeneous populations, the rapid assessment of sTREM-1 concentration could prove to be a valuable tool for the diagnosis of infection, particularly with regard to its plasma levels in sepsis and BAL fluid levels in pneumonia. Although promising, the therapeutic manipulation of the TREM-1 signalling pathway still warrants further studies, particularly in assessing whether such modulation does not bypass important steps in the physiological reaction to pathogens.

Competing interests

Patent pending on sTREM-1 measurement.

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