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Chapter 4

TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives

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TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives

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A B S T R A C T

Triggering receptor expressed on myeloid cells-1 (TREM-1) is expressed on the majority of innate immune cells and to a lesser extent on parenchymal cells. Upon activation, TREM-1 can directly amplify an inflammatory response. Although it was initially demonstrated that TREM-1 was predominantly associated with infectious diseases, recent evidences shed new light into its role in sterile inflammatory diseases. Indeed, TREM-1 receptor and its signaling pathways contribute to the pathology of several non-infectious acute and chronic inflammatory diseases, including atherosclerosis, ischemia reperfusion-induced tissue injury, colitis, fibrosis and cancer. This review, aims to give an extensive overview of TREM-1 in non-infectious diseases, with the focus on the therapeutic potential of TREM-1 intervention strategies herein. In addition, we provide the reader with a functional enrichment analysis of TREM-1 signaling pathway and potential TREM-1 ligands in these diseases, obtained via *in silico* approach. We discuss pre-clinical studies which show that TREM-1 inhibition, via synthetic soluble TREM-1 protein mimickers, is effective in treating (preventing) specific inflammatory disorders, without significant effects on antibacterial response. Further research aimed at identifying specific TREM-1 ligands, in different inflammatory disorders, is required to further unravel the role of this receptor, and explore new avenues to modulate its function.

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Abbreviations: AGA, acute gouty arthritis; AP-1, activator protein-1; BAL, bronchial alveolar lavage; BD, intestinal Behcet's disease; BPAR, biopsy proven acute rejection; CAD, coronary artery disease; CARD, caspase recruitment domain-containing protein; CAT, colitis-associated tumorigenesis; CD, Crohn's disease; CIA, collagen-induced arthritis; DAMPs, damage associated molecular patterns; DGF, delayed graft function; ERK, extracellular signal-regulated; HMGB1, high mobility group box-1; HSP, heat shock proteins; IBD, inflammatory bowel disease; IFTA, interstitial fibrosis/tubular atrophy; IR, ischemia-reperfusion; IRAK, IL-1R-associated kinases; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Myd88, myeloid differentiation primary response; Nf-κB, nuclear factor-κB; NLR, Nod-like receptor; NOD, nucleotide-binding oligomerization domain; NSCLC, non-small cell lung cancer; PAMPs, pathogen-associated molecular patterns; PBC, peripheral blood cells; PGN, peptidoglycan; PI3K, phosphatidylinositol 3-kinase; PRR, pattern recognition receptors; RA, rheumatoid arthritis; RIP2, receptor-interacting serine/threonine-protein 2; SNV, single nucleotide variants; sTREM-1, soluble TREM-1; TLR, Toll like receptor; TREM-1, triggering receptor expressed on myeloid cells-1; UC, ulcerative colitis; UO, ureteral obstruction.

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1. Introduction

Innate immune cells are key players in the recognition of invading pathogens or alarming the host during tissue damage. The magnitude of inflammation relies on the activation of pattern recognition receptors (PRR). One family of PRRs is the family of Toll-like receptors (TLRs), which are well known for their role in innate immunity during infectious and non-infectious diseases. More recently, another family of innate immune receptors was described to interact with TLRs and influence the extent of the inflammatory response: the triggering receptors expressed on myeloid cells (TREMs) (Arts, Joosten, van der Meer, & Netea, 2012; Klesney-Tait, Turnbull, & Colonna, 2006). The TREM-family comprises both activating and inhibitory receptors. Among these family members, TREM-1 represent the most studied activating receptor, whereas TREM-2 is widely known as an inhibitor of the inflammatory response. Activation of TREM-1 is known to trigger and amplify inflammation, especially through synergism with TLR signaling. Early after the discovery of TREM-1, it was believed that TREM-1 was merely involved in non-infectious diseases, and research was mainly focused on TREM-pathogen interaction as described in several elegant reviews (Gibot, 2005, 2006; Roe, Gibot, & Verma, 2014; Sharif & Knapp, 2008). However, more recent research shows that TREM-1 is also involved in non-infectious diseases and this review aims to give an overview of the expression, and function of TREM-1 receptor and its ligands herein. Moreover, intervention studies that examined TREM-1 receptor modulation in non-infectious diseases, were included. Lastly, because several research questions regarding the TREM-1 ligand and pathway synergism, are still unanswered, we performed an *in silico* analysis of putative TREM1-ligand expression and pathway enrichment in several sterile inflammatory diseases.

2. TREM-1 signaling pathway

2.1. TREM-1 pathway

First experiments performed by Colonna and colleagues showed that TREM-1 is mainly expressed on myeloid cells such as monocytes/macrophages and granulocytes. However, ongoing research shows that during inflammation, TREM-1 is also detected on parenchymal cell types such as bronchial, corneal, gastric epithelial cells, and hepatic endothelial cells (Barrow et al., 2004; Chen, Laskin, Gordon, & Laskin, 2008; Rigo et al., 2012; Schmausser et al., 2008). TREM-1 is present in 2 forms: as a membrane-bound receptor and as soluble protein (Figs. 1 and 2). Membrane TREM-1 features 3 distinct domains: an Ig-like structure (most likely responsible for ligand binding), a transmembrane part and a cytoplasmic tail which associates with the adaptor molecule TYROBP (TYRO protein tyrosine kinase-binding protein, more frequently called DAP12: DNAX activating protein of 12 kDa) (Colonna, 2003). This complex is stabilized through a unique electrostatic interaction between a negatively charged (−) aspartic acid in DAP12, and a positively charged (+) lysine in TREM-1 intracytoplasmic tail, which is necessary for signal transduction. Following TREM-1 engagement, the cytoplasmic part of DAP12 containing ITAMs (Immunoreceptor tyrosine-based activation motif) becomes phosphorylated at its tyrosine residue, providing a docking site for protein tyrosine kinases: ZAP70 (Zeta-chain-associated protein kinase 70) and SYK (Spleen Tyrosine Kinase). SYK promotes the recruitment and tyrosine phosphorylation of adaptor complexes that contain Cbl (Casitas B-lineage Lymphoma), SOS (Son of sevenless) and GRB2 (Growth Factor Receptor Binding Protein-2), which results in downstream signal transduction through PI3K, PLC-Gamma (Phospholipase-C-Gamma) and the ERK pathways. These pathways induce Ca^{2+} mobilization, rearrangement of the actin cytoskeleton and activation of transcription factors such as Elk1 (ETS domain-containing protein), NFAT (Nuclear Factor of Activated T-Cells), AP1, c-Fos, c-Jun and Nf-κB, which transcribe genes that encode pro-inflammatory cytokines, chemokines and cell-surface molecules. In addition, TREM1-induced PI3K and ERK pathway activation can promote mitochondrial integrity and cell survival by inactivating pro-apoptotic factors: BID (BH3-Interacting Domain Death agonist), BAD (BCL-2-Antagonist of cell Death) and BAX (BCL-2-Associated X-Protein) and inhibiting CytoC (Cytochrome-C) release from mitochondria (Yuanet al., 2014, 2016).

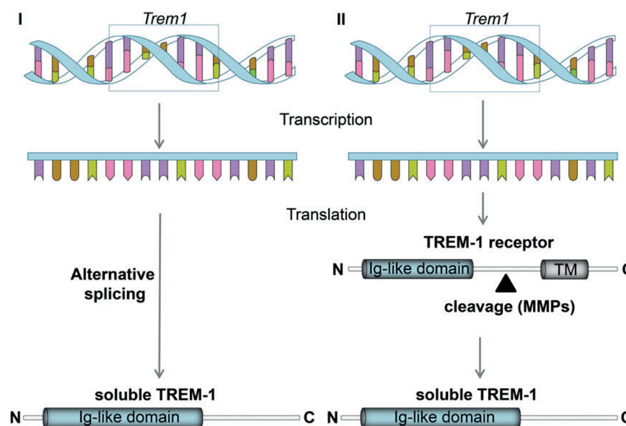

Fig. 1. TREM-1 receptor and soluble protein regulation. Graphical representation of the two hypotheses of soluble TREM-1 protein (sTREM-1) origins. Upon transcription, alternative splicing of the *Trem1* gene can result in the synthesis of a smaller protein, which contains only the immuoglobulin-like domain (Ig-like domain). This protein is referred to as sTREM-1 (I: left). The canonical translation process produces the TREM-1 receptor protein, which consists of the Ig-like domain and a transmembrane domain (TM). This receptor, upon proteolytic cleavage by metalloproteinases (MMPs), results in sTREM-1 protein generation (II: right).

Besides the membrane receptor, a soluble form of TREM-1 (sTREM-1) has been described (Klesney-Tait et al., 2006). Although the origin and function of sTREM-1 are still elusive, its relevance as both biomarker and therapeutic target is high, in both sterile and infectious disease settings. In terms of molecular structure, sTREM-1 lacks the transmembrane and intracellular domain; hence its lack of signal transduction properties. It exhibits the extracellular part of the receptor (Ig-like domain), also known as ectodomain, proposed as the site responsible for ligand binding. The firsts to propose the origin of sTREM-1 were Gingras et al., who described an alternatively spliced form of TREM-1 (TREM-1sv) (Gingras, Lapillonne, & Margolin, 2002). The protein produced in this case was 17.5 kDa in size, compared to the typical 27 kDa of TREM-1 membrane receptor. This isoform was isolated from CD34+ bone marrow cells and mature monocytes. Consistent with this study, a novel splice variant of TREM-1 was recently described in human neutrophil granules (Baruah et al., 2015). This isoform, with a molecular weight of 15 kDa, was isolated from the α and β neutrophil fractions, rich in primary and secondary granules, respectively. According to Baruah et al. the release of TREM-1sv may modulate the inflammatory response by inhibiting neutrophil migration regulated from membrane TREM-1, and by acting as a decoy receptor in the circulation (Baruah et al., 2015). Beside the splice variant hypothesis, another theory has emerged, which postulate that the proteolytic cleavage of membrane bound receptor by metallo-proteinases (MMPs) is an alternative source of sTREM-1. Monocytes treated with Lipopolysaccharide (LPS) in the presence of a metalloproteinases inhibitor displayed increased expression of the membrane receptor while sTREM-1 levels decreased (Gomez-Pina et al., 2007). Thus far, the main function of sTREM-1 appears to be the neutralization of TREM-1 inflammatory activity (Roe et al., 2014). Once secreted, sTREM-1 can be detected in biological fluid, during infection or inflammation. In sterile

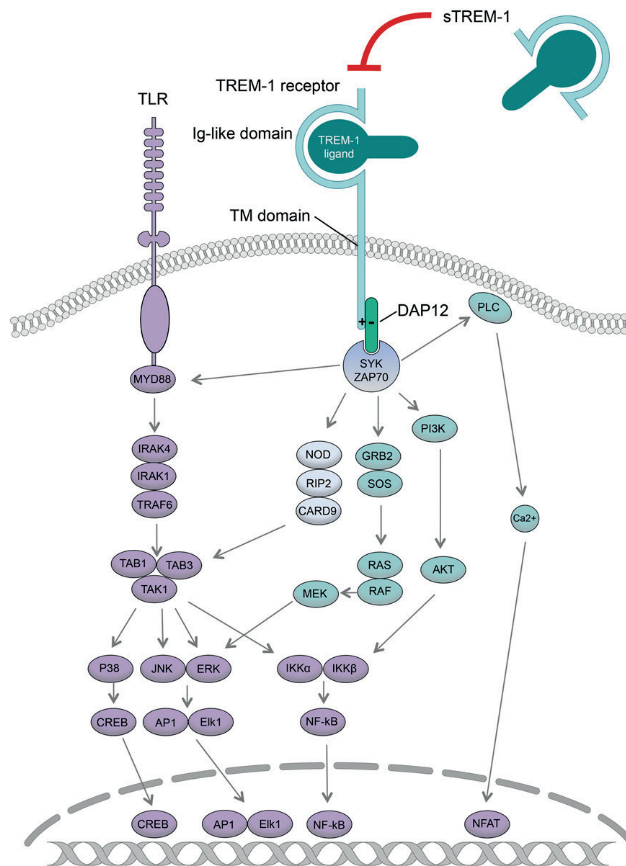

Fig. 2. TREM-1/TLR/sTREM-1 pathway interaction. Schematic illustration of TREM-1, TLR and NLR combined signaling pathways. TREM-1 belongs to the immunoglobulin superfamily of receptors and is located on the plasma membrane. The Immunoglobulin-like (Ig) domain, in the extracellular part, is responsible for ligand binding, while the transmembrane (TM) domain associates with the adaper molecule DAP12. Upon binding to DAP12, the protein tyrosine kinases SYK and ZAP70 are recruited, leading to the activation of PLC, PI3K and ERK pathways, upstream regulators of inflammatory gene transcription. The tyrosine kinases SYK/ZAP70 can also activate the NLR pathway, which will merge with the TLR pathway, initiated by Myd88. The TREM-1 pathway can synergize with the TLR pathway (in) dependently of Myd88. Engagement of Myd88 stimulates downstream signaling pathways that involve IRAKs and another family of adaptor molecules, namely TNF receptor-associated factors (TRAFs). This leads to the activation of the MAPKs, Janus N-terminal kinase (JNK) and p38, as well as the activation of transcription factors. The main families of transcription factors that are activated downstream of TLR signaling, are NF-κB, cyclic AMP-responsive element-binding protein (CREB), AP, and Elk, responsible for the transcription of proinflammatory cytokines and chemokines. sTREM-1 by scavenging TREM-1 ligand is able to inhibit the perpetuation of this inflammatory pathway.

inflammation, sTREM-1 has been described to increase during renal IR, chronic kidney disease patients on hemodialysis, myocardial infarction, inflammatory bowel disease, acute gouty inflammation and rheumatoid arthritis, suggesting the potential for biological activity of the soluble receptor (Table 1). However, the biological relevance of sTREM-1 in sterile inflammation is still unclear in contrast to its significance as predictor of infection and inflammation in infectious diseases. Whether sTREM-1 may be a novel biomarker candidate, experiments to unveil its function in sterile inflammation are necessary.

2.2. TREM-1-TLR connection

TLRs are PRRs that recognize distinct pathogen-associated molecular patterns (PAMPs) that play a critical role in innate immune responses. The fact that TLRs also recognize endogenous ligands released after sterile tissue injury (called damage-associated molecular patterns; DAMPs) led to new mechanistic insights on the role of TLRs in the induction of inflammation. Activation of TLRs leads to association of the cytoplasmic Toll/IL-1 receptor (TIR) domain with a TIR domain-containing adaptor, MyD88 that recruits

Table 1.
(Soluble) Trem-1 expression in mice and human.

		Expression upon inflammation *vs* control 1) Soluble form, 2) Circulating leukocytes, 3) Tissue	
Location	**Model/Disease**	**Mouse**	**Human**
Circulation	Atherosclerosis	1. Increased during high cholesterol/high fat diet (Joffre, Potteaux, et al., 2016; Zysset et al., 2016) 2. Enhanced expression on myeloid cells after high cholesterol/high fat diet (Joffre, Potteaux, et al., 2016; Zysset et al., 2016) 3. Enhanced mRNA in aortic arch after high cholesterol/high fat diet (Zysset et al., 2016)	1. n.d. 2. Enhanced expression on monocytes with oxLDL (Joffre, Potteaux, et al., 2016; Zysset et al., 2016) 3. Enhanced mRNA and protein in severe condition (Joffre, Potteaux, et al., 2016; Zysset et al., 2016)
Heart	Myocardial IR	1. Increased 2. Increased 3. Enhanced mRNA/protein by infiltrating granulocytes (Boufenzer et al., 2015)	1. Enhanced and associated with mortality and recurrence of cardiovascular events (Boufenzer et al., 2015) 2. n.d. 3. Enhanced number of TREM-1-expressing granulocytes (Boufenzer et al., 2015)
Intestine	IBD/Colitis	1. n.d. 2. Enhanced number of Trem-1 expressing granulocytes (Liu et al., 2015) 3. Enhanced mRNA/protein (Schenk et al., 2007)	1. n.d. 2. n.d. 3. Enhanced number of TREM-1-expressing macrophages (Schenk et al., 2007)
	Mesenteric IR	1. Elevated in plasma (Gibot et al., 2008) 2. n.d. 3. n.d.	1. n.d. 2. n.d. 3. n.d.
Joint	Gout (AGA)	1. n.d. 2. n.d. 3. n.d.	1. Elevated in synovial fluid (Lee et al., 2016) 2. Enhanced expression on monocytes in synovial fluid (Lee et al., 2016) 3. Presence of TREM-1-expressing macrophages in tophaceous tissue (Lee et al., 2016)
	RA	1. n.d. 2. n.d. 3. n.d.	1. n.d. 2. Enhanced mRNA expression (PBC) (Lee, Sugino, Aoki, & Nishimoto, 2011)* 3. Presence of TREM-1-expressing macrophages in synovial tissue (Murakami et al., 2009)
Kidney	Renal fibrosis (UO/IFTA)	1. n.d. 2. n.d. 3. Enhanced mRNA/protein (Campanholle et al., 2013; Lech et al., 2012; Lo et al., 2014; Tammaro et al., 2013)	1. n.d. 2. n.d. 3. Enhanced mRNA (Maluf et al., 2014)*. Presence of TREM-1-expressing cells (Lo et al., 2014; Tammaro et al., 2013)
	Renal IR	1. Elevated in plasma (Tammaro et al., 2016) 2. Increased on monocytes (Tammaro et al., 2016) 3. Enhanced mRNA/protein (Campanholle et al., 2013; Lech et al., 2012; Liu et al., 2014; Tammaro et al., 2016)	1. n.d. 2. n.d. 3. mRNA n.s. [ATN (Famulski et al., 2012)*]
Lung	Cystic fibrosis	1. n.d. 2. n.d. 3. n.d.	1. Similar (Del et al., 2008) 2. Reduced expression on monocytes (Del et al., 2008) 3. n.d.
	NSCLS	1. n.d. 2. n.d. 3. n.d.	1. Elevated in pleural effusion (Ho et al., 2008) 2. n.d. 3. Enhanced mRNA in tissue and number of TREM-1-expressing macrophages (Ho et al., 2008; Kadara et al., 2014; Yuan, Mehta, et al., 2014)*
	Pulmonary contusion	1. n.d. 2. n.d. 3. n.d.	1. Elevated in BAL: higher in severe *vs* no/mild/moderate (Bingold et al., 2011) 2. n.d. 3. n.d.
Pancreas	Pancreatitis	1. Elevated in plasma (Dang, Shen, Yin, & Zhang, 2012; Kamei et al., 2010) 2. n.d. 3. Enhanced mRNA/protein (Dang et al., 2012; Kamei et al., 2010)	1. Elevated in plasma: higher in non-survivors *vs* survivors (Ferat-Osorio et al., 2009; Yasuda et al., 2008) 2. Enhanced expression on monocytes (Ferat-Osorio et al., 2009) 3. Enhanced mRNA/protein (Wang, Qin, Liu, Gupta, & Chang, 2004)
Skin	Psoriasis	1. n.d. 2. Increased in splenic mononuclear cells (Joffre, Hau, et al., 2016) 3. Enhanced mRNA expression (Suarez-Farinas et al., 2013)	1. Elevated in plasma (Hyder et al., 2013) 2. n.d. 3. Enhanced number of TREM-1 expressing myeloid cells (Hyder et al., 2013)

n.d.: not determined, n.s.: not significant (or unknown). * Data was obtained through Nextbio.

IL-1 receptor-associated kinase-4 (IRAK-4). This leads to IRAK-1 phosphorylation and association with TRAF6, thereby activating the IKK complex and leading to activation of MAP kinases (JNK, p38 MAPK). Finally, nuclear translocation of CREB, Nf-κB and AP1 leads to induction of diverse gene transcription, including inflammatory mediators (Fig. 2). TLR activation also leads to upregulation of TREM-1 expression which is MyD88-dependent and involves transcription factors Nf-kB, PU.1 and AP1 (Bouchon, Dietrich, & Colonna, 2000; Gibot et al., 2005; Knapp et al., 2004). Following LPS stimulation of neutrophils, TREM-1 was found to be recruited to M1-lipid rafts and co-localized with TLR4 (Fortin, Lesur, & Fulop, 2007). TREM-1 knock down induces down-regulation of several genes implicated in the TLR4 pathway including MyD88, CD14 and IκBα, even when TLR4 expression is unaltered (Ornatowska et al., 2007; Tammaro et al., 2016). Simultaneous activation of TREM-1 and TLR4 leads to synergistic production of pro-inflammatory mediators through common signaling pathway activation including PI3K, ERK1/2, IRAK1 and Nf-κB activation (Tessarz & Cerwenka, 2008) (Fig. 2). TREM-1 inhibition by means of inhibitory peptides is linked to a decrease in the production of LPS-induced cytokines including MCP-1, IL-8 and IL-10 (Derive et al., 2012; Ornatowska et al., 2007; Zeng, Ornatowska, Joo, & Sadikot, 2007). Interestingly, TREM-1 ligation alone does not lead to sustained inflammation, which confirms that TREM-1 amplifies the inflammatory response initiated by TLR engagement.

2.3. TREM-1-NLR connection

Next to TLRs, NLRs (nucleotide-binding oligomerization domain – NOD - receptors) are members of the PRR family that can detect microbial infection, but also sterile tissue damage. NLRs can cooperate with TLRs and regulate inflammatory and apoptotic responses. Among the NLRs, NOD1 and NOD2 are two well defined cytosolic PRR that specifically recognize diaminopimelatic acid (DAP)-containing muropeptide found primarily in Gram-negative bacteria (NOD1) and muramyl dipeptide (MDP) moieties (NOD2). However, NOD1/2 are also activated by molecules induced from dead or dying cells, as consequence of endoplasmic reticulum stress, through an intracellular pathway involving the activating receptor EIF1 and TRAF2. Many conditions that impose stress on cells, including hypoxia, starvation and changes in secretory needs, promote endoplasmic reticulum stress (Iurlaro & Muñoz-Pinedo, 2016). Thus, this finding provides a new link between inflammatory disease which involve ER stress and NOD1/2 (Keestra-Gounder et al., 2016). Both NOD1 and NOD2 contain ligand binding regions LRR (C-terminal leucine-rich repeats), a central nucleotide-binding oligomerization domain (NACHT) and N-terminal caspase recruitment domains (CARDs). NOD activation results in oligomerization of the NACHT domains, which results in CARD binding of downstream proteins such as RIP2 (receptor-interacting protein 2) which is important for NOD-induced Nf-κB activation. Very little is known about the effect of TREM-1 and NLRs. TREM-1 has a synergistic effect on the production of pro-inflammatory mediators induced by NOD1 and NOD2 ligands (Netea et al., 2006; Prufer et al., 2014). Monocytes stimulation with TREM-1 agonist and TLR or NOD1/2 ligands leads to phosphorylation of Akt and MAPK p38 and synergistic production of cytokines (Netea et al., 2006; Prufer et al., 2014). Mechanistically, TREM-1 activation/crosslinking can lead to enhanced NOD2 expression, caspase activation and RIP2/CARD9 related NF-kB activation and cytokine production like IL-1β which in addition induces other pro-inflammatory mediators like IL-6 in an autocrine loop (Netea et al., 2006) (Fig. 2). It remains to be determined if and how TREM-1 and (other) NLRs interact in different inflammatory diseases and if this interaction is of significance compared to other synergistic pathways with TREM-1.

3. Identifying TREM-1 ligands

3.1. Methods

The identification of TREM-1 ligands remains a difficult, yet essential task. The reason behind this lies in the low affinity and rapid dissociation between receptor and ligand, common phenomena for innate immune receptors. Most of the functional studies on TREM-1 were performed in murine models. A crystallography study has shown that, unlike human TREM-1, mouse TREM-1 extracellular immunoglobulin domain, lacks conserved residues at the complementary determining region (CDR), crucial domain part for ligand binding. Hence, this means that no obvious binding sites for the ligand are observed. This raises the question as to whether the CDR regions are involved in direct ligand recognition and binding (Kelker, Debler, & Wilson, 2004). It is not excluded indeed, that indirectly, *via* a carrier molecule for example, TREM-1 ligand can engage the receptor. In order to describe low affinity ligands, several strategies have been proposed either by immunoprecipitation assays, mass spectroscopy and/or by using cell reporter assays (Read et al., 2015). Some of these methods have been successfully used to propose two candidates as TREM-1 ligands (see below).

3.1.1. Method: TREM-1 fluorescent-labeled tetramer

This method uses a construct in which the extracellular domain of TREM-1 is biotinylated and subsequently assembled to a scaffold of streptavidin, conjugated with a fluorescent dye. As streptavidin contains 4 binding sites for biotin, the resulting molecule is a TREM-1 tetramer with 4 available ectodomains; hence this strategy is expected to increase the affinity for TREM-1 ligand.

3.1.2. Method: functional assay with TREM-1 reporter cells

A cell-based TREM-1 reporter has been described, which can be used to identify TREM-1 activating factor/s. This cell line was derived from BWZ.36 mouse T cell lymphoma line and contains a NFAT-driven LacZ reporter gene. These cells were stably transfected with a TREM-1/DAP12 chimera protein composed of a fusion between TREM-1 (aa1-200) and DAP12. These cells were shown to be robustly activated by the agonistic anti-TREM-1 antibody MAB1278 (R&D Systems) (Campanholle et al., 2013).

3.1.3. Method: surface plasmon resonance (SPR)

This is a powerful technique used to study ligand-binding interactions with membrane proteins. SPR is capable of measuring real-time binding affinities and kinetics of two interacting molecules. It is based on the immobilization of one of the components to a chip that is connected to a sensor, whereas the other interacting molecule is allowed to flow over the surface of the sensor. The detection of the binding is based on the optical phenomena, that measure the changes in the refractive index at the surface of the sensor. Whenever an interaction is detected there will be a change in signal intensity. The increase of the signal is expressed in response unit (RU). SPR is performed using the Biacore technology (Patching, 2014).

TREM-1 receptor can be immobilized on the sensor chip and solutions with putative ligands may be flowed over it. Two recent works have identified HMGB1 and PGLRP1 as potential ligands of TREM-1 receptor using this technique (see below) (Read et al., 2015; Wu et al., 2012).

3.2. High mobility group Box 1 (HMGB1)

HMGB1 is a ubiquitous nuclear protein, that interacts with nucleosomes, transcription factors and histones, to regulate transcription. During inflammation, HMGB1 is actively secreted by activated myeloid cells and is released by dying and necrotic cells (Magna & Pisetsky, 2014; Stephenson, Herzig, & Zychlinsky, 2016; Tsung, Tohme, & Billiar, 2014; Venereau, Ceriotti, & Bianchi, 2015). HMGB1 has been shown to signal

Box 1

Future research questions

- Which ligand is responsible for TREM-1 signaling in different inflammatory diseases?
- Does the TREM-1 and TLR signaling pathways work synergistically or independently in acute and chronic inflammatory disease? Does TREM-1 signal through other (innate) PRRs?
- During inflammation, is TREM-1 expressed on other non-myeloid cells, besides those that are currently known?
- What is the phenotype of TREM-1 polymorphisms in humans?
- Is sTREM-1 a biomarker for non-infectious diseases?

through several receptors, including TLRs and RAGE (receptor for advanced glycation end products), to induce a pathogenic inflammatory response, thereby functioning as a DAMP molecule. HMGB1 has been suggested as a TREM-1 ligand. Indeed, a direct interaction between TREM-1 and HMGB1 was observed by Wu and colleagues, using different approaches like immunoprecipitation and cross-linking assays (Wu et al., 2012). Necrotic cell lysates (NCL), from activated human monocyte cell line THP1, and murine hepatocytes, containing HMGB1, were able to induce an inflammatory response that was blocked by recombinant, soluble TREM-1 (El Mezayen et al., 2007; Wu et al., 2012). However, HMGB1 alone seems unable to trigger TREM-1 activation and possibly needs co-activating molecules (Lo et al., 2014). HMGB1 is considered an inducer of inflammation through activation of diverse PRR and its expression in inflammatory diseases has been observed during colitis, ischemia, arthritis, fibrosis, pancreatitis and cancer (Table 2). Currently, several clinical trials are ongoing to determine its association with outcome and potential for intervention strategies (https://clinicaltrials.gov). It is unknown if HMGB1-TREM1 signaling is of significance compared to signaling of HMGB1 through other PRRs in non-infectious diseases. An additional level of complexity in HMGB1 biology is that it is a redox-sensitive protein containing three conserved cysteine residues, whose redox status dictates the extracellular chemokine- or cytokine-inducing properties of the protein. Ample evidence suggests that HMGB1 is released as a mixture of several isoforms, with distinct post-translational modifications (Yang, Antoine, Andersson, & Tracey, 2013).

3.3. Peptidoglycan recognition receptor 1 (PGLYRP1)

Recently, Read and colleagues identified PGLYRP1 as a potential TREM-1 ligand (Read et al., 2015). The PGLYRP1 protein is primarily found in the granules of granulocytes and is known for its bactericidal properties, namely binding to peptidoglycan (PGN) and other microbial cell wall components such as LPS, and inducing lethal membrane depolarization and oxidative stress in bacteria. It was already known that neutrophils activated by bacteria can release a TREM-1 ligand (Gibot, Buonsanti, et al., 2006). Supernatants from neutrophils activated by PGN (but not by other TLR/NOD-ligands) were able to activate the TREM-1 reporter cell line and this was inhibited with TREM-1 blocking antibodies (Read et al., 2015). Since PGN by itself did not induce activation of the TREM-1 reporter cell line, the authors further investigated the presumed TREM-1 ligand on PGN-activated neutrophils using immunoprecipitation with a tetramer of TREM-1 and mass spectrometry. PGLYRP1 was found to bind TREM-1 (Read et al., 2015). Interestingly, stimulation with soluble PGLYRP1 alone did not induce TREM-1 activation and crosslinking of PGLYRP1 by PGN was required to stimulate TREM-1, suggesting a role of PGLYRP1 as a TREM-1 ligand in infectious diseases. Similar to TREM-1, PGLYRP1 mRNA expression is upregulated in tissue following diverse inflammatory diseases; however its protein expression has not yet been investigated (Table 2). PGLYRP1 was proven to be beneficial in several inflammatory diseases. Mice lacking *Pglyrp1* are more sensitive to colitis and variants in the *PGLYRP1* gene are associated with ulcerative colitis in patients (Saha et al., 2010; Zulfiqar et al., 2013). It remains to be investigated if this is due to the bactericidal properties of PGLYRP1 or its signaling *via* TREM-1 (Yashin et al., 2015).

3.4. Heat shock protein 70-kDA (Hsp70)

Endogenous danger signals released by stressed or necrotic cells, are represented mostly by HMGB1 and HSP70. Eukariotics Hsps are evolutionarily conserved molecules present in all intracellular organelles. They mainly function as molecular chaperones and participate in maintenance of protein homeostasis in normal and stressful conditions. Hsp70 is one of the most frequently studied Hsps because of its potential anti-inflammatory properties. NCLs prepared from LPS-treated THP-1 cells, contain endogenous danger signals, including Hsp70, which are able to induce pro-inflammatory response (El Mezayen et al., 2007; Wu et al., 2012). This effect was reduced by blocking Hsp70 and/or TREM-1, most likely due to a reduction of the p38 MAPK (and not Nf-κB) pathway (El Mezayen et al., 2007). In contrast to the direct interaction observed with HMGB1, Hsp70 did not directly bind TREM-1 (Wu et al., 2012), suggesting that possibly HMGB1 has stronger affinity and might compete for the binding. Of note, Hsp70 seems to display beneficial properties in several pathophysiological conditions; colitis was ameliorated in Hsp70 transgenic mice compared to normal mice, and variants in *HSP70* are associated with Crohn's disease and ulcerative colitis (Debler et al., 2003; Esaki et al., 1999; Klausz et al., 2005; Nam et al., 2007; Tanaka et al., 2007). In addition, Hsp70 plays a protective role in IR-induced injury, fibrosis, cancer and pancreatitis (Bellaye, Burgy, Causse, Garrido, & Bonniaud, 2014; Feng & Li, 2010; Jones, Voegeli, Li, Chen, & Currie, 2011; Murphy, 2013; O'Neill, Harrison, Ross, Wigmore, & Hughes, 2014; Sherman & Gabai, 2015). In conclusion, it appears that only blocking antibodies experiments support the hypothesis that TREM-1 and HSP70 are functionally related (El Mezayen et al., 2007). Further experiments are required to confirm whether TREM-1 activation, in other models of non-infectious diseases, is HSP70-dependent.

3.5. Ligand on platelets

Platelets (thrombocytes) are blood components without nuclei that play a major role in coagulation. Platelets express a wide range of receptors including TLT-1 (TREM-Like Transcript-1, see below) (Haselmayer, Grosse-Hovest, von Landenberg, Schild, & Radsak, 2007). Authors used a recombinant soluble fusion protein consisting of the extracellular domain of human TREM-1 fused to the Fc part of human IgG (rsTREM-1), to demonstrate the binding of TREM-1 on human platelets. In addition to the receptor, platelets also express a TREM-1 ligand on the surface of platelets, both in resting condition and after thrombin activation. Binding of the fusion protein to platelets was inhibited by competing soluble protein or LP17 (Haselmayer et al., 2007). However, the soluble TREM-1 construct did not activate platelets as determined by platelet-dependent aggregation and degranulation (Haselmayer et al., 2007). Interestingly, and in contrast to earlier reports (Gibot, Alauzet, et al., 2006; Gibot, Buonsanti, et al., 2006), TREM-1 ligand was not found on neutrophils.

4. TREM-1 deficient mice

In absence of a specific TREM-1 ligand in sterile inflammatory disorders, genetic models with TREM-1 deficiency represent a valid tool to unveil TREM-1 function *in vivo*. To study this, two different genetic modified murine models are available: TREM1/3 double KO and TREM-1 single KO. Colonna and colleagues postulated that murine *Trem1* lies adjacent to *Trem3* gene and that the 2 genes are likely to have complementary functions. Additionally *TREM-3* is a pseudogene

Table 2
Role for Trem-1 in murine non-infectious disease and potential ligands.

Location	Model	Modulation: phenotype (species, dose)	Potential TREM-1 ligands
Circulation	Hemorrhagic shock	- LP17: protective (rat: 1.0 mg) (Gibot et al., 2009)	- HMGB1 (Yang & Tracey, 2010) - HSP70 (Yang & Tracey, 2010), HSP70 (mRNA lung/liver ↑) (Feinman et al., 2007; Moran et al., 2011)* - CD177 (mRNA liver ↑, lung/liver n.s.) (Edmonds et al., 2011; Feinman et al., 2007; Moran et al., 2011) * - PGLYRP1 (mRNA lung/liver ↑) (Feinman et al., 2007; Moran et al., 2011)*
	Atherosclerosis	- Apoe/Trem-1 KO mice: protective compared to Apoe KO mice (Joffre, Potteaux, et al., 2016; Zysset et al., 2016) - Ldlr/Trem-1 KO mice: protected compared to Ldlr KO mice (Joffre, Potteaux, et al., 2016) - LR12: protective (mice: 5 mg/kg) (Joffre, Potteaux, et al., 2016)	- HMGB1 (Kanellakis et al., 2011) - HSP70 (Xie et al., 2016; Zhou et al., 2004) - CD177 (mRNA n.s.) (Beer et al., 2011; Grabner et al., 2009; Yin et al., 2015)* - PGLYRP1 (mRNA n.s.) (Beer et al., 2011; Grabner et al., 2009; Yin et al., 2015)*
Heart	Myocardial IR	- LR12: protective (mice: 5 mg/kg) (Boufenzer et al., 2015) - Trem-1 KO: Protective (Boufenzer et al., 2015) (mice)	- HMGB1 (de Haan, Smeets, Pasterkamp, & Arslan, 2013) - HSP70 (de Haan et al., 2013) - CD177 (mRNA n.s.) (Harpster et al., 2006; Hunt et al., 2012; Roy et al., 2006; Tarnavski et al., 2004)* - PGLYRP1 (mRNA↑) (Harpster et al., 2006; Hunt et al., 2012; Tarnavski et al., 2004)*
Intestine	Colitis/CAT	- LP17: protective (mice: 0.2 mg, rat: 1.0 mg) (Schenk et al., 2007; Zhou et al., 2013)	- HMGB1 (Hu et al., 2015; Yang & Tracey, 2010) - HSP70 (Tanaka et al., 2007) - CD177 (mRNA ↑; GSE64658)* - PGLYRP1 (mRNA ↑; GSE64658) (Gao et al., 2013)*
	Mesenteric IR	- LP17: protective (rat: 3.5 mg/kg) (Gibot et al., 2008)	- HMGB1 (Kojima et al., 2012)
Joint	CIA	- LP17: protective (mice: 0.2 mg) (Murakami et al., 2009) - Trem1-adenovirus: protective (mice: Trem1 adenovirus 10e8–9 PFU) (Murakami et al., 2009)	- HMGB1 (Yang & Tracey, 2010) - HSP70 (mRNA ↑ Tarsal joints) (Denninger et al., 2015)* - CD177 (mRNA n.s Tarsal Joints) (Denninger et al., 2015)* - PGLYRP1 (mRNA ↑ n.s. Tarsal Joints) (Denninger et al., 2015)*
Kidney	Renal fibrosis	- Trem-1 KO: protective (Lo et al., 2014) - Trem-1/3 KO: mild phenotype (Tammaro et al., 2013)	- HMGB1 (Yang & Tracey, 2010) - HSP70 (mRNA ↓) (Wu & Brooks, 2012) * - CD177 (mRNA n.s.) (Wu & Brooks, 2012)* - PGLYRP1 (mRNA ↑) (Wu & Brooks, 2012)*
	Renal IR	- LP17: no effect (mice; 0.05–0.2 mg) (Tammaro et al., 2016) - LR12: no effect (mice: 0.2 mg) (Tammaro et al., 2016) - Fc Trem1: no effect (mice; 0.04–5 mg) (Campanholle et al., 2013; Tammaro et al., 2016)	- HMGB1 (Leemans, Kors, Anders, & Florquin, 2014; Yang & Tracey, 2010) - HSP70 (mRNA n.s.) (Liu et al., 2014)* - CD177 (mRNA ↑ interstitial/vascular) (Liu et al., 2014)* - PGLYRP1 (mRNA myeloid cells↑) (Liu et al., 2014)*
Liver	Hepatocellular Carcinoma	- Trem-1 KO: protective (Wu et al., 2012)	- HMGB1 (Wang et al., 2015; Zhou et al., 2014) - HSP70 (mRNA ↑) (Dapito et al., 2012)* - CD177 (mRNA n.s.) (Dapito et al., 2012)* - PGLYRP1 (mRNA ↑) (Dapito et al., 2012)*
Pancreas	Pancreatitis	- LP17: protective (Rat; 1.0 mg) (Dang et al., 2012; Kamei et al., 2010)	- HMGB1 (Yang & Tracey, 2010) - HSP70 (mRNA ↑) (Siveke et al., 2008; Ulmasov, Oshima, Rodriguez, Cox, & Neuschwander-Tetri, 2013)* - CD177 (mRNA n.s.) (Siveke et al., 2008; Ulmasov et al., 2013)* - PGLYRP1 (mRNA n.s.) (Siveke et al., 2008; Ulmasov et al., 2013)
Skin	Psoriasis	- Trem-1 KO: no effect (Joffre, Hau, et al., 2016)	- HMGB1 (mRNA ↓ or n.s.) (Swindell et al., 2011)* (Suarez-Farinas et al., 2013)* - HSP70 (mRNA ↓ or n.s.) (Swindell et al., 2011)* (Suarez-Farinas et al., 2013)* - CD177 (mRNA n.s or (Swindell et al., 2011)* (Suarez-Farinas et al., 2013)* - PGLYRP1 (mRNA ↑) (Swindell et al., 2011)* (Suarez-Farinas et al., 2013)*

n.s.: not significant (or unknown).* Data obtained from Nextbio.

in human, hence mice lacking TREM1/3 receptors bear strong resemblance to the human TREM-1 system regulation *in vivo* (Klesney-Tait et al., 2013; Tammaro et al., 2013). In the context of sterile inflammatory disorders, genetic deletion of TREM1/3 showed a milder protective phenotype following obstructive-induced nephropathy compared to TREM-1 single knockout mice, indicating indeed a potential role for TREM-3 herein (Lo et al., 2014; Tammaro et al., 2013). Additionally, *Trem-1* single KO mice display protection, mostly due to decreased recruitment of inflammatory cells, in models of atherosclerosis, myocardial IR, and hepatocellular carcinoma (Boufenzer et al., 2015; Joffre, Hau, et al., 2016; Joffre, Potteaux, et al., 2016; Wu et al., 2012Zysset et al., 2016). If phenotypes are different in TREM1/3 double KO mice remains to be determined. Given the difference in phenotype observed in the 2 genetic mice, especially in the context of renal fibrosis, one could speculate that knockdown of both receptors *in vivo* might affect the expression of other receptors, located in proximity of the genes, such as TREM-2, known to have opposite functions compared to TREM-1. However, it cannot be excluded that TREM-3 plays any role in inflammation, which remains yet to be elucidated. In addition, differences in phenotype between TREM-1 and TREM1/3 KO mice during renal fibrosis could be related to different operators, different facility and local mice microbiota, with or without genetic background differences (Jenq et al., 2012). We would like to refer to Table 2 for the overview of TREM-1 function in these studies.

5. TREM-1 as a promising therapeutic target in non-infectious disease

Although the pathophysiological role of TREM-1 was first identified during infectious diseases (Bouchon et al., 2000), TREM-1 is, today,

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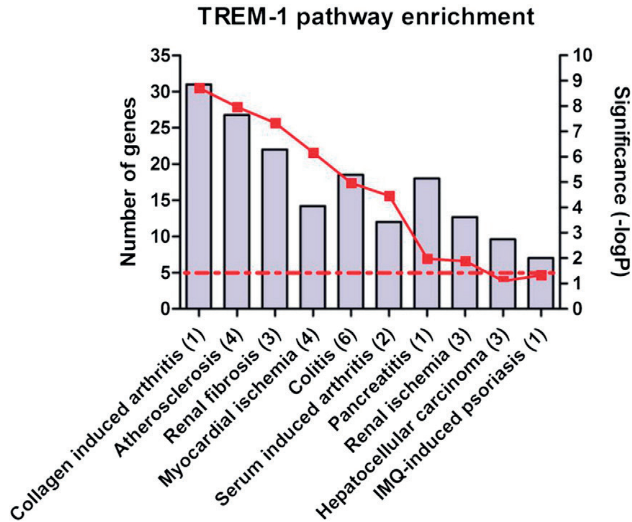

Fig. 3. TREM-1 signaling pathway enrichment in non-infectious diseases. A set of 45 genes related to TREM-1 signaling pathway (provided by Ingenuity® Pathway Analysis, Qiagen; www.ingenuity.com) was uploaded as bioset, and analyzed *in silico* by Nextbio (Illumina; www.nextbio.com). TREM-1 signaling pathway enrichment and number of overlapping genes were determined between the TREM-1 bioset and murine genome datasets related to non-infectious diseases. Number of studies included is displayed in brackets. Purple bar shows average of overlapping genes between TREM-1 bioset and selected genome datasets (× out of 45 genes, left Y-axis). Red line indicates average of significance for TREM-1 signaling pathway enrichment (−LogP; right Y-axis). Red dotted line indicates threshold for significance (−logP = 1.3). See supplementary methods for further details.

believed to play a crucial role in, both acute (ischemia-reperfusion, hemorrhagic shock,pancreatitis) and chronic (inflammatory bowel diseases, rheumatic diseases, atherosclerosis, psoriasis and cystic fibrosis) forms of aseptic inflammation. In line, DAMPs that emerged from the danger theory (Matzinger, 1994) and PAMPs are the primary triggers of non-infectious and infectious inflammation and are both recognized by PRRs. This may explain the similarities observed in the cascade of events occurring during sterile or infectious inflammation, including the activation of TREM-1 as an amplifier of TLRs. In Tables 1 and 2 we summarize current knowledge about TREM-1 expression and function respectively in the non-infectious diseases discussed in this review. In addition, we determined TREM-1 signaling pathway enrichment in several published array datasets related to acute and chronic non-infectious diseases in mice. Based on Ingenuity® Pathway Analysis (IPA, Qiagen; www.ingenuity.com), 45 genes associated to TREM-1 signaling pathway (presented in supplementary Table S1) were uploaded to Nextbio (Illumina; www.nextbio.com) and TREM-1 signaling pathway enrichment was analyzed *in silico*, in available genome datasets curated by Illumina. Relevant selected public datasets used for the analysis are displayed in supplementary Table S2. For comprehensive methods please see the supplementary methods section. Based on *in silico* analysis, we show that TREM-1 signaling pathway is enriched and TREM-1 putative ligands are expressed in multiple non-infectious diseases which suggest that TREM-1 intervention strategies could affect the diseased phenotype through interference with receptor-ligand interaction (Table 2 and Fig. 3). Moreover in Table 3, we describe the association of TREM-1 single nucleotide variance (SNVs) and non-infectious diseases, in humans.

5.1. Acute inflammatory diseases

5.1.1. Ischemia reperfusion (IR)

Reperfusion injury that follows an ischemic event is the consequence of a complex cascade of events that first implicates the components of the innate immune response and subsequently leads to an acute inflammatory reaction. IR-induced cellular and molecular processes show similarities among different organs including the release of DAMPs and inflammatory cytokines (such as HMGB-1, TNFα, IL-1β and IL-6), activation of PRRs and leukocytes influx. Although these

Table 3
Trem-1 polymorphisms in non-infectious disease.

Disease	SNV	Country (population)	Cohort (N)	Effect
CAD	rs2234237 (A/A) rs4711668 (T/T) rs6910730 (G/G) rs9471535 (C/C)	Russia (Caucasian)	CAD (702) Healthy control (300)	Elevated risk (Golovkin et al., 2014)
IBD	rs2234237 (A allele) rs3789205 (G allele) rs9471535 (C allele)	Korea	CD (202) UC (265) BD (138) Ctrl (234)	Elevated risk for BD, not CD or UC (Jung et al., 2011)
Kidney transplantation	rs2234237 (A allele)	Netherlands (Caucasian)	Donor (1260) Recipient (1260)	No effect on DGF, BPAR, graft loss, patient survival (Tammaro et al., 2016)

signals initiate the mechanisms of tissue repair, excessive and prolonged inflammation may lead to deleterious fibrosis and organ dysfunction.

5.1.1.1. Kidney: In a model of renal IR, we and others have shown an increase in TREM-1 expression as well as sTREM-1 in renal tissue 24 h after the event that was mainly due to the recruitment and *in situ* accumulation of TREM-1 expressing granulocytes (Campanholle et al., 2013; Tammaro et al., 2016). In the repair phase following renal IR, Campanholle and colleagues observed an increased expression of TREM-1 in macrophages (Campanholle et al., 2013). While TLR2/4 and MyD88 pathway impairment was associated with a decrease in monocytic TREM-1 expression and displayed beneficial effects, TREM-1 inhibition by the administration of a recombinant soluble form of TREM-1 had no impact on disease severity or macrophage activation (Campanholle et al., 2013). Similarly, we observed no effect of TREM-1 inhibition in the prevention of renal IR induced injury (Tammaro et al., 2016). In addition, we did not observe any association with allograft or host-related TREM-1 SNVs with delayed graft function, generally caused by IR induced injury (Tammaro et al., 2016). Apparently, any pathophysiological contribution of TREM-1 in IR is time-dependent and organ specific.

5.1.1.2. Intestine: During intestinal IR, the *in situ* inflammatory reaction may contribute to ischemic injury in the intestinal epithelium, which in turn might allow for the translocation of bacteria and bacterial products from the gut lumen to the blood compartment, accounting for distant organ failure with high mortality (Harward, Brooks, Flynn, & Seeger, 1993; Oldenburg, Lau, Rodenberg, Edmonds, & Burger, 2004; Panes & Granger, 1998; Swank & Deitch, 1996). In preclinical models, the pharmacological inhibition of TREM-1 by the use of synthetic peptide LP17 was shown to be associated with reduced leukocyte recruitment/activation and systemic inflammatory reaction, thus limiting intestinal injury and bacterial translocation as well as distant organ failure, and finally improving survival (Gibot et al., 2008). These results were confirmed in a similar model by the inhibition of Syk, a component of the TREM-1/DAP12 signaling pathway (Pamuk et al., 2010).

5.1.1.3. Heart: Considerable work has been performed to decipher cellular or molecular targets in myocardial infarction (MI) clinical trials, though with disappointing results (Armstrong & Granger, 2007; Christia & Frangogiannis, 2013; Faxon et al., 2002). Interestingly, the inhibition of TREM-1 is associated with protection against hyper-responsiveness without a complete abrogation of the immune response (Bouchon, Facchetti, Weigand, & Colonna, 2001; Derive et al., 2012). A crucial determinant of infarct healing and scar formation resides into the delicate balance between the type and amount of recruited leukocytes (Libby, Nahrendorf, & Swirski, 2016; Swirski & Nahrendorf, 2013). In a recent preclinical study, we demonstrated that the genetic invalidation and the pharmacological inhibition of TREM-1 using the synthetic peptide LR12 limit the recruitment of inflammatory leukocytes to the infarcted myocardium and their activation (Boufenzer et al., 2015). More precisely, TREM-1 inhibition limited neutrophil recruitment and MCP-1 production, thus reducing the mobilization of Ly6C^{high} inflammatory monocytes to the heart, but without impacting the trafficking of pro-resolving Ly6C^{low} monocytes. This strongly suggests that TREM-1 is a central player in the inflammatory reactions following MI that regulates leukocyte recruitment, both quantitatively and qualitatively. In a more clinically relevant model of cardiogenic shock in mini-pigs, the administration of LR12 improved hemodynamic parameters and cardiac function, concomitantly, with a reduction in infarct size as evaluated by the reduction of circulating creatinine phosphokinase and troponin I within the first hours following reperfusion (Lemarie et al., 2015).

5.1.2. Hemorrhagic shock

Hemorrhagic shock, following major trauma, results in a global IR injury. This may lead to multiple organ failure, a process in which the innate immune system plays a significant role. Numerous studies have shown that the early activation of leukocytes is responsible for the systemic release of inflammatory mediators, leading to multiple organ failure after severe hemorrhagic shock (Hierholzer et al., 1998; Meldrum et al., 1997; Meng, Dyer, Billiar, & Tweardy, 2001). Bacterial and endotoxin translocation also appear to be implicated in the development of organ failure after hemorrhagic shock and resuscitation, *via* the release of HMGB1 and activation of TLR4 of the intestinal epithelium (Benhamou et al., 2009; Kao et al., 2014; Shimizu et al., 2002; Sodhi et al., 2015). Pharmacological inhibition of TREM-1 in rats by the administration of the TREM-1inhibitor, LP17, attenuated the hemodynamic compromise and the development of lactic acidosis, prevented cytokine production, organ dysfunction and, finally, improved survival (Gibot, Massin, et al., 2009).

5.1.3. Pancreatitis

The initial injury in acute pancreatitis is characteristically sterile and results in release of DAMPs and activation of innate immunity. Severe acute pancreatitis can readily progress from a localized inflammation in the pancreas to multiple organ failure. In a mouse model for mild and severe acute pancreatitis, membrane TREM-1 on neutrophils, as well as sTREM-1 plasma levels, were correlated to severity of the model (Liu, Wu, Zhao, Feng, & Wang, 2015). Similar results were observed in patients with acute pancreatitis, in which sTREM-1 plasma levels were higher in non-survivors as compared to survivors and may be a predictive marker of organ failure (Ferat-Osorio et al., 2009; Yasuda et al., 2008). In mice, TREM-1 upregulation in injured major organs was observed in the early phases following acute pancreatitis, and inhibition of TREM1 by LP17, displayed protective effects by lowering aspartate transaminase and blood urea nitrogen (Kamei et al., 2010).

5.2. Chronic inflammatory diseases

5.2.1. Inflammatory bowel disease(IBD)

Innate immune sensors such as TLRs, possibly in collaboration with TREM-1, may be considered as an interface between the intestinal epithelial barrier and innate immunity. TREM-1 appears to be crucially involved in chronic inflammatory bowel diseases and experimental colitis. It was shown to be upregulated in CD14^{high} macrophages from IBD patients. TREM-1 expression could not be detected in macrophages derived from healthy intestine. This expression correlated with an enhanced release of inflammatory mediators such as TNFα, IL-6, IL-8 and MCP-1, and enhanced disease activity (Schenk, Bouchon, Seibold, & Mueller, 2007). In parallel, serum sTREM-1 concentrations are significantly enhanced in patients with IBD (Park et al., 2009). In 2 distinct mouse models of colitis, blocking TREM-1, by LP17 administration attenuated intestinal inflammation and histological alterations. Interestingly, both early and late LP17 treatment showed the same efficacy (Schenk et al., 2007). Trem-1 KO mice displayed significantly attenuated disease that was associated with reduced inflammatory infiltrates and diminished expression of pro-inflammatory cytokines (Weber et al., 2014).

Although the phenotype of TREM-1 SNVs is not yet known, SNVs within the *TREM-1* gene are associated with elevated risk for intestinal Behçet's disease, another form of multisystemic, chronic, relapsing intestinal inflammatory disorder (Jung et al., 2011). These data suggest that TREM-1 may be a potential therapeutic target in chronic intestinal inflammatory diseases.

5.2.2. Rheumatoid arthritis/gout

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune and inflammatory syndrome characterized by the destruction of synovial joints. The synovium of established RA patients is characterized by

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marked synovial intimal lining hyperplasia, increased vascularity and accumulation of inflammatory cells (Smolen, Aletaha, & McInnes, 2016). TREM-1 expression was shown to be increased in $CD45^{+}$/$CD14^{+}$ cells, both in synovial samples from patients with RA and from mice with collagen-II-induced arthritis, but was not detectable in synoviocytes (Collins et al., 2009; Kuai et al., 2009). Its engagement was linked to an increase in inflammatory mediators. Indeed, *in vitro* stimulation of human primary synovial cells, isolated from RA patients (comprising synoviocytes but also infiltrated leukocytes), with an agonistic anti-TREM-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 TREM-1 activation could downregulate excessive chemokine and cytokine production. This was further demonstrated in an experimental model of collagen type II-induced arthritis model in mice: TREM-1 fusion protein or LP17 administration was associated with a sharp reduction in clinical signs, in a dose-dependent manner (Yousuke Murakami et al., 2009). Recently Fan et al. showed that Triplotide, an active component isolated from the *Tripterygium wilfordii* Hook F plant, was able to modulate the expression of several inflammatory pathways including the TREM-1 pathway. This was linked to a reduction in the arthritic score in a rat model of collagen-induced arthritis (Fan et al., 2016).

Gout is a prototype of crystal-induced acute inflammation, caused by the deposition of MonoSodium Urate Monohydrate (MSU) crystals. The innate immune system initiates inflammatory responses through the recognition of MSU crystals as a danger signal (Shi, Evans, & Rock, 2003). In line, phagocytes up-regulate TREM-1 after stimulation with MSU crystals and its engagement synergistically enhanced production of pro-inflammatory cytokines by macrophages such as IL-1β and MCP-1 (Murakami et al., 2006). TREM-1 expression was significantly upregulated among infiltrating cells in a murine air pouch model of MSU crystal-induced acute inflammation (Murakami et al., 2006). In acute gouty arthritis patients, TREM-1 expression was higher in the synovial fluid mononuclear cells than in PBMCs and profound sTREM-1 levels were measured in gout synovial fluid. Interestingly, MSU crystals were able to induce TREM-1 in PBMCs from acute gout patients, but not from chronic gout patients (Lee et al., 2016). These data show that TREM-1 is induced and participates in the amplification of inflammation in acute gouty arthritis.

5.2.3. Fibrosis

Kidney: Renal fibrosis is the hallmark of progressive renal disease and has been investigated in mice using the unilateral ureteral obstruction (UUO) model. During UUO, macrophage and TLR signaling play a role in the infiltration of several inflammatory myeloid cells into the tissue (Leemans et al., 2009; Pulskens et al., 2010). Lo and colleagues demonstrated that TREM-1 was implicated in the polarization of monocytes through classically activated (M1) macrophages, and that UUO-injured kidneys contain a TREM-1-ligand that triggers M1 macrophage differentiation. We, and others, have shown that TREM-1 is highly upregulated in renal tissue following UUO, and that TREM1 KO and TREM-1/3 double KO mice showed less tissue inflammation as compared to WT mice (Lo et al., 2014; Tammaro et al., 2013). In humans, TREM-1 expression on inflammatory cells was observed in obstructive nephropathy which was correlated with disease progression (Lo et al., 2014; Tammaro et al., 2013).

5.2.4. Atherosclerosis

Atherosclerosis is a chronic inflammatory response of the arterial wall initiated by a lesion in the endothelium, which evolve in progressive damage sustained by interaction between lipoproteins, monocyte-derived macrophages and other constituents of the arterial wall. Monocytes-derived macrophages promote atherosclerotic plaque initiation, by differentiation into foam cells, which play a role in further plaque progression and rupture (Libby & Hansson, 2015). TREM-1 controls the production of IL-8 and TNFα in U937 foam cells, suggesting a critical role for TREM-1 in the pathogenesis of atherosclerosis (Wang, Li, & Zhao, 2012). Indeed silencing of TREM-1, by means of shRNA or inhibitory peptides, leads to reduced oxLDL phagocytosis and a reduction in the release of pro-inflammatory mediators by macrophages (Li, Hong, Pan, Lei, & Yan, 2016). Recently, pre-clinical models of atherosclerosis, in unison, confirmed a pro-atherogenic role of TREM-1 receptor (Joffre, Potteaux, et al., 2016; Zysset et al., 2016). The genetic invalidation of TREM-1 in mice models of atherosclerosis (ApoE −/− and Ldlr −/−) was linked to a strong reduction in atherosclerotic plaque size, inflammation and macrophage infiltration, possibly due to decreased CXCR1 expression on circulating non-classical monocyte (Joffre, Potteaux, et al., 2016). This led to a decrease in monocyte recruitment and accumulation in plaques, a decrease in TLR4-initiated pro-inflammatory macrophages response, and decreased foam cell formation (Joffre, Potteaux, et al., 2016; Zysset et al., 2016). Macrophage with TREM-1 deletion or inhibition displayed a pronounced anti-atherogenic phenotype as displayed by CD36 decrease. Targeting TREM-1, according to the authors, would be beneficial as a double hit therapy against both and TLR and CD36 (Joffre, Potteaux, et al., 2016). However, it is still unclear how TREM-1 directly regulates CXCR1 and CD36 pathways in atherosclerosis. Unraveling these mechanisms would set the bases for a next research step.

The role of TREM-1 during atherogenesis is supported by clinical data, showing increased levels of TREM-1 in atheromatous human atherosclerotic lesions, and a significant association between high levels of circulating sTREM-1 and major adverse cardiovascular events in patients with acute myocardial infarction (Boufenzer et al., 2015; Zysset et al., 2016). Additionally, TREM-1 was reported to control plaque vulnerability. Patients with symptomatic plaque display increased TREM-1 expression on dendritic cells, vascular smooth muscle cells and macrophages, possibly as consequence of the inflammatory milieu present in the atheroma. TREM-1 activates MMPs to induce collagen degradation and hence plaque instability (Rai, Rao, Shao, & Agrawal, 2016; Rao, Rai, Stoupa, Subramanian, & Agrawal, 2016a; Rao, Rai, Stoupa, Subramanian, & Agrawal, 2016b). Although these encouraging results strongly suggest TREM-1 involvement in the initiation and progression of atherosclerosis, additional studies are required to determine the exact molecular mechanism underlying protection in absence of TREM-1.

5.2.5. Psoriasis

Psoriasis is a common inflammatory skin disease, of unknown etiology, and dendritic cells (DCs) are thought to play an important role in the pathogenesis of skin lesions (Lowes, Bowcock, & Krueger, 2007). The TREM-1 signaling pathway was identified as the third highest canonical pathway enriched in the transcriptome of inflammatory DCs derived from psoriatic lesions. The *in situ* upregulation of TREM-1 was confirmed by immunohistochemistry on $CD11c^{+}$ DCs and $CD31^{+}$ cells. Moreover, psoriatic patients displayed elevated levels of circulating sTREM-1 (Hyder et al., 2013). In psoriatic patients subjected to a 6 weeks treatment with narrow-band ultraviolet radiation (NB-UVB), the number of TREM-1^{+} dermal cells was increased in non-responder patients. Contrary, a decrease in TREM-1+ dermal cells and mRNA levels, were observed in patients classified as responders to the NB-UVB treatment. In both, responders and non-responders patients, circulating levels of sTREM-1 show no profile differences (Hyder et al., 2013). In an *in vitro* and *ex vivo* model, TREM-1 blockade by TREM-1 fusion protein was linked to a decreased Th17 response, suggesting that TREM-1 blockade could reduce the effect of psoriatic DC activation of Th17 cells (Hyder et al., 2013). However, in a model of imiqimod (IMQ)-induced psoriatic lesion, an acute model of inflammation, TREM-1 deficiency did not induce any difference in T cell infiltration in the lesion as well as pathological features of psoriasis (Joffre, Hau, et al., 2016). In line, *in silico* analysis in Nextbio we show that TREM-1 signaling pathway enrichment was not significant in a dataset of skin tissue from the

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back of IMQ-treated wild type mice (Swindell et al., 2011) (Fig. 2). This question whether TREM-3 might compensate for TREM-1 absence or other TLRs are predominant in this model. Of note, significance for TREM-1 signaling pathway enrichment was highly variable in datasets between different types of murine models for psoriasis that are used, including spontaneous lesions in specific knockout mice, IL-23 or IMQ-induced psoriasis (Suarez-Farinas et al., 2013; Swindell et al., 2011). In many of these murine models for psoriasis, each model displayed similarities but also differences among each other and in comparison to human psoriasis (Swindell et al., 2011). Altogether, these results suggest that future research should be conducted to investigate whether TREM-1 is a potential target in psoriasis.

5.2.6. Cystic fibrosis

Cystic fibrosis (*CF*) results from abnormalities in the gene that codes for the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), which is implicated in the regulation of chloride flux across cell membranes. In this disease, an impaired inflammatory response allows for a huge bacterial colonization of the airways (Cantin, Hartl, Konstan, & Chmiel, 2015; Montgomery, Mall, Kicic, & Stick, 2017). While lung resident macrophages and circulating monocytes from *CF* patients express levels of TLR4/MD2 similar to those of control subjects, TREM-1 expression is profoundly downregulated (Del et al., 2008). These data suggest that monocytes are maintained in a LPS-tolerant state, due to repression of TREM-1, associated with a failure to generate an appropriate inflammatory response (Del et al., 2008; Fresno et al., 2009). The active form of Vitamin D, $1,25(OH)_2D_3$, is known to strongly upregulate TREM-1 expression of in human monocytes and macrophages (Kim et al., 2013). The use of $1,25(OH)_2D_3$ to stimulate airway epithelial cells from patients with *CF* was able to induce TREM-1 expression, without affecting the expression of TREM-2, which is known to antagonize TREM-1, through DAP12 (Rigo et al., 2012). Altogether these studies demonstrate that TREM-1 repression during cystic fibrosis can lead to an impaired response to infection following TLR engagement.

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5.3. Other diseases

5.3.1. Cancer

Numerous studies support the concept that inflammation is a critical component of tumor progression. Tumor cells can use the innate immune system for migration, invasion, angiogenesis, all of which enable metastasis. It has become evident that the inflammatory response observed in or around developing neoplasms can regulate tumor development. Moreover, tumor cells have co-opted some of the signaling molecules of the innate immune system (Coussens & Werb, 2002; van Kempen, de Visser, & Coussens, 2006). TREM-1 was shown to be upregulated on Tumor-Associated Macrophages (TAMs) under the control of COX2/PGE2 axis, and its engagement promotes cells' invasive ability (Ho et al., 2008; Yuan, Mehta, et al., 2014). Levels of sTREM-1 were increased in malignant pleural effusion from patients with non-small cell lung cancer (NSCLC), without infection, making sTREM-1 an independent predictor of patient survival. Moreover, an increased TREM-1 expression in tumor tissue was associated with poor outcome (Ho et al., 2008).

IBD is associated with an increased risk for the development of colorectal and small bowel cancer (Rubin, Shaker, & Levin, 2012). TREM-1 inhibition, by LP17, attenuated colitis-associated tumorigenesis in a murine model (Zhou et al., 2013). Hepatocellular carcinoma (HCC) is a well-known type of inflammation-related cancer. Deletion of *Trem1* in a murine diethylnitrosamine (DEN)-induced hepatocellular carcinogenesis model attenuated Kupffer cell activation and HCC development suggesting that TREM-1 is a pivotal determinant of Kupffer cell activation in liver carcinogenesis (Wu et al., 2012). TREM-1 expression by HCC cells has been confirmed in humans and was shown to increase the migratory ability of cells in culture (Wu et al., 2012), confirming the role of TREM-1 in cancer metastasis. The TREM-1 expression in HCC cells correlated with worse survival (Liao et al., 2012). These data underline the potential therapeutic interest in TREM-1 modulation during cancer in order to prevent tumor progression.

6. Pharmacological TREM-1 inhibitors: a novel therapeutic approach

Several strategies have been developed to inhibit TREM-1 receptor activation using small molecules and peptides. Soluble TREM-1 is used as the blueprint for the development of inhibitory peptides, that work as a scavenger receptors to bind TREM-1 ligands, and thereby prevent TREM-1 activation. Currently, these peptides are in an experimental phase, and some are under modification for potential use in clinical trials. Herein, we discuss the two potential peptides actually tested in preclinical studies.

6.1. LP17

Based on murine and human TREM-1 sequences, a highly conserved sequence was found in the ectodomain and is therefore suitable as a sTREM-1 mimics (Gibot et al., 2004).

This peptide (LQVTDSGLYRCVIYHPP), called LP17, was shown to reduce an inflammatory response in monocytes, induced by LPS or TREM-1 agonistic antibody (Gibot et al., 2004). Further studies in mice show that LPS- and caecal ligation puncture-induced mortality was reduced by LP17 treatment, prompting an interest for clinical application (Gibot, Buonsanti, et al., 2006; Gibot et al., 2004). Since then, the therapeutic potential of LP17 in several infectious and non-infectious diseases has been investigated in experimental models (Table 2).

6.2. LR12

The LR12 peptide is another pharmacological approach to inhibit TREM-1. LR12 (LQEEDAGEYGCM) represents a conserved motif from TREM-like transcript (TLT)-1. TLT-1 is a member of the TREM gene cluster 6p21.1, but more importantly, is expressed exclusively in the platelet lineage and localized in α-granules of resting platelets (Morales et al., 2010). Therefore, TLT-1 is exposed at the membrane upon platelet activation and could promote platelet aggregation by crosslinking fibrinogen (Derive et al., 2012; Morales et al., 2010; Washington et al., 2009). A soluble form of TLT-1 (sTLT-1) was found in the sera of septic shock patients which correlated with mortality (Washington et al., 2009). Moreover, mice lacking TLT-1 (Treml-1 ko mice) were more susceptible to LPS- or caecal ligation puncture-induced inflammation. In these models, administration of the LR12 peptide was able to restore a balanced immune response and improve survival (Derive et al., 2012; Washington et al., 2009). sTLT-1 controls leukocyte activation, and displays immunomodulatory properties through the specific inhibition of TREM-1 (Derive et al., 2012). The TLT-1-derived peptide LR12, was able to reduce the LPS-induced inflammatory responses and to prevent sepsis-induced tissue abnormalities and dysfunctions, which translated into a gain of survival, even in case of administration after the onset of sepsis (Derive et al., 2012). Moreover, pharmacological inhibition of TREM-1, by the use of LR12, was associated with a reduced need for norepinephrine and improved cardiac parameters in a mini-pig model of peritonitis. During experimental monkey endotoxemia, the administration of LR12 attenuated the endotoxin-induced blood pressure decrease and release of several inflammatory cytokines in blood (Derive, Boufenzer, & Gibot, 2014; Derive et al., 2013). In all preclinical models of septic shock, the administration of LR12 was linked to a strong protective effect of the cardiovascular system and an improvement in survival.

Pharmacological inhibition of TREM-1 in preclinical models of non-infectious diseases was also linked to markedly beneficial effects. Indeed, LR12 administration during acute myocardial infarction was able to control leukocyte recruitment to the infarcted area, thus limiting *in situ* inflammation, excessive cardiac remodeling and infarct size

(Boufenzer et al., 2015). Even more interesting, a recent study shows that pharmacological blockade of Trem-1 in ApoE−/− mice, using LR-12 peptide, significantly reduced the development of atherosclerosis throughout the vascular tree, and lessened plaque inflammation (Joffre, Potteaux, et al., 2016). These studies strengthen the hypothesis that LR12 exhibits potent immunomodulatory properties, by inhibiting the TREM-1 inflammatory amplification loop, which dampened, but did not completely abrogate, the inflammatory reaction. Safety and pharmacokinetics of LR12 (www.inotrem.com; MOTREM™, INN: Nangibotide, CAS number 2014384-91-7) are being evaluated in a First-in-Man study before further clinical trials in patients.

7. Conclusion and clinical perspectives

The discovery of the relatively novel TREM family has increased our repertoire of PRRs and understanding of innate immunity. Although no TREM-1 *specific* (endogenous) ligand has been discovered, TREM-1 appears to be activated by several DAMPs that are shared by other PRRs. It is unknown why these ligands, specifically, share TREM-1 activation. Neither it is known what they have in common, but this information could certainly be of use in the determination of new specific ligands.

We now know that TREM-1 is not only involved in infectious disease, but also in several non-infectious diseases. In general, TREM-1 amplifies the inflammatory response triggered by DAMPs, released upon tissue injury or stress. In many cases, TREM-1 intervention proved to be beneficial in non-infectious diseases. However, accumulating data shows that the contribution of TREM-1 in the pathophysiology can be organ or disease specific. Although, in many cases, TREM-1 intervention shows a beneficial effect in murine studies, the precise mechanism that underlies protection is unknown and mechanism-of-action should be further investigated. Several scientific questions are still unanswered and are of interest for the future use of TREM-1 as a target for intervention therapy (see Box 1).

Thus far, TREM-1 inhibitory proteins, such as LP17 and LR12 are a promising class of compounds for the inhibition of unwanted inflammation during several (non)-infectious diseases as shown in several preclinical studies in different murine models. Needless to say, this is only the beginning of the drug development cycle and more research, and money, is needed to go from first-in-man study to further clinical trials. Of the described TREM-1 inhibitors, LR12 is most developed and shown to be well tolerated in human without side effects in a double-blind, randomized, placebo-controlled phase I clinical trial. Currently, LR12 is being investigated in phase I trial for septic shock and acute myocardial infarction (www.inotrem.com). Based on pre-clinical studies, many more applications for LR12 are thinkable. Importantly, the main advantage of pharmacologically targeting TREM-1 over other PRRs, such as TLRs, is that such an approach does not fully abolish the inflammatory response required for a proper immune response against bacteria, pathogenic or not. This feature contributes to the safety for TREM-1 inhibitors in treating inflammatory diseases.

Conflict of interests

Marc Derive and Sebastien Gibot are co-founders of INOTREM, a company developing TREM-1 inhibitors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pharmthera.2017.02.043.

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Supplementary Information

TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives

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Supplementary Methods

TREM-1 signaling pathway enrichment based on Ingenuity Pathway Analysis

Using IPA (Ingenuity® Pathway Analysis, Qiagen; www.ingenuity.com) we obtained a report of 45 genes related to TREM-1 signaling pathway, chosen by Qiagen. This list of genes was uploaded to Nextbio (Illumina; www.nextbio.com) as a bioset, analyzed and compared to public genome data sets curated by Illumina. Obtained studies were further selected based on several filters including species (mus musculus), keywords relative to acute or chronic non-infectious diseases, genetic background (exclusion of knockout animals), RNA profiling performed in the tissue related to specific disease and acceptable sample size. The keywords to identify the studies included ischemia, colitis, arthritis, fibrosis, hepatocellular carcinoma, pancreatitis or atherosclerosis. The source and GSE numbers of the final selected studies are displayed in supplementary Table S2. Among the selected studies, Nextbio analyzed the number of overlapping genes between the TREM-1 bioset and selected genome datasets (x out of 45 genes). Purple bars show the number of genes belonging to TREM-1 signaling pathway which overlap with the gene dataset of each selected study. The significance for the enrichment is calculated using the Fisher's exact test for the overlap between the two datasets. The average of overlapping genes and significance for pathway enrichment was calculated if multiple datasets per disease group were available.

Supplementary Table S1: TREM-1 signaling pathway. Genes related to TREM-1 signaling pathway provided by IPA (Ingenuity® Pathway Analysis, Qiagen; www.ingenuity.com/).

Official symbol	Entrez Gene Name
AKT1	v-akt murine thymoma viral oncogene homolog 1
DEFB4A	defensin beta 4A
CASP1	caspase 1
CASP5	caspase 5
ITGB1	integrin subunit beta 1
FCGR2A	Fc fragment of IgG receptor IIa
FCGR2B	Fc fragment of IgG receptor IIb
FCGR2C	Fc fragment of IgG receptor IIc
CD40	CD40 molecule
ICAM1	intercellular adhesion molecule 1
CD83	CD83 molecule
CD86	CD86 molecule
ITGAX	integrin subunit alpha X
ITGA5	integrin subunit alpha 5
TYROBP	TYRO protein tyrosine kinase binding protein
MAPK3	mitogen-activated protein kinase 3
MAPK1	mitogen-activated protein kinase 1
CSF2	colony stimulating factor 2
GRB2	growth factor receptor bound protein 2
IL6	interleukin 6
CXCL8	chemokine (C-X-C motif) ligand 8
IL10	interleukin 10
IL18	interleukin 18
IL1B	interleukin 1 beta
IRAK1	interleukin 1 receptor associated kinase 1
JAK2	Janus kinase 2
CCL2	chemokine (C-C motif) ligand 2
CCL7	chemokine (C-C motif) ligand 7
CXCL3	chemokine (C-X-C motif) ligand 3
CCL3	chemokine (C-C motif) ligand 3
MPO	myeloperoxidase
MYD88	myeloid differentiation primary response 88
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2
NOD2	nucleotide binding oligomerization domain containing 2
LAT2	linker for activation of T-cells family member 2
SIGIRR	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain
IL1RL1	interleukin 1 receptor like 1
STAT3	signal transducer and activator of transcription 3
STAT5A	signal transducer and activator of transcription 5A
STAT5B	signal transducer and activator of transcription 5B
TLR2	toll-like receptor 2
TLR4	toll-like receptor 4
TNF	tumor necrosis factor
TREM1	triggering receptor expressed on myeloid cells 1

Supplementary Table S2: Datasets selected for TREM-1 signaling pathway enrichment. GSE number of public genome datasets selected for in silico analysis available in Nextbio (Illumina; www.nextbio.com). Available studies were filtered by species (mus musculus), keywords related to specific disease, genetic background (exclusion of knockout animals), acceptable sample size and when RNA profiling was done in tissue related to specific disease. GSE: Genomic Spatial Event database

Tissue	Disease	GSE
Heart	Myocardial ischemia	GSE775
		GSE4105
		GSE23294
		GSE19322
Circulation	Atherosclerosis	GSE21419
		GSE38574
		GSE40156
		GSE10000
Intestine	IBD / Colitis	GSE9293
		GSE18163
		GSE64658
		GSE36806
		GSE9281
		GSE19793
Joint	Collagen-induced arthritis	GSE13071
	Serum-induced arthritis	GSE22971
		GSE27492
Kidney	Renal fibrosis	GSE36496
		GSE38117
		GSE55808
	Renal ischemia	GSE39548
		GSE52004
		GSE34351
Liver	Hepatocellular carcinoma	GSE32244
		GSE50431
		GSE37129
		GSE44356
		GSE33446
Pancreas	Pancreatitis	GSE41418
Skin	Imiquimod-induced Psoriasis	GSE2768

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