Triggering receptor expressed on myeloid cells-1 as a new therapeutic target during inflammatory diseases

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The Triggering Receptor Expressed on Myeloid cells (TREM)-1 is a recently identified molecule involved in monocytic activation and inflammatory response. It belongs to a family related to Natural Killer cell-receptors and is expressed on neutrophils, mature monocytes and macrophages. The engagement of TREM-1 synergizes with several Toll Like Receptors (TLR) and/or NOD Like Receptors (NLR) activation in amplifying the inflammatory response mediated by microbial components or danger signals. The implication of TREM-1 during experimental models of acute or chronic inflammatory conditions, as well as during cancer, begins to understand. Furthermore, the modulation of the TREM-1 signalling pathway by the use of small synthetic peptides derived from its extracellular moiety confers interesting survival advantages during experimental murine septic shock and protects from organ damage during other inflammatory diseases. This review summarizes the recent advances on TREM-1 biology and highlights the promises of its therapeutic modulation.

Introduction

Stimulatory immunoreceptors have a central role in allowing the recognition of foreign antigens or pathogens by the immune system.[1] Typical examples of immunoreceptors are the B cell receptor (BCR) and the T cell receptor (TCR), structures used by B and T cells to discriminate between self and non-self. Stimulatory immunoreceptors are composed of ligand-binding sites and associated transmembrane adaptor proteins. The cytoplasmic domain of adaptor proteins contains an immunoreceptor tyrosine-based activation motif (ITAM) with the consensus sequence $YxxL/Ix_{6-8}YxxL/I$ (x representing any amino acid). Among these ITAM-containing adaptor proteins are CD3ζ, FcRγ and DAP12 (DNA activating protein 12, also called KARAP).[1] Several immunoglobulin (Ig)-like activating receptors have been characterized including paired Ig receptors,[2] NKp44 [3] and the SHPS-1 family.[4] Recently, a new family of receptors expressed on myeloid cells, distantly related to NKp44, has been described: the Triggering Receptor Expressed on Myeloid cells (TREM) family.[5,6] The TREM isoforms share low sequence homology to each other or to other IgSF members and are characterized by having only one Ig-like domain. Five *trem* genes have been identified with four encoding putative functional type I transmembrane glycoproteins.[7] The *trem* genes are clustered on human chromosome 6 (and mouse chromosome 17). All TREMs associate with the adaptor DAP12[5-8] for signalling.

Among this family, TREM-1 has been identified on both human and murine polymorphonuclear cells and mature monocytes. Its expression by these effector cells is dramatically increased in skin, biological fluids and tissues infected by gram-positive or gram-negative bacteria as well as by fungi.[9,10] By 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.[9] In mice, the engagement of TREM-1 with agonist monoclonal antibodies has been shown to stimulate the production of such proinflammatory cytokines and chemokines[5,11] as interleukin-8 (IL-8), monocyte chemoattractant proteins 1 and 3 and macrophage inflammatory protein 1α, along with rapid neutrophil degranulation and oxidative burst.[12] The activation of TREM-1 in the presence of Toll-like receptor 2 (TLR2) or TLR4 ligands amplifies the production of proinflammatory cytokines (tumor necrosis factor alpha [TNFα], IL-1β, granulocyte-macrophage colony stimulating factor), together with the inhibition of IL-10 release.[11] In addition, activation of these TLRs upregulates TREM-1 expression.[5] Thus, TREM-1 and TLRs appear to cooperate in producing an inflammatory response.

The role of TREM-1 as an amplifier of the inflammatory response has been confirmed in a mouse model of septic shock in which blocking signalling through TREM-1 partially protected animals from death.[9,13] Both in vitro and in vivo, synthetic peptides mimicking short highly interspecies-conserved domains of TREM-1 attenuated the cytokine production of human monocytes and protected septic animals from hyperresponsiveness and death.[14] These peptides were efficient not only in preventing but also in down-modulating the deleterious effects of proinflammatory cytokines.[13]

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The implication of TREM-1 in acute or chronic inflammatory disorders begins to be better understood. We will discuss here the promising therapeutic potential of modulating TREM-1 activation.

TREM-1 Structure and Function

Human TREM-1 (hTREM-1) consists of an extracellular region of 194 amino acid (aa) residues, a membrane spanning region of 29 aa and a short cytoplasmic tail of 5 aa. The extracellular Ig-like domain contains the motif DxGxYxC which corresponds to a V-type Ig-domain. The Ig domain is connected to the transmembrane region by a 60-aa portion containing three *N*-glycosylation sites. The spanning region contains a Lys residue which forms a salt-bridge with an Asp residue of the transmembrane domain of DAP12, allowing the association between TREM-1 and its adaptor protein.[5,14] Engagement of TREMs triggers a signalling pathway involving ZAP70 (ζ-chain-associated protein 70) and SYK (spleen tyrosine kinase) and an ensuing recruitment and tyrosine phosphorylation of adaptor molecules such as GRB2 (growth factor receptor binding protein 2), the activation of PI3K (phosphatidylinositol 3-kinase), PLC-γ (phospholipase C-γ), ERK-1, -2 (extracellular-signal-regulated kinase) and p38 MAPK (p38 mitogen-associated protein kinase), Akt serine/threonine kinase, STAT5 (signal transducer and activator of transcription 5)[15-19] and CARD9-MALT1-BCL10 complex formation.[20,21]

The activation of these pathways ultimately leads to a mobilization of intracellular calcium, a rearrangement of the actin cytoskeleton and activation of transcriptional factors such as NFκB. This finally results in production of metalloproteases,[22] pro-inflammatory cytokines and chemokines,[11-13,16,23] including MCP-1, MIP1-α, IL-1β, IL-6, IL-8, TNFα, along with rapid neutrophil degranulation and oxidative burst,[12,21] with a parallel negative regulation of anti-inflammatory IL-10.[16]

Of note, although crystallographic analyses[24,25] can predict TREM-1 recognition by using antibody-equivalent complementary determining regions (CDR) loops (such as TCRs, CD8 and CTLA-4), its natural ligand has yet to be determined.

Nod-Like Receptors (NLRs) and TLRs engagement upregulates TREM-1 expression and membrane exposition.[5,26-28] The regulation of TREM-1 by these Pattern recognition receptors (PRRs) is independent of MyD88 but TRIF-dependent,[29] and involves transcription factors NFκB (p65), PU.1 and AP1.[30] Co-engagement of PRRs and TREM-1 results in higher cytokines production than the sum of the responses of either TREM-1 or PRRs alone.[9] Importantly, TREM-1 engagement alone by the mean of a monoclonal agonist antibody does not lead to sustained inflammation. TREM-1 silencing on LPS-activated myeloid cells induces a decrease in expression of several TLR-pathway key mediators.[23] Indeed, the TREM-1 function is to modulate rather than to activate/initiate inflammation and these data suggest that TREM-1 cooperates with PRRs in a synergistic way to trigger an exuberant immune response.

Besides its membrane-bound form, a soluble form of TREM-1 is liberated by cleavage of its extracellular domain.[31] Soluble TREM-1 acts as a decoy receptor, sequestering TREM-1-ligand,

that may exist in soluble form in the sera of septic patients,[32] and dampening TREM-1 activation.[9,17] To counteract excessive inflammatory reaction, several mechanisms exist, one of which involving another TREM member. Hamerman et al. suggested that one or more DAP12-associated receptors could negatively regulate TLRs signalling.[33] One of these receptors could be TREM-2: when expressed on monocytes/macrophages, its activation downregulates TLRs signalling through DAP12.[34] These data suggest that immune cells are able to integrate the sum of different signals through sensor receptors, like TREM-1 and TREM-2, in order to induce a balanced inflammatory response.

TREM-1 is also implicated in the platelet/neutrophil dialogue. Indeed, a TREM-1-ligand is constitutively expressed on platelets and megacaryocytes.[17] Although the TREM-1-ligand (expressed on platelets) interaction with the TREM-1 receptor (expressed on neutrophils) is not responsible for platelet/neutrophil complex formation, it mediates platelet-induced neutrophils activation.

Considering the role of TREM-1 as an amplifier of the inflammatory response, it is therefore tempting to try to modulate its activation in conditions during which excessive inflammation is thought to be deleterious.

How to Modulate the TREM-1 Pathway

Although crystallographic analyses[24,25] can predict TREM-1 recognition by using antibody-equivalent complementary determining regions (CDR) loops (such as TCRs, CD8 and CTLA-4), its natural ligand has yet to be determined.

We therefore synthesized several TREM-1 peptides matching the following criteria (i) highest homology between human and mouse and rat TREM-1 and lowest homology with TREM-2 (TREM-1 sequence in GenBank/EMBL/DDBJ accession numbers XM217336, AF287008 and AF241219), (ii) peptides spanning the CDRs of TREM-1. One peptide (P1) was designed in the CDR2 region, LP17 in the CDR3 and P3 in the neck region. Competition experiments suggested a direct interaction of P1 and LP17 with TREM-1 ligand.[35] Therefore, these two peptides could act as decoy receptors and block TREM-1 interactions with its ligand. An additional effect of LP17 on the TREM-1 pathway could stem from its overlap with the "F" β strand of the extracellular domain of TREM-1. The "F" β strand contains a tyrosine residue mediating a putative homo-dimerization and LP17 could thus impair the TREM-1 dimerization necessary for its engagement. The hypothesis of these peptides acting in impairing the TREM-1 signaling upon binding to its ligand yielded us to investigate whether the TREM-1 ligand could be expressed on the myeloid cells' surface. We indeed found high level of TREM-1 ligand expression on the neutrophils infiltrating the peritoneum 5 hours after the completion of peritonitis in mice. By contrast, neutrophils isolated from sham-operated animals did not express the TREM-1 ligand. These findings clearly indicate a selective expression of the TREM-1 ligand during infection. The pattern expression of the TREM-1 ligand appeared to be delayed on peripheral blood granulocytes. This might reflect both a recirculation of peritoneal neutrophils as well

as systemic spreading of bacteria. Another mechanism by which the TREM-1 peptides could exert their action has recently been suggested by Hamerman et al.[33] These authors demonstrated that DAP12-deficient macrophages produced higher levels of inflammatory cytokines in response to diverse microbial stimuli. Moreover, DAP12-deficient mice were more susceptible to endotoxemia and had enhanced resistance to infection by *Listeria monocytogenes*. These data suggest the existence of DAP12-pairing receptor(s) that negatively regulate the TLR-mediated signaling. One of these could be a specific receptor for sTREM-1 (and then recognize the TREM-1 peptides), since many DAP12-paired receptors have a related inhibitory receptor.[33]

Effects of the Trem-1 Pathway Modulation during Inflammatory Disorders

Acute inflammatory disorders. *Sepsis.* Septic shock, a complex clinical syndrome resulting from a harmful and damaging host response to infection, is the leading cause of mortality in intensive care units. Sepsis develops when the initial appropriate host response to systemic infection becomes dysregulated and over-amplified with an intimate crosstalk between inflammation and coagulation.

Implication of TREM-1 as an amplifier of the host immune response to microbial infection was firstly described by Bouchon et al.[5,9] In this study, infected tissues were infiltrated by neutrophils and macrophages that express high levels of TREM-1. In vitro, PMN and monocytes stimulated with LPS and αTREM-1, a monoclonal antibody against TREM-1 used as specific agonist, were over-activated as compared to LPS-challenge only. Finally, TREM-1 blockade by a chimeric protein composed of an Fc fragment and the extracellular portion of murine TREM-1 (mTREM-1/IgG1) protected septic mice from death.[9]

LP17—a TREM-1 peptide antagonist—administration to septic mice resulted in a decreased plasma concentration of several pro-inflammatory cytokines. LP-17 treated animals were also protected against organ failure,[36] hemodynamic disorders[35] and finally against death. Moreover, unpublished data recently obtained in our laboratory confirm that TREM-1 modulation confers cardiovascular protection during polymicrobial sepsis.

Interestingly, while partial in vivo *trem-1* silencing showed the same protective effects as LP-17 treatment, complete in vivo *trem-1* silencing was associated with bacterial clearance impairment and a decreased survival during polymicrobial sepsis.[37] Crucially, however, this silencing *decreased* mortality in the endotoxemic mice, the converse outcome to that seen in mice with polymicrobial sepsis. This indicates a beneficial role of TREM-1 during a non-bacterial form of shock and underlines the point that injection of endotoxin is an artificial challenge which does not reflect the complex events occurring during human sepsis. These data therefore highlight the crucial role of TREM-1 in mounting a sufficient inflammatory response during polymicrobial sepsis that is necessary for bacterial control and host survival.

In humans, respiratory tract infections are the leading cause of sepsis. Concentrations of sTREM-1, as well as TNFα and IL-1β, are increased in the broncho-alveolar lavage fluid (BALF) from patients with community-acquired or ventilator-associated pneumonia and sTREM-1 determination may constitute an interesting biomarker in this context.[38] Pathogenesis of pneumonia is now becoming better understood and a greater comprehension of the complex network of immune, inflammatory and haematological mediators involved in this disorder now allows for the development of rational and novel therapies. Among them, TREM-1 modulation proved to be beneficial. In a rat model of *Pseudomonas aeruginosa*-induced pneumonia, LP17 treatment was associated with hemodynamic improvement, as well as tissue and systemic inflammatory responses dampening and a decrease of coagulation activation. In fine, LP17 treatment improved survival.[36]

In Southeast-Asia and northern-Australia the Gram-negative bacillus *Burkholderia pseudomallei* is an important cause of community-acquired sepsis. More than half of these cases of melioidosis, as this severe infection is named, habitually present with pneumonia, frequently associated with bacterial dissemination to distant sites.

Wiersinga et al.[39] showed that during melioidosis, TREM-1 expression was upregulated in peripheral monocytes and granulocytes, followed by increased plasma and BALF (Broncho-alveolar lavage fluid) sTREM-1 concentrations. Soluble TREM-1 levels were initially higher in non-surviving patients than in survivors, suggesting that sTREM-1 may constitute a prognostic biomarker during this disease. Similar results were obtained in a mouse model of melioidosis induced by tracheal instillation of *B. pseudomallei*. In this model, LP17 treatment partially protected animals from death and bacteriema.[39]

Hemorrhagic shock. Severe hemorrhagic shock (HS) leads to an exaggerated production of inflammatory mediators, such as cytokines and chemokines, which may play a significant role in the development of multiple organ failure (MOF) under those conditions.[40-42] Numerous studies have shown that leukocytes are activated early during HS and are responsible for cytokine production and tissue injury.[43,44] Moreover, in rats, bacterial translocation has also been involved in the development of organ failure.[45] Indeed, several attempts in down-modulating HS-associated inflammation by using various compounds have been promising, supporting the inflammatory hypothesis of HS-induced organ failure.[46-48] Considering the particularly high mortality associated with MOF, the development of specific interventions that could prevent both local and distant organ injury that follow HS is obviously needed. In a rat model of HS, the TREM-1 modulation by LP17 attenuated the haemodynamic compromise, the development of lactic acidosis, prevented form cytokine production, organ dysfunction and finally improved survival.[49]

Ischemia-reperfusion. Acute mesenteric ischemia is a medico-surgical emergency associated with 60 to 90% mortality.[50] While ischemia induced little damages by itself, reperfusion leads to a systemic release of several proinflammatory cytokines (TNFα, IL-1β and IL-6) in parallel of leukocyte activation and bacterial translocation, believed to play a crucial role in the induction of local and remote organ failure. Recent evidences show that those phenomena are dependant or TLR/MyD88 signalling pathway[51] and that a NFκB inhibitor prevents organ injury.[52] As TREM-1

is known to amplify TLR pathway, we showed in a precedent study that modulation of TREM-1 during ischemia/reperfusion in rats is beneficial in terms of systemic inflammation, lactatemia, hemodynamic deterioration, activation of hepatic NFκB, bacterial translocation and then mortality.[53] These results have recently been confirmed by Pamuk et al. who demonstrated that inhibition of Syk, involved in TREM-1/DAP12 pathway, provides similar protective effects.[54]

Pancreatitis. During acute pancreatitis (AP) humoral mediators released by monocytes/macrophages and PMN may lead to aggravation of inflammation and remote organ injury. Mortality of severe AP remains very high: 25% to 50%.[55,56] In patients with AP, plasma sTREM-1 concentrations were higher in non-survivors than in survivors,[55] and may be helpful to early predict the development of organ dysfunction.[57] Expression of TREM-1 on myeloid cells in these patients is upregulated and correlates to disease severity.[58] TREM-1 upregulation and elevated levels of plasma sTREM-1 were also found in a rat model of severe AP, in which peritoneal macrophage depletion resulted in a decrease in serum sTREM-1 levels. Using this model, Kamei et al. were able to demonstrate a salutary effect of TREM-1 modulation with an LP17-associated organ dysfunction improvement.[59]

Chronic inflammatory disorders. *Inflammatory bowel diseases.* TREM-1 appears to be crucially implicated in inflammatory bowel diseases (IBD).[60,61] TREM-1 expression on myeloid cells in IBD patients or animals is upregulated and correlates with disease severity.[60] The number of TREM-1+ macrophages in the inflamed intestine of patients with both acute and chronic IBD is increased as compared to controls in which resident intestinal macrophages express very low level of TREM-1. This aberrant TREM-1 expression mediates enhanced secretion of pro-inflammatory chemokines and cytokines.[60] In parallel, serum sTREM-1 concentrations are significantly enhanced in patients with IBD.[61] As expected, LP17 treatment attenuated intestinal inflammation in an animal model of colitis.[60] Interestingly, both early and late LP17 treatment showed the same efficacy.

These data suggest that TREM-1 may be a potential therapeutic target during chronic intestinal inflammatory diseases.

Rheumatic diseases. Recent studies show that TREM-1 could be implicated in the development and evolution of rheumatoid arthritis (RA), an autoimmune inflammatory disease, as well as during septic arthritis. Indeed, infiltrated leucocytes in inflammatory synovia expressed high levels of TREM-1.[62] Moreover, patients with RA or septic arthritis showed elevated levels of sTREM-1 in synovial fluid that correlated with TNFα concentration and with the number of infiltrated leucocytes. TREM-1 expressing cells were of myelomonocytic lineage and synoviocytes didn't express TREM-1.[63,64] Soluble TREM-1 was also more elevated in the plasma from RA patients than controls.[62] In vitro stimulation of human primary synovial cells isolated from RA patients (comprising synoviocytes but also infiltrated leucocytes) by αTREM-1 showed increased cytokine production (TNFα, IL-8, IL-1β, GM-CSF) as compared to controls. These data suggest that TREM-1 could play a role in amplifying inflammation during arthritis and that modulating the TREM-1

activation could downregulate excessive chemokines and cytokine production.

This was further demonstrated in an experimental *c*ollagen type II induced arthritis in mice: TREM-1 fusion protein or LP17 administration were associated with a sharp reduction of clinical signs in a dose-dependant manner.[64]

Finally, a recent study also found a TREM-1 upregulation in peripheral blood cells from patients suffering from to spondylarthropathy.[65]

Thus, while TREM-1 role in acute inflammation, especially during sepsis, is today better understood, its function during chronic rheumatic diseases only begins to be elicited.

Cystic fibrosis. Cystic dibrosis (CF), also called mucoviscidosis, results from abnormalities in the gene that codes for the CFTR (Cystic Fibriosis Transmembrane Conductance Regulator) which is implicated in the regulation of chloride flux across cell membranes. It affects all exocrine epithelia and leads to injuries of primary organs (pancreas, sinus, liver, intestine and exocrine pancreas). Mortality is often the result of repetitive lung infections and persistent inflammation that leads to profound lung function alteration.[66] In this disease, a deregulation of the immune system is pointed out that allows for a huge bacterial colonization of the airways. TREM-1 was shown to be expressed at low levels in lung resident macrophages and circulating monocytes from CF patients. This phenomena was associated with a failure to mount an appropriate inflammatory response,[67,68] along with an impairment of antigen presentation properties. Those monocytes expressed elevated levels of PU.1, a transcription factor that counteracts the TLRs-induced TREM-1 upregulation.[30] These data suggest that both resident macrophages and circulating monocytes were maintained in a LPS-tolerant state due to TREM-1 repression during CF. Therefore a restauration of TREM-1 expression is likely to improve TLRs responsiveness that is crucial for bacterial clearance.

Cancer. Tumour cells can use the innate immune system signalling pathway for migration, invasion, angiogenesis and thus metastasis. It has become evident that the inflammatory response observed in or around developing neoplasm can regulate tumour development. Moreover, tumour cells have co-opted some of the signalling molecules of the innate immune system.[69,70] Clinical data suggest that enhanced innate immunity is a significant factor influencing malignant outcome and thus manipulation of the immune system could constitute an approach for anti-tumour therapy.[71] Ho et al.[72] showed that in humans sTREM-1 concentration was elevated in malignant pleural effusions and correlated with poor outcomes, making sTREM-1 an independent predictor of patient survival. Second, TREM-1 expression was upregulated in tumour-associated macrophages (TAMs), but not in cancer cells. Co-culture of blood monocytes and lung cancer cells leads to monocytic TREM-1 upregulation both at the gene and the protein levels. Finally, TREM-1 engagement by the mean of a αTREM-1 facilitated the metastasis process while TAMs *Trem-1* silencing resulted in the loss of cancer cells invasiveness. These data underline the potential therapeutic interest of the TREM-1 modulation during lung cancer in order to prevent cancer progression.

Conclusion

TREM-1 appears to work as a sensor for extracellular danger-associated molecular patterns that emerged from the Matzinger's danger theory.[73] Such sensors integrate different signals that further defined organism response.

A large number of experimental studies dealing both with acute or chronic inflammatory diseases, of infectious aetiology or not, have demonstrated the role of TREM-1 in amplifying the inflammatory response triggered by the aggression. In all these studies, the modulation of the TREM-1 pathway proved beneficial. The advantage of modulating TREM-1 is that such an approach does not totally abrogate the inflammatory response which is essential for bacterial clearance, control of cancer progression. Thus, peptides like LP17 may represent a new promising class of anti-inflammatory compounds able to modulate rather than to inhibit inflammation during numerous inflammatory diseases.

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