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(54) Title: THERAPEUTIC PEPTIDES COMPRISING SEQUENCES DERIVED FROM CDR2 OR CDR3 OF TREM-1 AND USES THEREOF TO INHIBIT SEPSIS

(57) Abstract: A polypeptide comprising one or more sequences derived from CDR2 or CDR3 of a TREM-1 protein, characterised by the ability to treat, ameliorate, or lessen the symptoms of sepsis, septic shock or sepsis-like conditions.



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THERAPEUTIC PEPTIDES COMPRISING SEQUENCES DERIVED FROM CDR2 OR CDR3 OF TREM-1
AND USES THEREOF TO INHIBIT SEPSIS

The present invention relates to the field of immunology. More particularly, the present invention relates to inflammation and the use of proteins and peptides containing certain
5 sequences of the TREM-1 protein and their functional equivalents (referred to herein as TREM1-peptides) in the treatment of disease, for example, sepsis and septic shock.

Sepsis constitutes a significant consumption of intensive care resources and remains an ever-present problem in the intensive care unit. It has been estimated that between 400 000 and 500 000 patients are so affected each year in both the USA and Europe. Morbidity and mortality
10 have remained high despite improvements in both supportive and anti-microbial therapies. Mortality rates vary from 40% for uncomplicated sepsis to 80% in those suffering from septic shock and multi-organ dysfunction. The pathogenesis of the conditions is now becoming better understood. Greater understanding of the complex network of immune, inflammatory and haematological mediators may allow the development of rational and novel therapies.

Following an infection, innate and cognitive immune responses develop in sequential phases that build-up in specificity and complexity, resulting ultimately in the clearance of infectious agents and restoration of homeostasis. The innate immune response serves as the first line of defence and is initiated upon activation of pattern recognition receptors, such as Toll-like receptors (TLRs) (1, 2), by various pathogen-associated microbial patterns (PAMPs) (3).
20 Activation of the TLRs triggers the release of large quantities of such cytokines as TNF- α and IL-1 β , which, in case of such massive infections as sepsis, can precipitate tissue injury and lethal shock (4, 5). Although antagonists of TNF- α and IL-1 β appeared in this context as possibly interesting therapeutic agents of sepsis, they have unfortunately shown limited efficacy in clinical trials (6-8). This could be due to the fact that these cytokines are necessary for the
25 clearance of infections, and that their removal would allow for fatal bacterial growth (9-11).

Another receptor involved in, inter alia, response to infection, triggering receptor expressed on myeloid cells-1 (TREM-1) is a member of a recently discovered family of receptors, the TREM family, expressed on the surface of neutrophils and a subset of monocytes. TREM receptors activate myeloid cells via association with the adaptor molecule
30 DAP12. Engagement of TREM-1 has been reported to trigger the synthesis of pro-inflammatory cytokines in the presence of microbial products.

The triggering receptor expressed on myeloid cells (TREM)-1 is a recently discovered cell-surface molecule that has been identified both on human and murine polymorphonuclear neutrophils and mature monocytes (12). It belongs to the immunoglobulin superfamily and
35 activates downstream signalling pathways with the help of an adapter protein called DAP12 (12-15). Bouchon and co-workers have shown that the expression of TREM-1 was greatly up-regulated on neutrophils and monocytes in the presence of such bacteria as *Pseudomonas*

aeruginosa or *Staphylococcus aureus*, both in cell culture and in tissue samples from patients with infection (16). In striking contrast, TREM-1 was not up-regulated in samples from patients with non-infectious inflammatory diseases such as psoriasis, ulcerative colitis or vasculitis caused by immune complexes (16). Moreover, when TREM-1 is bound to its ligand, there is a synergistic effect of LPS and an amplified synthesis of the pro-inflammatory cytokines TNF- α and GM-CSF, together with an inhibition of IL-10 production (17). In a murine model of LPS-induced septic shock, blockade of TREM-1 signalling protected the animals from death, further highlighting the crucial role of this molecule (13, 16).

Recent studies demonstrate that TREM-1 plays a critical role in the inflammatory response to infection (see BOUCHON et al. (2000) J. Immunol. 164:4991-4995). Expression of TREM-1 is increased on myeloid cells in response to both bacterial and fungal infections in humans. Similarly, in mice the induction of shock by lipopolysaccharide (LPS) is associated with increased expression of TREM-1. Further, treatment of mice with a soluble TREM-1/Ig fusion protein, as a 'decoy' receptor, protects mice from death due to LPS or E.coli.

US 6,420,526 entitled "186 Secreted Proteins" claims unspecified and unexemplified isolated fragments of TREM-1 containing at least 30 contiguous amino acids of human TREM-1. No biological data relating to such fragments are provided.

As described in US2003165875A, fusion proteins between human IgG1 constant region and the extracellular domain of mouse TREM-1 or that of human TREM-1 show an effect against endotoxemia in mice.

The inventors have surprisingly found that certain peptides derived from the TREM-1 protein are capable of acting as antagonists of the TREM-1 protein and therefore have applications in the treatment of sepsis and septic shock. The Inventors further demonstrate that the same peptides also modulate *in vivo* the pro-inflammatory cascade triggered by infection, thus inhibiting hyper-responsiveness and death in an animal model of sepsis.

Previously, the Inventors have identified a soluble form of TREM-1 (sTREM-1) and observed significant levels in serum samples from septic shock patients but not controls. As also described herein the Inventors have investigated its putative role in the modulation of inflammation during sepsis (see Gibot et al. (2004) Ann. Intern. Med. 141(1):9-15 and Gibot et al. (2004) N. Engl. J. Med. 350(5):451-8).

As described herein the Inventors show that a soluble form of TREM-1 (sTREM-1) is released in the peripheral blood during infectious aggression in mouse. The Inventors also confirm monocytes as a major source of sTREM, and show that synthetic peptides mimicking a part of the extra-cellular domain of TREM-1 can modulate cytokine production by activated monocytes *in vitro*.

The Inventors have observed that sTREM-1 is secreted by monocytes activated *in vitro* by LPS, as well as in the serum of animals involved in an experimental model of septic shock.

Both *in vitro* and *in vivo*, synthetic peptides mimicking a short highly conserved domain of sTREM-1 attenuate cytokine production by human monocytes and protect septic animals from hyper-responsiveness and death. These peptides are efficient not only in preventing but also in down-regulating the deleterious effects of pro-inflammatory cytokines. These data demonstrate that *in vivo* modulation of TREM-1 by TREM-1 peptides is a valuable therapeutic tool for the treatment of infection, for example sepsis or septic shock or for the treatment of sepsis-like conditions

Accordingly, the present invention provides methods and compositions for the treatment of infectious disease, in particular, sepsis and septic shock or for the treatment of sepsis-like conditions

As described herein, the Inventors have determined that several peptides of the extracellular portion of the TREM-1 protein (see Table 1), which incorporate sequences from "CDR2" and "CDR3" surprisingly have activity similar to previously described fusion proteins of IgG1 constant region and the extracellular domain of TREM-1 in models of sepsis. These peptides also have advantages over the protein particularly in terms of cost of manufacture.

Thus, the invention provides polypeptides comprising one or more sequences derived from CDR2 or CDR3 of a TREM-1 protein. Preferably, said polypeptides comprise less than 30 contiguous amino acids of said TREM-1 protein.

As shown in Table 1, examples of such peptides or polypeptides, contain or comprise for example 15-25 amino acid ("AA") peptides from the TREM-1 protein and contain or comprise all or part of a CDR domain (3-6 AAs) of the receptor flanked by natural sequences from the protein that can vary in length so long as function of the CDR-like domain is not lost. Such peptides are derived from the TREM-1 receptor protein amino acid sequence for example, as shown in Table 2 (human) and Table 3 (mouse).

Table 1 shows peptides derived from mouse TREM-1 "mPX" (NCBI Reference Sequences (RefSeq) NP_067381) or human TREM-1 "hPX" (NCBI Reference Sequences (RefSeq) NP_061113). Underlined amino acids span the human TREM-1 Complementarity Determining Regions (CDR), as described by Radaev et al. 2003 Structure (Camb.) 11 (12), 1527-1535 (2003).

Table 2 shows the human TREM-1 amino acid sequence NP_061113. Underlined amino acids span the human TREM-1 Complementarity Determining Regions (CDR) 2 (RPSKNS; [SEQ ID NO:20]) and 3 (QPPKE [SEQ ID NO:21]), as described by Radaev et al. 2003 Structure (Camb.) 11 (12), 1527-1535 (2003).

Table 3 shows the mouse TREM-1 amino acid sequence NP_067381. Underlined amino acids span the mouse TREM-1 Complementarity Determining Regions (CDR) 2 (RPFTRP; [SEQ ID NO:22]) and 3 (HPPND; [SEQ ID NO:23]).

Table 1. Peptides including sequences from human and mouse TREM-1 CDR 2 and CDR 3

hCDR 2	
mP1 (67-89) : [SEQ ID NO:3]	LVVTQRPFTRPSEVHMGKFTLKH
hP1 (67-89) : [SEQ ID NO:16]	LACTERPSKNSHPVQVGRILED
hCDR 3	
mP2 (114-136) : [SEQ ID NO:4]	VIYHPPNDPVVLFHPVRLVVTKG
mP4 (103-123) : [SEQ ID NO:6]	LQVTDGSLYRCVIYHPPNDPV
mP5 (103-119) : [SEQ ID NO:7]	LQVTDGSLYRCVIYHPP
hP2 (114-136) : [SEQ ID NO:17]	VIYQPPKEPHMLFDRIRLVVTKG
hP4 (103-123) : [SEQ ID NO:18]	LQVEDSGLYQCVIYQPPKEPH
hP5 (103-119) : [SEQ ID NO:19]	LQVEDSGLYQCVIYQPP

Table 2 Human TREM-1 amino acid sequence NP_061113

1	MRKTRLWGLL	WMLFVSELRA	ATKLTEEKYE	LKEGQTLDVK	CDYTLEKFAS	SQKAWQIIRD
61	GEMPKTLACT	ERPSKNSHPV	QVGRILEDY	HDHGLLRVM	VNLQVEDSGL	YQCVIYQPPK
121	EPHMLFDRIR	LVVTGFGSGT	PGSNENSTQN	VYKIPPTTK	ALCPLYTSPR	TVTQAPPKST
181	ADVSTPDSEI	NLTNVTDIIR	VPVFNIVILL	AGGFLSKSLV	FSVLFAVTLR	SFVP
	[SEQ ID NO:1]					

5 Table 3 Mouse TREM-1 amino acid sequence NP_067381

1	MRKAGLWGLL	CVFFVSEVKA	AIVLEEERYD	LVEGQTLTVK	CPFNIMKYAN	SQKAWQRLPD
61	GKEPLTLVVT	QRPFTRPSEV	HMGKFTLKHD	PSEAMLQVQM	TDLQVTDGSL	YRCVIYHPPN
121	DPVVLVFPVR	LVVTGSSDV	FTPVIIPITR	LTERPILITT	KYSPSDTTT	RSLPKPTAVV
181	SSPGLGVTII	NGTDADSVST	SSVTISVICG	LLSKSLVFII	LFIVTKRTFG	[SEQ ID NO:2]

Accordingly, the invention provides isolated or recombinantly prepared polypeptides or peptides comprising or consisting essentially of one or more sequences derived from CDR2 or CDR3 of a TREM-1 protein, or fragments, homologues, derivatives, fusion proteins or variants of such polypeptides, as defined herein, which are herein collectively referred to as "polypeptides or peptides of the invention" or "TREM-1 peptides or TREM-1 polypeptides", preferably such entities comprise less than 30 contiguous amino acids of a TREM-1 protein, for example as shown in Table 2 or Table 3. Generally where polypeptides or proteins of the invention or fragments, homologues, derivatives, or variants thereof are intended for use (for

example treatment) in a particular species, the sequences of CDR2 or CDR3 of a TREM-1 protein are chosen from the TREM-1 protein amino acid sequence of that species, or if the sequence is not known, an analogous species. For example, polypeptides or proteins of the invention for the treatment of human disease, in particular sepsis, septic shock or sepsis-like conditions, will comprise one or more sequences comprising all or part of CDR2 or CDR3 from the human TREM-1 protein.

Furthermore, the invention provides isolated polypeptides or proteins comprising an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:20, 21, 22, 23 or fragments, homologues, derivatives, or variants thereof. The invention also provides isolated peptides, polypeptides or proteins comprising an amino acid sequence that comprises or consists of at least about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 or more contiguous amino acids of a TREM-1 protein of which 3 or more contiguous amino acids are derived from the sequences of SEQ ID NO:20, 21, 22 or 23 (in other words a sequence representing all, or part of CDR2 or CDR3 of a TREM-1 protein is present in the peptide, polypeptide or protein), or fragments, homologues, derivatives, or variants thereof. In preferred embodiments, such peptides, polypeptides or proteins, or fragments, homologues, derivatives or variants thereof have a biological activity of a TREM-1 full-length protein, such as antigenicity, immunogenicity, triggering of proinflammatory chemokines and cytokines, mobilization of cytosolic Ca^{2+} , protein tyrosine-phosphorylation, mediator release, and other activities readily assayable. Generally, such peptides, polypeptides or proteins or fragments, homologues, derivatives or variants thereof are capable of treating sepsis, septic shock or sepsis-like conditions, or are active in experimental models of sepsis, septic shock or sepsis-like conditions, for example by acting as antagonists of the activity of the TREM-1 receptor. Such peptides, polypeptides or proteins or fragments, homologues, derivatives or variants thereof are characterised by the ability to treat, ameliorate, or lessen the symptoms of sepsis, septic shock or sepsis-like conditions.

In particular, the invention provides, a TREM-1 polypeptide having activity against sepsis, septic shock or sepsis-like conditions which consists of (i) a contiguous sequence of 5 to 29, for example 15-25, amino acids corresponding to the native TREM-1 protein sequence which includes at least 3 amino acids from the CDR2 or CDR3 sequences; or (ii) such a sequence in which one or more amino acids are substituted conservatively with another amino acid provided, however that at least 3 amino acids from the CDR2 or CDR3 sequences are not substituted; or (iii) a sequence of (i) or (ii) linked at one or both of its N and C termini to a heterologous polypeptide. For example, in a polypeptide wherein the native TREM-1 protein sequence is the human sequence identified as [SEQ ID NO: 1], the CDR2 and CDR3 sequences are RPSKNS and QPPKE respectively. In such polypeptides, the at least 3 amino

acids from the CDR2 or CDR3 sequences can be QPP, PPK, PKE, RPS, PSK, SKN or KNS. Such polypeptides may comprise the sequence QPPK, QPPKE or RPSKNS. For example, in a polypeptide wherein the native TREM-1 protein sequence is the mouse sequence identified as [SEQ ID NO: 2] the CDR2 and CDR3 sequences are RPFTRP and HPPND respectively. In
5 such polypeptides, the at least 3 amino acids from the CDR2 or CDR3 sequences can be HPP, PPN, PND, RPF, PFT, FTR or TRP. Such polypeptides may comprise the sequences HPP, HPPN, HPPND or RPFTRP.

In certain embodiments, the polypeptide of the invention is or comprises SEQ ID No. 7 which is disclosed in Gibot et al (2004) J Exp Med 200, 1419-1426..

10 In certain embodiments the polypeptide of the invention neither is nor comprises SEQ ID No. 7.

In certain embodiments the polypeptide of the invention is or comprises a sequence selected from SEQ ID Nos. 3, 4 and 6.

15 In certain embodiments the polypeptide of the invention is or comprises a sequence selected from SEQ ID Nos. 16, 17, 18 and 19.

In certain embodiments the polypeptide of the invention is or comprises a sequence derived from CDR2.

In certain embodiments the polypeptide of the invention is or comprises a sequence derived from CDR3.

20 The polypeptides or peptides of the invention are provided for use in therapy, in particular in the treatment of sepsis, septic shock and sepsis-like conditions, and for use in the manufacture of a medicament for the treatment of sepsis, septic shock and sepsis-like conditions. Further provided are compositions and pharmaceutical compositions containing polypeptides or peptides of the invention and methods of treatment of sepsis, septic shock and
25 sepsis-like conditions using polypeptides or peptides of the invention. In addition the polypeptides or peptides of the invention are provided for use in therapy to restore haemodynamic parameters in sepsis, septic shock and sepsis-like conditions and for use in the manufacture of a medicament for the treatment of aberrant haemodynamic parameters in sepsis, septic shock and sepsis-like conditions.

30 The term "triggering receptor expressed on myeloid cells" or "TREM" refers to a group of activating receptors which are selectively expressed on different types of myeloid cells, such as mast cells, monocytes, macrophages, dendritic cells (DCs), and neutrophils, and may have a predominant role in immune and inflammatory responses. TREMs are primarily transmembrane glycoproteins with a Ig-type fold in their extracellular domain and, hence, belong to the Ig-SF.
35 These receptors contain a short intracellular domain, but lack docking motifs for signaling mediators and require adapter proteins, such as DAP12, for cell activation.

The term "myeloid cells" as used herein refers to a series of bone marrow-derived cell lineages including granulocytes (neutrophils, eosinophils, and basophils), monocytes, macrophages, and mast cells. Furthermore, peripheral blood dendritic cells of myeloid origin, and dendritic cells and macrophages derived in vitro from monocytes in the presence of
5 appropriate culture conditions, are also included.

The term "sepsis, septic shock" or "sepsis or septic shock" as defined herein, refers to sub-groups of systemic inflammatory response syndrome (SIRS). The term "sepsis" is generally reserved for SIRS when infection is suspected or proven. A pattern of physiological variables have been shown in critically ill patients in response to a range of insults including; trauma,
10 burns, pancreatitis and infection. These include inflammatory responses, leucocytosis or severe leucopaenia, hyperthermia or hypothermia, tachycardia and tachypnoea and have been collectively termed the systemic inflammatory response syndrome (SIRS). This definition emphasises the importance of the inflammatory process in these conditions regardless of the presence of infection. Sepsis is further stratified into severe sepsis when there is evidence of
15 organ hypoperfusion, made evident by signs of organ dysfunction such as hypoxaemia, oliguria, lactic acidosis or altered cerebral function. "Septic shock" is severe sepsis usually complicated by hypotension, defined in humans as systolic blood pressure less than 90 mmHg despite adequate fluid resuscitation. Sepsis and SIRS may be complicated by the failure of two or more organs, termed multiple organ failure (MOF), due to disordered organ perfusion and
20 oxygenation. In addition to systemic effects of infection, a systemic inflammatory response may occur in severe inflammatory conditions such as pancreatitis and burns. The appearance of signs of an inflammatory response is less well defined following traumatic insults. In the intensive care unit, gram-negative bacteria are implicated in 50 to 60% of sepsis cases with gram-positive bacteria accounting for a further 35 to 40% of cases. The remainder of cases are
25 due to the less common causes of fungi, viruses and protozoa.

The term "sepsis-like conditions" as used herein refers to those states in which a patient presents with symptoms similar to sepsis or septic shock but where an infectious agent is not the primary or initial cause of a similar cascade of inflammatory mediators and/or change in haemodynamic parameters as seen in cases of sepsis, for example in patients with acute or
30 chronic liver failure (see Wasmuth HE, et al. *J Hepatol.* 2005 Feb;42(2):195-201), in cases of post-resuscitation disease after cardiac arrest (see Adrie C et al. *Curr Opin Crit Care.* 2004 Jun;10(3):208-12) in the treatment of sepsis-like symptoms after cancer chemotherapy (see Tsuji E et al. *Int J Cancer.* 2003 Nov 1;107(2):303-8) in patients undergoing hyperthermic isolated limb perfusion with recombinant TNF-alpha or similar treatments (see Zwaveling JH et al. *Crit Care Med.* 1996 May;24(5):765-70) or sepsis-like illness in neonates (see Griffin MP et al. *Pediatr Res.* 2003 Jun;53(6):920-6).

The term "activity against sepsis, septic shock or sepsis-like conditions" as used herein refers to the capability of a molecule, for example a peptide, polypeptide or engineered antibody, to treat sepsis, septic shock or sepsis-like conditions, or be active in experimental models of sepsis, septic shock or sepsis-like conditions, for example by acting as an antagonist
5 of the activity of the TREM-1 receptor.

Typically the indication for polypeptides of the invention is sepsis or septic-shock.

The term "substantial sequence identity", when used in connection with peptides/amino acid sequences, refers to peptides/amino acid sequences which are substantially identical to or similar in sequence, giving rise to a homology in conformation and thus to similar biological
10 activity. The term is not intended to imply a common evolution of the sequences.

Typically, peptides/amino acid sequences having "substantial sequence identity" are sequences that are at least 50%, more preferably at least 80%, identical in sequence, at least over any regions known to be involved in the desired activity. Most preferably, no more than five residues, other than at the termini, are different. Preferably, the divergence in sequence, at
15 least in the aforementioned regions, is in the form of "conservative modifications"

To determine the percent sequence identity of two peptides/ amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for
20 comparison purposes). For example, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the first amino acid sequence which has for example 100 amino acid residues, at least 30, preferably at least 40,
25 more preferably at least 50, even more preferably at least 60, and even more preferably at least 70, 80 or 90 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position
30 (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences
35 can be accomplished using a mathematical algorithm. In one embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the

GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4, and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at

5 <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80, and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*CABIOS*, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12, and a

10 gap penalty of 4. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to identify, for example, other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100,

15 wordlength = 12 to obtain nucleotide sequences homologous to NIP2b, NIP2cL, and NIP2cS nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to NIP2b, NIP2cL, and NIP2cS protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997)

20 *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The terms "protein" and "polypeptide" are used interchangeably herein. The term "peptide" is used herein to refer to a chain of two or more amino acids or amino acid analogues

25 (including non-naturally occurring amino acids), with adjacent amino acids joined by peptide (-NHCO-) bonds. Thus, the peptides of the invention include oligopeptides, polypeptides, proteins, mimetopes and peptidomimetics. Methods for preparing mimetopes and peptidomimetics are known in the art.

The terms "mimetope" and "peptidomimetic" are used interchangeably herein. A

30 "mimetope" of a compound X refers to a compound in which chemical structures of X necessary for functional activity of X have been replaced with other chemical structures which mimic the conformation of X. Examples of peptidomimetics include peptidic compounds in which the peptide backbone is substituted with one or more benzodiazepine molecules (see *e.g.*, James, G.L. *et al.* (1993) *Science* 260:1937-1942) and "retro-inverso" peptides (see U.S. Patent No.

35 4,522,752 to Sisto). The terms "mimetope" and "peptidomimetic" also refer to a moiety, other than a naturally occurring amino acid, that conformationally and functionally serves as a substitute for a particular amino acid in a peptide-containing compound without adversely

interfering to a significant extent with the function of the peptide. Examples of amino acid mimetics include D-amino acids. Peptides substituted with one or more D-amino acids may be made using well known peptide synthesis procedures. Additional substitutions include amino acid analogues having variant side chains with functional groups, for example, *b*-cyanoalanine, 5 canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxyphenylalanine, 5-hydroxytryptophan, 1-methylhistidine, or 3-methylhistidine

As used herein an "analogue" of a compound X refers to a compound which retains chemical structures of X necessary for functional activity of X, yet which also contains certain chemical structures which differ from X. An example of an analogue of a naturally-occurring peptide is a peptide which includes one or more non-naturally-occurring amino acids. The term "analogue" is also intended to include modified mimetopes and/or peptidomimetics, modified peptides and polypeptides, and allelic variants of peptides and polypeptides. Analogues of a peptide will therefore produce a peptide analogue that is substantially homologous or, in other words, has substantial sequence identity to the original peptide. The term "amino acid" includes its art recognized meaning and broadly encompasses compounds of formula I:



Preferred amino acids include the naturally occurring amino acids, as well as synthetic derivatives, and amino acids derived from proteins, *e.g.*, proteins such as casein, *i.e.*, casamino acids, or enzymatic or chemical digests of, *e.g.*, yeast, an animal product, *e.g.*, a meat digest, or a plant product, *e.g.*, soy protein, cottonseed protein, or a corn steep liquor (see, *e.g.*, Traders' Guide to Fermentation Media, Traders Protein, Memphis, TN (1988), Biotechnology: A Textbook of Industrial Microbiology, Sinauer Associates, Sunderland, MA (1989), and Product Data Sheet for Corn Steep Liquor, Grain Processing Corp., IO).

The term "naturally occurring amino acid" includes any of the 20 amino acid residues which commonly comprise most polypeptides in living systems, rarer amino acids found in fibrous proteins (*e.g.*, 4-hydroxyproline, 5-hydroxylysine, -N-methyllysine, 3-methylhistidine, desmosine, isodesmosine), and naturally occurring amino acids not found in proteins (*e.g.*, -aminobutyric acid, homocysteine, homoserine, citrulline, ornithine, canavanine, djenkolic acid, and -cyanoalanine).

The term "side chain of a naturally occurring amino acid" is intended to include the side chain of any of the naturally occurring amino acids, as represented by R in formula I. One skilled in the art will understand that the structure of formula I is intended to encompass amino

acids such as proline where the side chain is a cyclic or heterocyclic structure (*e.g.*, in proline R group and the amino group form a five-membered heterocyclic ring.

The term "homologue," as used herein refers to any member of a series of peptides or polypeptides having a common biological activity, including antigenicity/immunogenicity and inflammation regulatory activity, and/or structural domain and having sufficient amino acid as defined herein. Such homologues can be from either the same or different species of animals.

The term "variant" as used herein refers either to a naturally occurring allelic variation of a given peptide or a recombinantly prepared variation of a given peptide or protein in which one or more amino acid residues have been modified by amino acid substitution, addition, or deletion.

The term "derivative" as used herein refers to a variation of given peptide or protein that are otherwise modified, *i.e.*, by covalent attachment of any type of molecule, preferably having bioactivity, to the peptide or protein, including non-naturally occurring amino acids.

Preferably, such homologues, variants and derivatives are capable of treating of sepsis, septic shock or sepsis-like conditions, or are active in experimental models of sepsis, septic shock or sepsis-like conditions, for example by acting as antagonists of the activity of the TREM-1 receptor.

An "isolated" or "purified" peptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized.

The language "substantially free of cellular material" includes preparations of a polypeptide/protein in which the polypeptide/protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a polypeptide/protein that is substantially free of cellular material includes preparations of the polypeptide/protein having less than about 30%, 20%, 10%, 5%, 2.5%, or 1%, (by dry weight) of contaminating protein. When the polypeptide/protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When polypeptide/protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the polypeptide/protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than polypeptide/protein fragment of interest. In a preferred embodiment of the present invention, polypeptides/proteins are isolated or purified.

In addition to the polypeptides described above, polypeptides of the invention also encompass those polypeptides having a common biological activity and/or structural domain and having sufficient amino acid identity (homologues) as defined herein. These homologues

can be from either the same or different species of animal, preferably from mammals, more preferably from rodents, such as mouse and rat, and most preferably from human. Preferably, they exhibit at least one structural and/or functional feature of TREM-1, and are preferably, capable of treating sepsis, septic shock or sepsis-like conditions, for example by acting as
5 antagonists of the activity of the TREM-1 receptor. Such modifications include amino acid substitution, deletion, and/or insertion. Amino acid modifications can be made by any method known in the art and various methods are available to and routine for those skilled in the art.

Additionally, in making amino acid substitutions, generally the amino acid residue to be substituted can be a conservative amino acid substitution (i.e. "substituted conservatively"), for
10 example, a polar residue is substituted with a polar residue, a hydrophilic residue with a hydrophilic residue, hydrophobic residue with a hydrophobic residue, a positively charged residue with a positively charged residue, or a negatively charged residue with a negatively charged residue. Moreover, generally, the amino acid residue to be modified is not highly or completely conserved across species and/or is critical to maintain the biological activities of the
15 peptide and/or the protein it derives from.

Peptides of the invention may be directly synthesised in any convenient way. Generally the reactive groups present (for example amino, thiol and/or carboxyl) will be protected during overall synthesis. A proportion of the peptides of the invention, i. e. those wherein the comprised amino acids are genetically coded amino acids, will be capable of being expressed in
20 prokaryotic and eukaryotic hosts by expression systems well known to the man skilled in the art. Methods for the isolation and purification of e. g. microbially expressed peptides are also well known. Polynucleotides which encode these peptides of the invention constitute further aspects of the present invention. As used herein, "polynucleotide" refers to a polymer of
25 deoxyribonucleotides or ribonucleotides, in the form of a separate fragment or as a component of a larger construct, e. g. an expression vector such as a plasmid. Polynucleotide sequences of the invention include DNA, RNA and cDNA sequences. Due to the degeneracy of the genetic code, of course more than one polynucleotide is capable of encoding a particular peptide according to the invention. When a bacterial host is chosen for expression of a peptide, it may be necessary to take steps to protect the host from the expressed anti-bacterial peptide. Such
30 techniques are known in the art and include the use of a bacterial strain which is resistant to the particular peptide being expressed or the expression of a fusion peptide with sections at one or both ends which disable the antibiotic activity of the peptide according to the invention. In the latter case, the peptide can be cleaved after harvesting to produce the active peptide. If the peptide incorporates a chemical modification then the activity/stability of the expressed peptide
35 may be low, and is only modulated by post-synthetic chemical modification

Furthermore, the invention also encompasses derivatives of the polypeptides of the invention. For example, but not by way of limitation, derivatives may include peptides or

proteins that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific
5 chemical cleavage, acetylation, formylation, etc. Additionally, the derivative may contain one or more non-classical amino acids. Those skilled in the art will be aware of various methods for modifying peptides to increase potency, prolong activity and/or increase half-life. In one example (WO0210195) the modification is made via coupling through an amide bond with at least one conformationally rigid substituent, either at the N-terminal of the peptide, the C-
10 terminal of the peptide, or on a free amino or carboxyl group along the peptide chain. Other examples of peptide modifications with similar effects are described, for example, in WO2004029081, WO03086444, WO03049684, WO0145746, WO0103723 and WO9101743.

The invention further provides antibodies that comprise a peptide or polypeptide of the invention or that mimic the activity of peptides or polypeptides of the invention. Such antibodies
15 include, but are not limited to: polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs, and fragments containing either a VL or VH domain or even a complementary determining region (CDR) that specifically binds to a polypeptide of the invention. In another embodiment, antibodies can also be generated using various phage
20 display methods known in the art. Techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax, et al., *BioTechniques*, 12(6):864-869, 1992; and Sawai, et al., 1995, *AJRI* 34:26-34; and Better, et al., 1988, *Science* 240:1041-1043 (each of which is incorporated by reference in its entirety). Examples of techniques that can be used to
25 produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston, et al., 1991, *Methods in Enzymology* 203:46-88; Shu, et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:7995-7999; and Skerra, et al., 1988, *Science* 240:1038-1040. For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a
30 molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art. See, e.g., Morrison, 1985, *Science* 229:1202; Oi, et al., 1986, *BioTechniques* 4:214; Gillies, et al., 1989, *J. Immunol. Methods* 125:191-202; U.S. Patent Nos.
35 5,807,715; 4,816,567; and 4,816,397; which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-

human species and framework regions from a human immunoglobulin molecule or in the case of the present invention, one or more CDRs derived from a TREM-1 protein. As known in the art, framework residues in the human framework regions can be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modelling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., Queen, et al., U.S. Patent No. 5,585,089; Riechmann, et al., 1988, Nature 332:323, 1988, which are incorporated herein by reference in their entireties.

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Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka, et al., 1994, Protein Engineering, 7(6):805-814; Roguska, et al., 1994, Proc Natl. Acad. Sci. USA 91:969-973, and chain shuffling (U.S. Patent No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety. Human antibodies can also be produced using transgenic mice (see Lonberg and Huszar (1995), Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598; which are incorporated by reference herein in their entireties. In addition, companies such as Abgenix, Inc. (Freemont, CA), Medarex (NJ) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., 1988, Bio/technology 12:899-903). Antibodies fused or conjugated to heterologous polypeptides may be used in in vitro immunoassays and in purification methods (e.g., affinity chromatography) well known in the art.

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See, e.g., PCT publication Number WO 93/21232; EP 439,095; Naramura, et al., 1994, Immunol. Lett. 39:91-99; U.S. Patent 5,474,981; Gillies, et al., 1992 Proc. Natl. Acad. Sci. USA 89:1428-1432; and Fell, et al., 1991, J. Immunol. 146:2446-2452, which are incorporated herein by reference in their entireties.

5 In another aspect, the present invention provides methods for identifying a compound or ligand that binds to or modulates the activity of a polypeptide of the invention. Such a method comprises measuring a biological activity of the polypeptide in the presence or absence of a test compound and identifying test compounds that alter (increase or decrease) the biological activity of the polypeptide.

10 In one embodiment, the invention provides a fusion protein comprising a bioactive molecule and one or more domains of a polypeptide of the invention or fragment thereof. In particular, the present invention provides fusion proteins comprising a bioactive molecule recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to one or more domains of a polypeptide of the invention or fragments thereof.

15 The present invention further encompasses fusion proteins in which the polypeptides of the invention or fragments thereof, are recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to heterologous polypeptides (i.e., an unrelated polypeptide or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the
20 polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In one example, a fusion protein in which a polypeptide of the invention or a fragment thereof can be fused to sequences derived from various types of immunoglobulins. For example, a polypeptide of the invention can be fused to a constant region (e.g., hinge, CH2, and
25 CH3 domains) of human IgG1 or IgM molecule, (for example, as described by Hudson & Souriauso (2003) Nature Medicine 9(1):129 – 134) so as to make the fused polypeptides or fragments thereof more soluble and stable *in vivo*. The short half-life of antibody fragments can also be extended by 'pegylation', that is, a fusion to polyethylene glycol (see Leong, S.R. et al. (2001) Cytokine 16:106-119). In one example of such fusions, described in WO0183525, Fc
30 domains are fused with biologically active peptides. A pharmacologically active compound is produced by covalently linking an Fc domain to at least one amino acid of a selected peptide. Linkage to the vehicle increases the half-life of the peptide, which otherwise could be quickly degraded *in vivo*

Alternatively, non-classical alternative protein scaffolds (for example see Nygren &
35 Skerra (2004) J Immunol Methods 290(1-2):3-28 or WO03049684) can be used to incorporate, and replicate the properties of, the peptides of the invention, for example by inserting peptide sequences derived from TREM-1 CDR2 or CDR3 into a protein framework to support

conformationally variable loops having structural/functional similarities to CDR2 or CDR3 in a fixed spatial arrangement

Such fusion proteins or scaffold based proteins can be used as an immunogen for the production of specific antibodies which recognize the polypeptides of the invention or fragments thereof. In another preferred embodiment, such fusion proteins or scaffold based proteins can be administered to a subject so as to inhibit interactions between a ligand and its receptors *in vivo*. Such inhibition of the interaction will block or suppress certain cellular responses involved in sepsis and septic shock.

In one aspect, the fusion protein comprises a polypeptide of the invention which is fused to a heterologous signal sequence at its N-terminus. Various signal sequences are commercially available. For example, the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, CA) are available as eukaryotic heterologous signal sequences. As examples of prokaryotic heterologous signal sequences, the *phoA* secretory signal (Sambrook, et al., supra; and Current Protocols in Molecular Biology, 1992, Ausubel, et al., eds., John Wiley & Sons) and the protein A secretory signal (Pharmacia Biotech; Piscataway, NJ) can be listed. Another example is the gp67 secretory sequence of the baculovirus envelope protein (Current Protocols in Molecular Biology, 1992, Ausubel, et al., eds., John Wiley & Sons).

In another embodiment, a polypeptide of the invention can be fused to tag sequences, e.g., a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz, et al., 1989, Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other examples of peptide tags are the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, et al., 1984, Cell 37:767) and the "flag" tag (Knappik, et al., 1994, Biotechniques 17(4):754-761). These tags are especially useful for purification of recombinantly produced polypeptides of the invention.

Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, e.g., by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, e.g., Current Protocols in Molecular Biology, 1992, Ausubel, et al., eds., John Wiley & Sons). The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Various

host-vector systems and selection systems are known. In a specific embodiment, the expression of a fusion protein is regulated by a constitutive promoter. In another embodiment, the expression of a fusion protein is regulated by an inducible promoter. In accordance with these embodiments, the promoter may be a tissue-specific promoter. Expression vectors

5 containing inserts of a gene encoding a fusion protein can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a fusion protein in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding the

10 fusion protein. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a nucleotide sequence encoding a fusion protein in the vector. For example, if the nucleotide sequence encoding the fusion

15 protein is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the fusion protein insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (i.e., fusion protein) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the fusion protein in in vitro assay

20 systems, e.g., binding with anti-fusion protein antibody. For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the fusion protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription

25 terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell

30 lines. This method may advantageously be used to engineer cell lines that express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein. Once a fusion protein of the invention has been produced by recombinant expression, it may be purified by any method known in the art

35 for purification of a protein, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antibody, and sizing column chromatography),

centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

The present invention also provides methods for treating a subject suffering from sepsis, septic shock or a sepsis-like condition by administering a peptide or polypeptide of the invention. In another embodiment, the modulator may be an antibody which mimics the activity of a polypeptide of the invention. In particular, the invention provides a method of treating or ameliorating sepsis, septic shock or a sepsis-like condition in a subject, comprising: administering a therapeutically effective amount of a peptide or polypeptide of any one of the preceding claims to a subject. In such methods, the peptide or polypeptide administered can have substantial sequence identity to sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19, is SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19, or an active fragment, analogue or derivative of SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 or has at least about 80% sequence identity to SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19

In one aspect, the invention provides a method for preventing sepsis, septic shock or sepsis-like conditions, by administering to the subject a peptide or polypeptide of the invention. Subjects at risk of sepsis or septic shock can be identified by, for example, any diagnostic or prognostic assays as known in the art (for particularly suitable methods of diagnosis, see WO2004081233, Gibot *et al.* (2004) *Ann Intern Med.* 141(1):9-15 and Gibot *et al.* (2004) *N Engl J Med.* 350(5):451-8. The prophylactic agents described herein, for example, can be used to treat a subject at risk of developing disorders such as those previously discussed. The methods of the invention are applicable to mammals, for example humans, non human primates, sheep, pigs, cows, horses, goats, dogs, cats and rodents, such as mouse and rat. Generally, the methods of the invention are to be used with human subjects.

Furthermore, the invention provides a pharmaceutical composition comprising a polypeptide of the present invention or an antibody or fragments thereof that mimics a polypeptide of the invention. The peptides, polypeptides and antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the peptide, protein, or antibody and a pharmaceutically acceptable carrier.

As used herein the language " pharmaceutically acceptable diluent, carrier or excipient " is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The invention includes methods for preparing pharmaceutical compositions containing a peptide or polypeptide of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with a peptide or polypeptide of the invention and one or more additional active compounds.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, intra-articular, intraperitoneal, and intrapleural, as well as oral, inhalation, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy injectability with a syringe exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle
5 which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

10 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and
15 the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient, such as starch or lactose; a disintegrating agent, such as alginic acid, Primogel, or corn starch; a lubricant, such as magnesium stearate or Sterotes; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; or a flavoring agent, such as peppermint, methyl
20 salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For
25 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into
30 ointments, salves, gels, or creams as generally known in the art. The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation,
35 including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will

be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

For antibodies, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank, et al., 1997, *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The invention further provides a kit containing a peptide or polypeptide of the invention of the present invention, or an antibody or fragments thereof mimicking a polypeptide of the invention, preferably with instructions for use, for example in the treatment of sepsis, septic shock or sepsis-like conditions.

The invention provides a method for identifying (or screening) modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which mimic a polypeptide of the invention or have a stimulatory or inhibitory effect on, for example, activity of a polypeptide of the invention. In particular, the invention provides a method

of screening compounds or compositions to treat sepsis, septic shock or sepsis-like conditions, comprising: providing a TREM-1 peptide; contacting an animal in a cecal ligation and puncture model (or using other assay or model as described herein or known in the art) with the TREM-1 peptide; determining if there was a modulation in the sepsis, for example wherein an increase in survival indicates that the TREM-1 peptide may be useful for treating sepsis, septic shock or sepsis-like conditions.

The invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Preferred features of each aspect of the invention are applicable to each other aspect, *mutatis mutandis*.

The present invention will now be described with reference to the following non-limiting examples, with reference to the figures, in which:

Figure 1A. shows a sequence alignment of TREM-1 and TREM-2 family members. Human TREM-1 was aligned with mouse TREM-1 and human and mouse TREM-2 using version 1.74 of CLUSTAL W. Secondary structure assignments correspond to the published human TREM-1 structure (arrows for β -strands and cylinder for α helices) (Radaev et al. (2003) Structure (Camb).Dec;11(12):1527-35). Residues involved in homo-heterodimer formation are shown in white on black background. Cysteine making disulfide bonds conserved for V-type Ig fold are in bold. Gaps are indicated with (-), identical residues with (*), similar with (: or .). An extended region of similarities between human and mouse TREM1 sequences is shown in boxes on grey background. TREM-1 peptide sequences used in the Examples herein are indicated underlined.

Figure 1B. shows a ribbon diagram of the published TREM-1 homodimeric structure (Kelker, et al. (2004) J Mol Biol. Sep 24;342(4):1237-48). Postulated binding sites that comprise the antibody equivalent Complementarity Determining Regions (CDRs) are in red.

Figure 2. shows that administration of TREM-1 peptides, 1 hour before LPS, reduces death induced by endotoxaemia. BALB/c mice (10 per group) were injected intraperitoneally with 200 μ g LPS. The TREM-1 peptides P1, P2, P3, or P5 (200 μ l of a 300 μ M solution per mouse) were injected intraperitoneally 1 hour before LPS. Viability of mice was monitored twice a day for 7 days. Statistical analysis was performed by Logrank test. Data from control mice represent

cumulative survival curves from two independent experiments performed under identical conditions.

Figure 3. shows that TREM-1 peptide P1 is able to effectively reduce death induced by endotoxaemia when injected at 4 hours after LPS. BALB/c mice (10 per group) were injected intraperitoneally with 200 µg LPS. TREM-1 peptide P1, 200 µl of a 300 µM solution per mouse was injected intraperitoneally 1 hour before or 4 hours after LPS. Viability of mice was monitored twice a day for 7 days. Statistical analysis was performed with the Logrank test. Data from control mice represent cumulative survival curves from two independent experiments performed under identical conditions

Figure 4. shows that administration of TREM-1 peptides, 4 hours after LPS, reduces death induced by endotoxaemia. BALB/c mice (10 per group) were injected intraperitoneally with 200 µg LPS. P1 peptide, 200 µl of a 150, 300 and 600 µM solution per mouse (dots) or P3, 200 µl of a 600 µM solution per mouse (filled squares) were injected intraperitoneally 4 hours after LPS. Viability of mice was monitored twice a day for 7 days. Statistical analysis was performed with the Logrank test

Figure 5. shows that TREM-1 peptide P1 protects against cecal ligation and puncture (CLP). CLP was induced in C57BL/6 mice (15 per group) as described in Materials and Methods. P1 peptide (empty dots) or P3 peptide (filled squares) (200 µl of a 600 µM solution per mouse) were injected intraperitoneally 5 and 24 hours after CLP induction. Viability of mice was monitored twice a day for 10 days. Statistical analysis was performed with the Logrank test.

Figure 6. shows that P1, P2 and P5 peptides, but not P3 peptide, inhibit the binding of soluble TREM-1/IgG1 to TREM-1 Ligand positive peritoneal exudate cells. Cytofluorimetric analysis of peritoneal exudate cells with 2 µg/ml of mouse TREM-1/hlgG1 in the presence of a 500µM solution per mouse (thin line), 100µM solution per mouse (dotted line) or absence (thick line) of the peptides is shown. The grey histogram represents immunostaining with human IgG1 as a control.

Figure 7A. shows the release of sTREM-1 from cultured monocytes after stimulation with LPS with and without proteases inhibitor. LPS stimulation induced the appearance of a 27-kD protein that was specifically recognized by an anti-TREM-1 mAb (inset). sTREM-1 levels in the conditioned culture medium were measured by reflectance of immunodots. Data are shown as mean ±SD (n=3).

Figure 7B. shows expression of TREM-1 mRNA in monocytes. Cultured monocytes were stimulated with LPS (1µg/mL) for 0, 1 and 16 hours as indicated. LPS induced TREM-1 mRNA production within 1 hour.

5

Figure 8A. shows the release of cytokines and sTREM-1 from cultured monocytes. For cell activation, primary monocytes were cultured in 24-well flat-bottom tissue culture plates in the presence of LPS (1µg/mL). In some experiments this stimulus was provided in combination with P5 (10 to 100 ng/mL), control peptide (10 to 100 ng/mL) or rIL-10 (500 U/mL). To activate monocytes through TREM-1, an agonist anti-TREM-1 mAb (10µg/mL) was added as indicated. Cell-free supernatants were analysed for production of TNF-α, IL-1β and sTREM-1 by ELISA or immunodot. All experiments were performed in triplicate and data are expressed as means (SEM).

- 15 a: Media
- b: P5 10 ng/mL
- c: Anti-TREM-1
- d: LPS
- e: LPS + Anti-TREM-1
- f: LPS + P5 10 ng/mL
- 20 g: LPS + P5 50 ng/mL
- h: LPS + P5 100 ng/mL
- i: LPS + IL10

Figure 8B. shows the effect of P5 on NFκB activation. Monocytes were cultured for 24 hours in the presence of *E.coli* LPS (O111:B4, 1µg/mL), anti-TREM-1 mAb (10µg/mL) and / or P5 (100 ng/mL) as indicated and the levels of NFκB p50 and p65 were determined using an ELISA-based assay. Experiments were performed in triplicate and data are expressed as means of optical densities (SEM).

30 Figure 9. shows accumulation of sTREM-1 in serum of LPS-treated mice.

Male Balb/C mice (20 to 23 g) were treated with LPS (LD₅₀, intraperitoneally). Serum was assayed for sTREM-1 by immunodot. Serum sTREM-1 was readily detectable 1 hour after LPS administration and was maintained at a plateau level from 4 to 6 hours.

35 Figure 10A. shows that P5 pre-treatment protects against LPS lethality in mice. Male Balb/C mice (20 to 23 g) were randomly grouped (10 mice per group) and treated with an LD₁₀₀ of LPS. P5 (50µg or 100µg) or control vector was administered 60 min before LPS.

Figure 10B. shows that delayed administration of P5 protects LPS lethality in mice. Male Balb/C mice (20 to 23 g) were randomly grouped (8 mice per group) and treated with an LD₁₀₀ of LPS. P5 (75µg) or control vector was administered 4 or 6 hours after LPS as indicated.

5

Figure 10C. shows that administration of agonist TREM-1 mAb is lethal to mice. Male Balb/C mice (20 to 23 g) were randomly grouped (8 mice per group) and treated with a combination of an LD₅₀ of LPS + control vector, LD₅₀ of LPS + anti-TREM-1 mAb (5µg) or LD₁₀₀ of LPS + control vector as indicated. Control vector and anti-TREM-1 mAb were administered 1 hour after LPS injection

10

Figure 11A. shows that P5 partially protects mice from CLP-induced lethality. Male Balb/C mice (20 to 23g) were randomly grouped and treated with normal saline (n=14) or the control peptide (n=14, 100µg) or with P5 (100µg) in a single infection at H0 (n=18), H+4 (n=18) or H+24 (n=18). The last group of mice (n=18) was treated with repeated injections of P5 (100µg) at H+4, H+8 and H+24.

15

Figure 11B. shows the dose effect of P5 on survival. Mice (n=15 per group) were treated with a single injection of normal saline or 10µg, 20µg, 50µg 100µg or 200µg of P5 at H0 after the CLP and monitored for survival

20

Figure 12. shows that P5 has no effect on bacterial counts during CLP. Mice (5 per group) were killed under anaesthesia at 24 hours after CLP. Bacterial counts in peritoneal lavage fluid and blood were determined and results are expressed as CFU per mL of blood and CFU per mouse for the peritoneal lavage.

25

Figure 13 shows TNF-α and IL-1β plasma concentration evolution after LPS (15mg/kg) administration in rats.

* p<0.05 P5-treated vs Control animals

30 § p<0.05 P5-treated vs P1-treated animals

Figure 14 shows Nitrite/Nitrate concentrations evolution after LPS (15mg/kg) administration in rats. * p<0.05 P5-treated vs Control and P1-treated animals

35 Figure 15 shows mean arterial pressure evolution during caecal ligation and puncture-induced peritonitis in rats.

* p<0.05 vs Control animals

Figure 16 shows TNF- α plasma concentration evolution during caecal ligation and puncture-induced peritonitis in rats.

* p<0.05 P5-treated vs Control animals

5 § p<0.05 P1-treated vs Control animals

§ p<0.05 P5 vs P1-treated animals

Figure 17 shows Nitrite/Nitrate concentration evolution during caecal ligation and puncture-induced peritonitis in rats.

10 * p<0.05 P5 and P1-treated vs Control animals

Example 1: TREM-1 peptides protect mice from death by septic shock.

TREM-1 peptides matching the following criteria were synthesized: i) highest homology
15 between human and mouse TREM-1 and lowest homology with TREM-2. ii) peptides spanning
the Complementarity Determining Regions (CDRs) of TREM-1. According to the published
crystal structure of TREM-1, and in analogy with antibodies, these residues are likely to be
involved in cognate ligand recognition (Radaev et al. (2003) Structure (Camb).Dec;11(12):1527-
35 & Kelker, et al. (2004) J Mol Biol. Sep 24;342(4):1237-48) (see Figure 1). One peptide (P1)
20 was designed in the CDR2 region and three peptides (P2, P4 and P5) in the CDR3 region. A
fourth peptide (P3) was designed in the neck region connecting the V-type immunoglobulin (Ig)-
like domain (Ig-V) to the trans-membrane domain. No peptide was designed in the CDR1
region due to high sequence homology between TREM-1 and TREM-2.

Thus, the following peptides of the TREM-1 protein were ordered from and were
25 synthesized and purified by the Protein and Peptide Chemistry Facility, Institute of Biochemistry,
University of Lausanne:

P1	(CDR2 67-89)	LVVTQRPFTRPSEVHMGKFTLKH	[SEQ ID NO:3]
P2	(CDR3 114-136)	VIYHPPNDPVVLFHPVRLVVTKG	[SEQ ID NO:4]
30	P3 (neck region 168-184)	TTTRSLPKPTAVVSSPG	[SEQ ID NO:5]
P4	(CDR3 103-123)	LQVTDGLYRCVIYHPPNDPV	[SEQ ID NO:6]
P5	(CDR3 103-119):	LQVTDGLYRCVIYHPP	[SEQ ID NO:7]
P1sc*	(P1 scrambled seq.)	LTPKHGQRSTHVTKFRVFEPVML	[SEQ ID NO:8]
P5sc*	(P5 scrambled seq.)	TDSRCVIGLYHPPLQVY	[SEQ ID NO:9]

35 * This is a control peptide and indeed does not protect

In the experiments of this example, the peptides were administered in a volume of 200 μ l of the solution molarity indicated. To assess the ability of TREM1-peptides to protect mice from LPS-induced endotoxaemia, the Inventors administered peptides P1, P2, P3 and P5 (300 μ M) 1 hour before a lethal dose of lipopolysaccharide (LPS) (Figure 2). Lethality was monitored over time and compared with animals that had received control injections of vehicle alone. P5 injection confers maximal protection, with 90% of the animals still alive 7 days after LPS injection, as compared with 10% of control mice ($p < 0.001$). 60% of the P1-treated mice and 50% of the P2 treated mice survived endotoxaemia as compared with 10% of control mice ($p < 0.01$ and $p < 0.05$ respectively). Interestingly, all P3-treated mice died within 4 days after LPS injection. These results indicate that peptides containing sequences of the extracellular portion of TREM-1 corresponding to the putative ligand binding site (CDR2 and CDR3) can protect mice from lethal shock.

In order to investigate whether TREM-1 peptide treatment could be delayed until after the administration of LPS, the Inventors injected the peptides at 4 hours after LPS injection. Only in the case of P1, this delayed treatment conferred significant protection against a lethal dose of LPS (Figure 3). 80 % of the mice injected with P1 4 hours after LPS survived endotoxaemia compared to 60 % of mice treated 1 h before LPS and 10 % of mice treated with vehicle alone ($p < 0.001$ and $p < 0.01$ respectively). Thus, P1 is effective even when injected after the outbreak of endotoxaemia. No late death occurred over one week, indicating that P1 did not merely delay the onset of LPS lethality, but provided lasting protection. P1 administration conferred maximal protection (80 %) when administered at 600 μ M ($p < 0.01$) and the level of protection dropped to 50 % at 300 μ M ($p < 0.05$) and further down to 30 % at 150 μ M as compared to 20% of control mice, indicating a dose dependent effect of P1 (Figure 4). The Inventors then investigated whether P1 protects against septic shock in the "CLP" model (Cecal Ligation and Puncture is a widely used experimental model of sepsis). Mice treated with two doses of P1 at 5 and 24 hours after CLP were protected from death as compared to control treated mice ($p = 0.0791$) although the difference was not statistically significant. 40 % of the mice injected with P1 at 5 days after CLP survived compared to 5 % of mice treated with P3 peptide. At 10 days after CLP, the treated mice were still alive, indicating that P1 did not merely delay mortality, but provided lasting protection (Figure 5).

Example 2: TREM-1 peptide P1 inhibits binding of soluble mouse TREM-1/IgG to TREM-1 ligand positive cells

Among TREM-1 derived peptides tested in CLP, peptides P1, P2 and P5 demonstrate a protective activity. A possible mechanism of action could be the ability of TREM-1 derived peptide to interfere with TREM-1/TREM-1 ligand interaction. To address this question the

Inventors performed competition experiments on TREM-1 ligand positive cells: PEC (Peritoneal Exudate Cells) from CLP treated mice.

Peritoneal exudate cells (PEC) from mice suffering from a caecal ligation and puncture (CLP)-induced peritonitis were subjected to flow cytometry analysis after incubation with a PE-
5 conjugated anti-human IgG1 (Jackson ImmunoResearch, Bar Harbor, USA). Competition with TREM-1 peptides was performed by pre-incubating cells with the indicated concentrations of peptides for 45 minutes on ice before adding mTREM-1-IgG1.

As shown in Figure 6, the P1 peptide, derived from the CDR2 region of mTREM-1, and the P2 and P5 peptides spanning the CDR3 region inhibit TREM-1 interaction with its ligand in a
10 dose dependent manner. Conversely the P3 peptide, derived from the neck region of TREM-1 connecting the IgG like portion to the transmembrane domain was ineffective.

Example 3: Additional studies on the modulation of the inflammatory response in murine sepsis by TREM-1 peptide P5

15

Methods

Preparation of monocytes from peripheral blood

Ten mL of peripheral blood samples were collected on EDTA-K from 5 healthy volunteer donors originating from laboratory staff. After dilution in RPMI (Life Technologies, Grand Island,
20 NY) v/v, blood was centrifuged for 30 min at room temperature over a Ficoll gradient (Amersham Pharmacia, Uppsala, Sweden) to isolate PBMC. The cells recovered above the gradient were washed and counted. In order to deplete the suspensions of lymphocytes, cells were then plated in 24-well flat-bottom tissue culture plates (Corning, Corning, NY) at a concentration of 5×10^6 /mL and allowed to adhere during 2 hours at 37°C. The resulting
25 lymphocyte suspension was discarded and the adhering monocytic cells were maintained in a 5% CO₂ incubator at 37°C in complete medium (RPMI 1640, 0.1 mM sodium pyruvate, 2 mM Penicillin, 50 µg/mL Streptomycin; Life Technologies) supplemented with 10% FCS (Invitrogen, Cergy, France).

30 *TREM-1 peptide*

Using the human TREM-1 sequence in Gen-Bank, accession #AF287008 and the mouse TREM-1 sequence #AF241219, a peptide "P5" (LQVTDSGLYRCVIYHPP; [SEQ ID NO:7]; was chemically synthesized as a C-terminally amidated peptide (Pepscan Systems, Lelystad, The Netherlands). The correct peptide was obtained in greater than 99% yield and
35 with measured mass of 1961 Da versus a calculated mass of 1962 Da and was homogeneous after preparative purification, as confirmed by mass spectrometry and analytic reversed phase-high performance liquid chromatography. A peptide "P5sc" containing the same amino-acids

than P5 but in a different sequence order (TDSRCVIGLYHPPLQVY; [SEQ ID NO:9]) was similarly synthesized and served as 'control peptide'.

In vitro stimulation of monocytes

5 For activation, monocytes were cultured in the presence of *E.coli* LPS (O111:B4, 1µg/mL, Sigma-Aldrich, La Verpillière, France). Cell viability was assessed by trypan blue exclusion and by measuring lactate dehydrogenase release. In some experiments, this stimulus was given in combination with TNF-α (5 to 100 ng/mL, R&D Systems, Lille, France), IL-1β (5 to 100 ng/mL, R&D Systems), rIFN-γ (up to 100 U/mL, R&D Systems), rIL-10 (500 U/ml, R&D
10 Systems) or up to 100 ng/mL of P5 or control peptide.

In order to activate monocytes through TREM-1, an anti-TREM-1 agonist monoclonal antibody (R&D Systems) was added as follows: flat-bottom plates were precoated with 10 µg/mL anti-TREM-1 per well. After thorough washing in phosphate buffered saline (PBS), the monocyte suspensions were added at a similar concentration as above. Some experiments were
15 performed in the presence of protease inhibitors (PMSF and Protease Cocktail Inhibitor; Invitrogen). Cell-free supernatants were assayed for the production of TNF-α and IL-1β by ELISA according to the recommendations of the manufacturer (BD Biosciences, San Diego, USA). To address the effect of P5 on NF-κB activity in monocytes, an ELISA-based assay was performed (BD Mercury™ Transfactor Kit, BD Biosciences). Monocytes were cultured for 24
20 hours in the presence of *E.coli* LPS (O111:B4, 1µg/mL), and / or an agonist anti-TREM-1 monoclonal antibody (10µg/mL), and / or P5 (100 ng/mL). Whole-cell extracts were then prepared and levels of NF-κB p50 and p65 were determined according to the recommendations of the manufacturer. All experiments were performed in triplicate and data are expressed as means (SEM).

25

Identification and quantitation of sTREM-1 release

Primary monocytes suspensions were cultured as described above. The cells were treated with *E.coli* LPS (O111:B4, 1µg/mL) for 24 hours at 37°C. Cell-conditioned medium was submitted to Western-blotting using an anti-TREM-1 monoclonal antibody (R&D Systems) in
30 order to confirm the presence of 27 kDa material recognized by anti-TREM-1. Soluble TREM-1 levels were measured by assessing the optical intensity of bands on immunodots by means of a reflectance scanner and the Quantity One Quantitation Software (Bio-Rad, Cergy, France) as reported elsewhere (18). Soluble TREM-1 concentration from each sample was determined by comparing the optical densities of the samples with reference to standard curves generated with
35 purified TREM-1. All measurements were performed in triplicate. The sensitivity of this technique allows the detection of sTREM-1 levels as low as 5pg/mL.

TREM-1 RT-PCR

Total mRNA was extracted from primary monocytes cultured in the presence of LPS using a TRIzol reagent (Invitrogen), and reverse transcribed using Superscript RT II (Invitrogen) to generate cDNA. RT-PCR conditions then used for all reactions were 94°C, 30s/65°C,
5 30s/68°C, 1 min for 30 cycles. Amplification was performed with 2.5 mM MgCl₂, 0.2 mM dNTP, 2.0 U *Taq* polymerase, and 20 pM 5' and 3' oligonucleotide primers (Proligos, Paris, France).

The sequences of the 5' and 3' primer pairs used were the following:

10 for TREM-1 (17)

TTGTCTCAGAACTCCGAGCTGC; [SEQ ID NO:10]

and

GAGACATCGGCAGTTGACTTGG; [SEQ ID NO:11]

15 for TREM-1sv (19)

GGACGGAGAGATGCCCAAGACC; [SEQ ID NO:12]

and

ACCAGCCAGGAGAATGACAATG; [SEQ ID NO:13]

20 for β -actin (used as housekeeping amplicon)

GGACGACATGGAGAAGATCTGG; [SEQ ID NO:14]

and

ATAGTAATGTCACGCACGATTTC; [SEQ ID NO:15]

25 PCR products were run on agarose gels and visualized by ethidium bromide staining.

LPS-induced endotoxemia in mice

After approval by the local ethical committee, male Balb/C mice (20 to 23g) were randomly grouped and treated with *E.coli* LPS intraperitoneally (i.p.) in combination with P5 (in
30 500 μ l normal saline) or control vector before or after LPS challenge. In some experiments, 5 μ g of an anti TREM-1 monoclonal antibody was administered i.p. one hour after LPS injection. The viability of mice was examined every hour, or animals were sacrificed at regular intervals. Serum samples were collected by cardiac puncture and assayed for TNF- α and IL-1 β by ELISA (BD Biosciences), and for sTREM-1 levels by immunodot.

35

CLP polymicrobial sepsis model

Male Balb/C mice (7 to 9 weeks, 20 to 23g) were anaesthetized by i.p. administration of ketamine and xylazine in 0.2 mL sterile pyrogen-free saline. The caecum was exposed through a 1.0 cm abdominal midline incision and subjected to a ligation of the distal half followed by two punctures with a G21 needle. A small amount of stool was expelled from the punctures to
5 ensure patency. The caecum was replaced into the peritoneal cavity and the abdominal incision closed in two layers. After surgery all mice were injected s.c. with 0.5 ml of physiologic saline solution for fluid resuscitation and s.c. every 12 h with 1.25 mg (i.e. 50 µg/g) of imipenem. The animals were randomly grouped and treated with normal saline (n=14), the control peptide (n=14, 100µg) or P5 (100µg) in a single injection at H0 (n=18), H+4 (n=18) or H+24 (n=18). The
10 last group of mice (n=18) was treated with repeated injections of P5 (100µg) at H+4, H+8 and H+24. All treatments were diluted into 500µl of normal saline and administered i.p. The inventors next sought to determine the effect of various doses of P5. For this purpose, mice (n=15 per group) were treated with a single injection of normal saline or 10µg, 20µg, 50µg, 100µg or 200µg of P5 at H0 after the CLP and monitored for survival. Five additional animals
15 per group were killed under anaesthesia 24 hours after CLP for the determination of bacterial count and cytokines levels. Peritoneal lavage fluid was obtained using 2mL RPMI 1640 (Life Technologies) and blood was collected by cardiac puncture. Concentrations of TNF-α and IL-1β in the serum were determined by ELISA (BD Biosciences). For the assessment of bacterial counts, blood and peritoneal lavage fluid were plated in serial log dilutions on tryptic soy supplemented with 5% sheep blood agar plates. After plating, tryptic soy agar plates were
20 incubated at 37°C aerobically for 24 hours, and anaerobically for 48 hours. Results are expressed as CFU per mL of blood and CFU per mouse for the peritoneal lavage.

Statistical analyses

25 Serum sTREM-1 and cytokines levels were expressed as mean (±SD). The protection against LPS lethality by P5 was assessed by comparison of survival curves using the Log-Rank test. All statistical analyses were completed with Statview software (Abacus Concepts, Berkeley CA) and a two-tailed P<0.05 was considered significant.

30

Results

A soluble form of TREM-1 is released from cultured human monocytes after stimulation with E.coli LPS

To identify the potential release of sTREM-1 *in vitro*, the inventors stimulated human
35 monocytes with LPS and analyzed the conditioned culture medium by SDS-PAGE. LPS stimulation induced the appearance of a 27-kDa protein in a time-dependent manner (Figure 7A). Western blotting analysis revealed that this protein was specifically recognized by a

monoclonal antibody directed against the extra-cellular domain of TREM-1 (Figure 7A). Cell viability was unaffected at LPS concentrations that induced the presence of sTREM-1 in conditioned medium, indicating that TREM-1 release was not due to cell death. Similarly, treatment of monocytes with protease inhibitors did not affect TREM-1 release (Figure 7A).

5 TREM-1 mRNA levels were increased upon LPS treatment (Figure 7B) whereas TREM-1sv mRNA levels remained undetectable. This suggests that TREM-1 release is likely to be linked to an increased transcription of the gene and unrelated to TREM-1sv expression.

Stimulation of monocytes for 16 hours with TNF- α (5 to 100 ng/mL) or IL-1 β (5 to 100 ng/mL) induced very small TREM-1 release in a cytokine dose-dependent manner. Stimulation with
10 IFN- γ did not induce TREM-1 release, even at concentrations of up to 100 U/mL.

LPS associated release of pro-inflammatory cytokines is attenuated by P5

Significant TNF- α and IL-1 β production was observed in the supernatant of monocytes cultured with LPS. TNF- α and IL-1 β production was even higher for cells cultured with both
15 TREM-1 mAb and LPS as compared with those cultured with mAb or LPS alone (Figure 8A). The inducible release of pro-inflammatory cytokines was significantly lower after LPS stimulation when the medium was supplemented with P5 or IL-10. P5 reduced, in a concentration-dependent manner, the TNF- α and IL-1 β production from cells cultured with LPS or with LPS and mAb and simultaneously increased the release of sTREM-1 from cells cultured with LPS.
20 The control peptide displayed no action on cytokines or sTREM-1 release (data not shown). In striking contrast, IL-10 totally inhibited the release of both TREM-1 and inflammatory cytokines (Figure 8A). Both LPS and TREM-1 mAb induced a strong activation of monocytic NF- κ B p50 and p65 and combined administration of LPS and TREM-1 mAb lead to a synergistic effect. P5 inhibited the NF- κ B activation induced by the engagement of TREM-1 but did not alter the effect
25 of LPS (Figure 8B).

Serum sTREM-1 levels of LPS-treated mice are increased

In order to determine whether sTREM-1 was released systemically during endotoxemia in mice, the Inventors measured serum sTREM-1 levels after LPS administration. Serum
30 sTREM-1 was readily detectable 1 hour after administration of an LD₅₀ dose of LPS and was maintained at peak plateau levels from 4 to 6 hours after LPS treatment (Figure 9).

TREM-1 peptide "P5" protects endotoxemic mice from lethality

Mice treated by a single dose of P5 60 min before a lethal dose (LD₁₀₀) of LPS were
35 prevented from death in a dose-dependent manner (Figure 10A). In order to investigate whether P5 treatment could be delayed until after the administration of LPS, the Inventors injected P5 beginning 4 or 6 hours after LPS injection. This delayed treatment up to 4 hours conferred

significant protection against a LD₁₀₀ dose of LPS (Figure 10B). No late death occurred over one week, indicating that P5 did not merely delay the onset of LPS lethality, but provided lasting protection. Control mice all developed lethargy, piloerection, and diarrhoea before death. By contrast, P5-treated mice remained well groomed and active, had no diarrhoea, and were lively.

5 To clarify the mechanism by which P5 protected mice from LPS lethality, the Inventors determined the serum levels of TNF- α , IL-1 β and sTREM-1 of endotoxemic mice at 2 and 4 hours. As compared to controls, pre-treatment by 100 μ g of P5 reduced cytokines levels by 30% and increased sTREM-1 levels by 2 fold as shown in Table 4:

10 Table 4. Serum concentrations of TNF- α , IL-1 β and sTREM-1 in endotoxemic mice.

	TNF- α (ng/mL)		IL-1 β (ng/mL)		sTREM-1 (ng/mL)	
	H2	H4	H2	H4	H2	H4
Control	3.3 \pm 1.0	0.4 \pm 0.1	0.3 \pm 0.1	1.5 \pm 0.2	249 \pm 48	139 \pm 8
P5 (100 μ g)	2.4 \pm 0.5	0.1 \pm 0.1	0.2 \pm 0.1	0.9 \pm 0.2	475 \pm 37	243 \pm 28

Engagement of TREM-1 is lethal to mice

To further highlight the role of TREM-1 engagement in LPS-mediated mortality, mice were treated with agonist anti-TREM-1 mAb in combination with the administration of an LD₅₀ dose of LPS. This induced a significant increase in mortality rate from 50% to 100% (Figure 10C).

P5 protects mice from CLP-induced lethality

To investigate the role of P5 in a more relevant model of septic shock, the Inventors performed CLP experiments (Figure 11A). The control groups comprised mice injected with normal saline or with the control peptide. In this model of polymicrobial sepsis, P5 still conferred a significant protection against lethality even when administered as late as 24 hours after the onset of sepsis. Interestingly, repeated injections of P5 had the more favourable effect on survival ($P < 0.01$). There was a dose response effect of P5 on survival (Figure 11B) and cytokine production (Table 5). P5 had no effect on bacterial clearance (Figure 12).

Table 5. Serum concentrations of TNF- α , IL-1 β and sTREM-1 at 24 hours after CLP.

TNF- α (pg/mL)	IL-1 β (pg/mL)	sTREM-1 (ng/mL)
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Control peptide	105±12	841±204	52±3
Control saline	118±8	792±198	35±5
P5 10µg	110±11	356±62	43±8
P5 20µg	89±10	324±58	58±8
P5 50µg	24±6	57±11	93±10
P5 100µg	20±3	31±3	118±12
P5 200µg	21±7	37±8	158±13

Sepsis exemplifies a complex clinical syndrome that results from a harmful or damaging host response to severe infection. Sepsis develops when the initial, appropriate host response to systemic infection becomes amplified, and then dysregulated (4, 5). Neutrophils and monocyte/macrophages exposed to LPS, for instance, are activated and release such pro-inflammatory cytokines as TNF- α and IL-1 β . Excessive production of these cytokines is widely believed to contribute to the multi-organ failure that is seen in septic patients (20-23).

TREM-1 is a recently identified molecule involved in monocytic activation and inflammatory response (12, 14). It belongs to a family related to NK cell receptors that activate downstream signalling events. The expression of TREM-1 on PNNs and monocytes/macrophages has been shown to be inducible by LPS (16, 17).

As described herein, the Inventors demonstrate that a soluble form of TREM-1 was released from cultured human monocytes after stimulation with *E.coli* LPS. Such a soluble form was also detectable in the serum of endotoxemic mice as early as 1 hour after LPS challenge. This is consistent with the implication of TREM-1 in the very early phases of the innate immune response to infection (14, 15, 24). The mechanism by which sTREM-1 is released is not clearly elucidated but seems to be related to an increased transcription of the TREM-1 gene. Nevertheless, although incubation with a protease inhibitor cocktail does not alter the sTREM-1 release, cleavage of the surface TREM-1 from the membrane cannot be totally excluded. Interestingly, stimulation of human monocytes with such pro-inflammatory cytokines as TNF- α , IL-1 β or IFN- γ induced very small sTREM-1 release unless LPS was added as a co-stimulus. The expression of an alternative mRNA TREM-1 splice variant (TREM-1sv) has been detected in monocytes that might translate into a soluble receptor (18) upon stimulation with cell wall fraction of *Mycobacterium bovis* BCG but not LPS (25). This was confirmed in this study as i) LPS did not increase the level of mRNA TREM-1sv in monocytes and ii) only a 27-kDa protein was released by monocytes upon LPS stimulation and not the 17.5-kDa variant.

Although its natural ligand has not been identified (13, 14), engagement of TREM-1 on monocytes with an agonist monoclonal antibody resulted in a further enhancement of pro-

inflammatory cytokines production, while P5 induced a decrease of these syntheses in a concentration-dependent manner, and IL-10 completely suppressed it.

Inflammatory cytokines, and especially TNF- α , are considered to be deleterious, yet they also possess beneficial effects in sepsis (5) as shown by the fatal issue of peritonitis in animals with impaired TNF- α responses (9-11). Moreover, in clinical trials, the inhibition of TNF- α increased mortality (8). Finally, the role of TNF- α in the clearance of infection has been highlighted by the finding that sepsis is a frequent complication in rheumatoid arthritis patients treated with TNF- α antagonists (26).

The mechanism by which P5 modulates cytokine production is not yet clear. P5 comprises the complementary determining region (CDR)-3 and the 'F' β strand of the extracellular domain of TREM-1. The latter contains a tyrosine residue mediating dimerization. Radaev et al postulated that TREM-1 captures its ligand with its CDR-equivalent loop regions (27). P5 could thus impair TREM-1 dimerization and / or compete with the natural ligand of TREM-1. Moreover, the increase of sTREM-1 release from monocytes mediated by P5 could prevent the engagement of membrane TREM-1, sTREM-1 acting as a decoy receptor, as in the TNF- α system (28, 29).

Activation of the transcription factor NF- κ B is a critical step in monocyte inflammatory cytokine production after exposure to bacterial stimuli such as LPS (30, 31). Among the various NF- κ B / Rel dimers, the p65 / p50 heterodimer is the prototypical form of LPS-inducible NF- κ B in monocytes (32). P5 abolishes the p65 / p50 NF- κ B over-activation induced by the engagement of TREM-1. This might at least partially explain the effects of P5 on cytokine production and the protection from lethality shown here to occur when the peptide was injected one hour before LPS-induced septic shock, or even up to 4 hours after.

Endotoxemia is simple to achieve experimentally, but imperfectly suited to reproduce human sepsis, while polymicrobial sepsis induced by CLP is a more complex but better model, including the use of fluid resuscitation and antibiotics. The latter was thus also used in this study, and confirmed the dose-dependent protection provided by P5, even when administered as late as 24 hours after the onset of sepsis. The favourable effect of P5 was however unrelated to an enhanced bacterial clearance.

One difficulty in the use of immunomodulatory therapies is that it is not possible to predict the development of sepsis, and, thus, patients receiving those treatments frequently already have well-established sepsis (6). Since P5 appeared to be effective even when injected after the outbreak of sepsis, it could thus constitute a realistic treatment (24, 33).

By contrast, engagement of TREM-1 by an agonist anti-TREM-1 monoclonal antibody mediated a dramatic increase of mortality rate in LPS-challenged mice: this further underscores the detrimental effect of TREM-1 engagement during septic shock.

Experimental septic shock reproduces human sepsis only in part. Indeed, our group recently showed that significant levels of sTREM-1 were released in the serum of critically ill patients with sepsis patients (34), the highest levels being observed in patients who survived. This is consistent with our experimental findings indicating that the more important sTREM-1 release, the more favourable is the outcome, and thus sustains, at least theoretically, the potential value of soluble TREM peptides as post-onset sepsis therapy.

TREM-1 appears to be a crucial player in the immediate immune response triggered by infection. In the early phase of infection, neutrophils and monocytes initiate the inflammatory response owing to the engagement of pattern recognition receptors by microbial products (3, 4). At the same time, bacterial products induce the up-regulation and the release of sTREM-1. Upon recognition of an unknown ligand, TREM-1 activates signalling pathways which amplify these inflammatory responses, notably in monocytes/macrophages. The modulation of TREM-1 signalling reduces, although without complete inhibition, cytokine production and protects septic animals from hyper-responsiveness and death. Modulation of TREM-1 engagement with such a peptide as P5 might be a suitable therapeutic tool for the treatment of sepsis, particularly because it seems to be active even after the onset of sepsis following infectious aggression.

Example 4: Haemodynamic studies in LPS treated and septic rats treated with P1 and P5

The role of TREM-1 peptides in further models of septic shock, was investigated by performing LPS and CLP (caecal ligation and puncture) experiments in rats.

Materials and Methods

LPS-induced Endotoxemia

Animals were randomly grouped (n=10-20) and treated with *Escherichia coli* LPS (O111:B4, Sigma-Aldrich, Lyon, France) i.p. in combination with the TREM-1 or scrambled peptides.

CLP Polymicrobial Sepsis Model

The procedure has been described in details elsewhere (see Mansart, A. et al. Shock 19:38-44 (2003)). Briefly, rats (n=6-10 per group) were anesthetized by i.p. administration of ketamine (150 mg/kg). The caecum was exposed through a 3.0-cm abdominal midline incision and subjected to a ligation of the distal half followed by two punctures with a G21 needle. A small amount of stool was expelled from the punctures to ensure potency. The caecum was replaced into the peritoneal cavity and the abdominal incision closed in two layers. After surgery, all rats were injected s.c. with 50 mL/kg of normal saline solution for fluid resuscitation. TREM-1 or scrambled peptides were then administered as above.

Haemodynamic Measurements in rats

Immediately after LPS administration as well as 16 hours after CLP, arterial BP (systolic, diastolic, and mean), heart rate, abdominal aortic blood flow, and mesenteric blood flow were recorded using a procedure described elsewhere (see Mansart, A. et al. Shock 19:38-44 (2003)). Briefly, the left carotid artery and the left jugular vein were cannulated with PE-50 tubing. Arterial BP was continuously monitored by a pressure transducer and an amplifier-recorder system (IOX EMKA Technologies, Paris, France). Perivascular probes (Transonic Systems, Ithaca, NY) wrapped up the upper abdominal aorta and mesenteric artery, allowed to monitor their respective flows by means of a flowmeter (Transonic Systems). After the last measurement (4th hour during LPS experiments and 24th hour after CLP), animals were sacrificed by an overdose of sodium thiopental i.v.

Biological Measurements

Blood was sequentially withdrawn from the left carotid artery. Arterial lactate concentrations and blood gases analyses were performed on an automatic blood gas analyser (ABL 735, Radiometer, Copenhagen, Denmark). Concentrations of TNF- α and IL-1 β in the plasma were determined by an ELISA test (Biosource, Nivelles, Belgium) according to the recommendations of the manufacturer. Plasmatic concentrations of nitrates/nitrites were measured using the Griess reaction (R&D Systems, Abingdon, UK).

Statistical analyses

Results are expressed as mean \pm SD. Between-group comparisons were performed using Student' *t* tests. All statistical analyses were completed with Statview software (Abacus Concepts, CA) and a two-tailed $P < 0.05$ was considered significant.

Results

Endotoxemia model

Following LPS administration, arterial pressures, aortic and mesenteric blood flows dropped rapidly in control animals (scrambled peptides treated rats) while the heart rate remained unchanged (Table 6). The decrease of arterial pressures and aortic blood flow was delayed until the second hour in TREM-1 peptide treated animals with significantly higher values by that time than in control animals. There was no difference between P1 and P5 treated groups. By contrast, none of these two peptides had any effect on the decrease of the mesenteric blood flow (Table 6).

Arterial pH remained constant over time until the fourth hour after LPS injection where it severely dropped in the control group only (Table 6). The significant arterial lactate level elevation present in control animals after the third hour was abolished by the TREM-1 peptides (Table 6). There was no difference between P1 and P5 with regard to pH, arterial bicarbonate and lactate concentrations.

As expected, a peak of TNF- α plasmatic concentration was induced by LPS between 30 minutes and 1 hour after injection followed by a progressive decline thereafter (Figure 13A). P1 peptide injection had no effect on this production, while P5 attenuated TNF- α production by ~30%.

P1 delayed the IL-1 β peak until the third hour after LPS injection, but without attenuation. By contrast, P5 strongly reduced IL-1 β release (Figure 13B).

Nitrite/nitrate concentrations increased rapidly after LPS administration in control and P1 treated animals but remained stable upon P5 treatment (Figure 14).

Table 6. Hemodynamic parameters during LPS-induced endotoxemia

		Heart Rate (bpm)	MAP (mmHg)	Aortic blood flow (mL/min)	Mesenteric blood flow (mL/min)	pH	Lactate (mmol/L)
Control	H0	486±13	123±21	45±7	13.6±3.4	7.31±0.03	3.3±0.8
	H1	522±16	103±25	25±8 ^a	9.6±3.3	7.28±0.03	4.2±0.3
	H2	516±13	98±23	12±5 ^{a,b}	8.0±3.7	7.29±0.03	5.9±0.6
	H3	490±20	78±8 ^{a,b}	8±3 ^{a,b}	5.8±1.1	7.26±0.01	7.9±1.8 ^{a,b}
	H4	510±18	67±9 ^{a,b}	6±1 ^{a,b}	4.1±0.8	7.03±0.10 ^{a,b}	11.5±0.7 ^{a,b}
P1	H0	464±25	116±10	49±11	12.0±3.7	7.32±0.04	2.7±0.1
	H1	492±26	119±14	39±12 ^a	10.5±1.7	7.29±0.04	4.9±1.1
	H2	492±26	113±21	26±14 ^a	7.7±2.7	7.30±0.01	5.0±0.9
	H3	480±30	97±29 ^a	22±8 ^a	5.0±1.0	7.26±0.06	5.7±0.7 ^a
	H4	480±20	92±7 ^a	16±6 ^a	4.8±0.9	7.26±0.08 ^a	7.9±1.7 ^a
P5	H0	474±49	115±16	48±9	12.8±6.4	7.33±0.04	3.4±1.5
	H1	498±26	99±22	32±8	11.4±2.7	7.28±0.06	5.4±1.4
	H2	510±42	101±18	23±4 ^b	9.2±1.9	7.32±0.07	5.5±1.6
	H3	517±62	93±21 ^b	20±7 ^b	6.0±0.8	7.29±0.11	5.9±1.7 ^b
	H4	510±26	89±10 ^b	15±6 ^b	5.0±1.0	7.28±0.12 ^b	7.4±1.8 ^b

^a p<0.05 P1 vs Controls

5 ^b p<0.05 P5 vs Controls

CLP Model

As the severity of the Inventors' model was at its highest 16 to 20 hours after the completion of the CLP, the Inventors chose to investigate animals by the 16th hour. Importantly, there were no deaths before this time point. Although all animals were fluid resuscitated, none received antibiotics in order to strictly consider the role of the peptides.

There was a dramatic decline in arterial pressure in the control animals over time, and by H24 systolic, diastolic and mean arterial pressures were 58 ± 7 mmHg, 25 ± 4 mmHg and 38 ± 2 mmHg respectively. This decrease was almost totally abolished with P1 or P5 treatment with no significant difference between H16 and H24 (Figure 15). There was no difference between P1 and P5 treated rats.

TREM-1 peptides also prevented the aortic and mesenteric blood flows decrease observed in control animals (Table 7). The protective effect on mesenteric blood flow alterations was even higher under P5 treatment. The relative preservation of blood flows was not related to an increased heart rate, since the latter was rather slower than in control animals (Table 7).

The progressive metabolic acidosis that developed in control rats was attenuated by the P1 peptide, and almost abrogated by P5. The same protective trend was observed for arterial lactate elevation with a more pronounced effect of P5 (Table 7).

Table 7. Hemodynamic and selected biochemical parameters during CLP polymicrobial sepsis

		Heart Rate (bpm)	Aortic blood flow (mL/min)	Mesenteric blood flow (mL/min)	pH	Bicarbonate (mmol/L)	Lactate (mmol/L)
Control	H16	$516\pm 44^{a,b}$	38 ± 10	10.6 ± 3.0^b	7.31 ± 0.07^b	16.9 ± 2.7	4.7 ± 1.5^b
	H20	$543\pm 35^{a,b}$	$19\pm 11^{a,b}$	4.3 ± 1.5^b	$7.23\pm 0.05^{a,b}$	$12.0\pm 5.6^{a,b}$	$8.5\pm 1.4^{a,b}$
	H24	480 ± 20	$14\pm 9^{a,b}$	2.5 ± 0.7^b	$7.17\pm 0.01^{a,b}$	10.3 ± 3.3^a	$10.8\pm 1.9^{a,b}$
P1	H16	462 ± 16^a	41 ± 12	13.5 ± 7.2	7.32 ± 0.04	16.8 ± 4.4	4.9 ± 0.4
	H20	480 ± 30^a	28 ± 17^a	5.3 ± 3.0^c	7.31 ± 0.18^a	16.0 ± 5.4^b	$5.3\pm 1.1^{a,c}$
	H24	420 ± 30	22 ± 16^a	4.5 ± 2.1^c	$7.24\pm 0.06^{a,c}$	11.2 ± 0.8^c	$6.8\pm 0.9^{a,c}$
P5	H16	460 ± 17^b	41 ± 14	15.3 ± 3.5^b	7.35 ± 0.01^b	18.6 ± 2.0	3.3 ± 0.4^b
	H20	500 ± 17^b	31 ± 5^b	$11.0\pm 6.9^{b,c}$	7.34 ± 0.01^b	18.0 ± 0.9^a	$3.6\pm 0.9^{b,c}$
	H24	510 ± 20	28 ± 8^b	$8.5\pm 3.5^{b,c}$	$7.36\pm 0.01^{b,c}$	$17.1\pm 0.9^{a,c}$	$4.9\pm 1.1^{b,c}$

^a $p < 0.05$ P1 vs Controls

^b $p < 0.05$ P5 vs Controls

^c $p < 0.05$ P5 vs P1

Both P1 and P5 induced a decrease in TNF- α production, again with a stronger effect of P5. By H20, plasmatic TNF- α was almost undetectable under P5 treatment whereas it remained elevated in the other groups of animals (Figure 16).

5 Nitrite/nitrate concentrations were increased in control animals but remained at a low level in both TREM-1 peptides treated groups (Figure 17).

A protective action of both P5 and P1 on hemodynamics was thus observed in septic rats. Both arterial pressure and blood flows were preserved, independently of heart rate. Moreover, modulation of TREM-1 signalling reduced, although not completely, cytokine
10 production and protected septic animals from hyper-responsiveness. The fact that the cytokine production was not totally inhibited is a crucial point. Indeed, although inflammatory cytokines such as TNF- α are considered deleterious, they also display beneficial effects in sepsis as underlined by the fatal issue of peritonitis models in animals with impaired TNF- α responses.

The activation of iNOS observed during septic shock leads to the production of large
15 amount of NO that partly explains some of the peripheral vascular disorders (notably vasodilation and hypotension). On the myocardium itself, most of the action of NO is mediated by an activation of the soluble guanylate-cyclase responsible for the production of cGMP which impairs the effect of cytosolic calcium on contraction. Cyclic GMP is also able to stimulate the activity of some phosphodiesterases. The subsequent decrease of intra-cellular cAMP levels
20 could explain the ability of NO to attenuate the effects of beta adrenergic stimulation. The preservation of arterial pressure could therefore be partly explained by a lessened production of NO, as reflected by the lower concentrations of plasma nitrite/nitrate in TREM-1 peptides treated animals.

The decrease in inflammatory cytokine production could partly explain the effect noted
25 on blood flows. Indeed, although the list of potential cytokine mediators of myocardial depression is long, TNF- α and IL-1 β have been shown to be good candidates. Both these latter cytokines depressed myocardial contractility in vitro or ex vivo. Moreover, the neutralization or removal of TNF- α or IL-1 β from human septic serum partly abrogates the myocardial depressant effect in vitro and in vivo. Although P1 and P5 had an identical action on blood flows
30 and arterial pressure during endotoxemia, their action on cytokine production differed with only a slight effect of P1 on plasma TNF- α and IL-1 β concentrations. The protective role of the TREM-1 peptides could therefore be only partly related to their action on cytokine release, or involve redundant pathways.

The modulation of the TREM-1 pathway by the use of small synthetic peptides had
35 beneficial effects on haemodynamic parameters during experimental septic shock in rats, along with an attenuation of inflammatory cytokine production.

In summary, these data show that the TREM-1 peptides of the invention 1) efficiently protect subject animals from sepsis-related hemodynamic deterioration; 2) attenuate the development of lactic acidosis; 3) modulate the production of such pro-inflammatory cytokines as TNF- α and IL-1 β and 4) decrease the generation of nitric oxide. Thus TREM-1 peptides are potentially useful in the restoration of haemodynamic parameters in patients with sepsis, septic shock or sepsis-like conditions and therefore constitute a potential treatment for the aforesaid conditions.

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CLAIMS

1. A polypeptide comprising one or more sequences derived from CDR2 or CDR3 of a
TREM-1 protein, characterised by the ability to treat, ameliorate, or lessen the symptoms of
5 sepsis, septic shock or sepsis-like conditions.
2. A polypeptide according to claim 1 wherein said polypeptide comprises fewer than 30
contiguous amino acids of said TREM-1 protein.
- 10 3. A TREM-1 polypeptide having anti-sepsis or anti-septic shock activity which consists of
(i) a contiguous sequence of 15-25 amino acids corresponding to a native TREM-1 protein
sequence which includes at least 3 amino acids from the CDR2 or CDR3 sequences; or (ii) such
a sequence in which one or more amino acids are substituted conservatively with another
amino acid provided, however that at least 3 amino acids from the CDR2 or CDR3 sequences
15 are not substituted; or (iii) a sequence of (i) or (ii) linked at one or both of its N and C termini to a
heterologous polypeptide.
4. A polypeptide according to claim 3 wherein the native TREM-1 protein sequence is the
human sequence identified as SEQ ID No 1 and the CDR2 and CDR3 sequences are RPSKNS
20 and QPPKE respectively.
5. A polypeptide according to claim 4 wherein the at least 3 amino acids from the CDR2 or
CDR3 sequences are QPP.
- 25 6. A polypeptide according to claim 4 wherein the at least 3 amino acids from the CDR2 or
CDR3 sequences are QPPK.
7. A polypeptide according to claim 4 wherein the at least 3 amino acids from the CDR2 or
CDR3 sequences are QPPKE.
30
8. A polypeptide according to claim 4 wherein the at least 3 amino acids from the CDR2 or
CDR3 sequences are RPSKNS.
9. A polypeptide according to claim 3 wherein the native TREM-1 protein sequence is the
35 mouse sequence identified as SEQ ID NO: 2 and the CDR2 and CDR3 sequences are RPFTRP
and HPPND respectively.

10. A polypeptide according to claim 9 wherein the at least 3 amino acids from the CDR2 or CDR3 sequences are HPP.
11. A polypeptide according to claim 9 wherein the at least 3 amino acids from the CDR2 or
5 CDR3 sequences are HPPN.
12. A polypeptide according to claim 9 wherein the at least 3 amino acids from the CDR2 or CDR3 sequences are HPPND.
- 10 13. A polypeptide according to claim 9 wherein the at least 3 amino acids from the CDR2 or CDR3 sequences are RPFTRP.
14. A polypeptide according to any of the preceding claims wherein said polypeptide is characterised by the ability to treat, ameliorate, or lessen the symptoms of sepsis, septic shock
15 or sepsis-like conditions.
15. A polypeptide having substantial sequence identity to sequence any one of SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 and characterised by the ability to treat, ameliorate, or lessen the symptoms of sepsis, septic shock or sepsis-like conditions
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16. A polypeptide of sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19, its active fragments, analogues, and derivatives, and characterised by the ability to treat, ameliorate, or lessen the symptoms of sepsis, septic shock or sepsis-like conditions.
- 25 17. A polypeptide having at least 80% sequence identity to the sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 and characterised by the ability to treat sepsis, septic shock or sepsis-like conditions and having at least two consecutive amino acids derived from CDR2 or CDR3 of a TREM-1 protein.
- 30 18. A polypeptide having at least 80% sequence identity to the sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 and characterised by the ability to treat sepsis, septic shock or sepsis-like conditions, wherein the peptide has at least three consecutive amino acids derived from CDR2 or CDR3 of a TREM-1 protein.
- 35 19. An isolated polynucleotide capable of encoding a polypeptide having substantial sequence identity to the sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 and characterised by the ability to treat sepsis, septic shock or sepsis-like conditions.

20. A vector which contains a polynucleotide capable of encoding a polypeptide having at least about 80% sequence identity to the sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 and characterised by the ability to treat sepsis, septic shock or sepsis-like conditions
- 5
21. A polypeptide according to any one of claims 15 to 20 wherein said polypeptide comprises less than 30 contiguous amino acids of a native TREM-1 protein.
22. A composition comprising a peptide or polypeptide of any one of the preceding claims
- 10
23. The peptide or polypeptide of any one of the preceding claims for use in therapy
24. The peptide or polypeptide of any one of the preceding claims for use in therapy in the treatment of sepsis, septic shock or sepsis-like conditions
- 15
25. Use of the peptide or polypeptide of any one of the preceding claims in the manufacture of a medicament for the treatment of sepsis, septic shock or sepsis-like conditions
26. A method of treating or ameliorating sepsis, septic shock or sepsis-like conditions in a subject, comprising:
- 20 administering a therapeutically effective amount of a peptide or polypeptide of any one of the preceding claims to a subject
27. The method of claim 26 where in said peptide or polypeptide has substantial sequence identity to sequence SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19.
- 25
28. The method of claim 26, wherein said peptide or polypeptide is SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19 or an active fragment, analogue or derivative of SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19
- 30
29. The method of claim 26, wherein said peptide or polypeptide has at least about 80% sequence identity to SEQ ID NOS: 3, 4, 6, 7, 16, 17, 18 or 19
30. A method of screening compounds or compositions to treat sepsis, septic shock or sepsis-like conditions, comprising:
- 35 providing a TREM-1 peptide or TREM-1 polypeptide;
contacting an animal in a suitable model with the TREM-1 peptide;

determining if there was a modulation in the sepsis, wherein an increase in survival indicates that the TREM-1 peptide may be useful for treating sepsis, septic shock or sepsis-like conditions.

- 5 31. A polypeptide, vector use, method or composition according to any one of the preceding claims wherein the indication is sepsis or septic-shock.

Figure 2

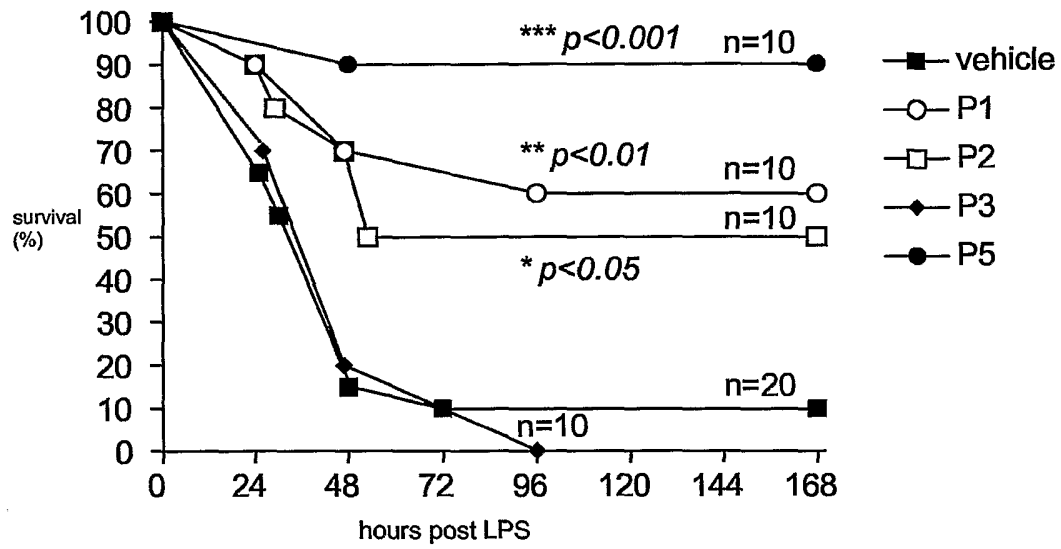


Figure 3

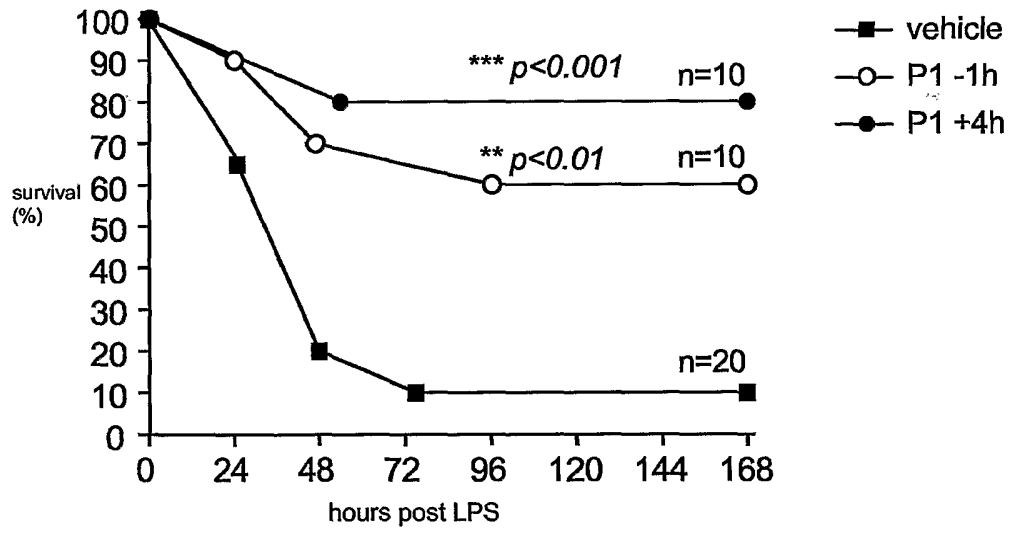


Figure 4

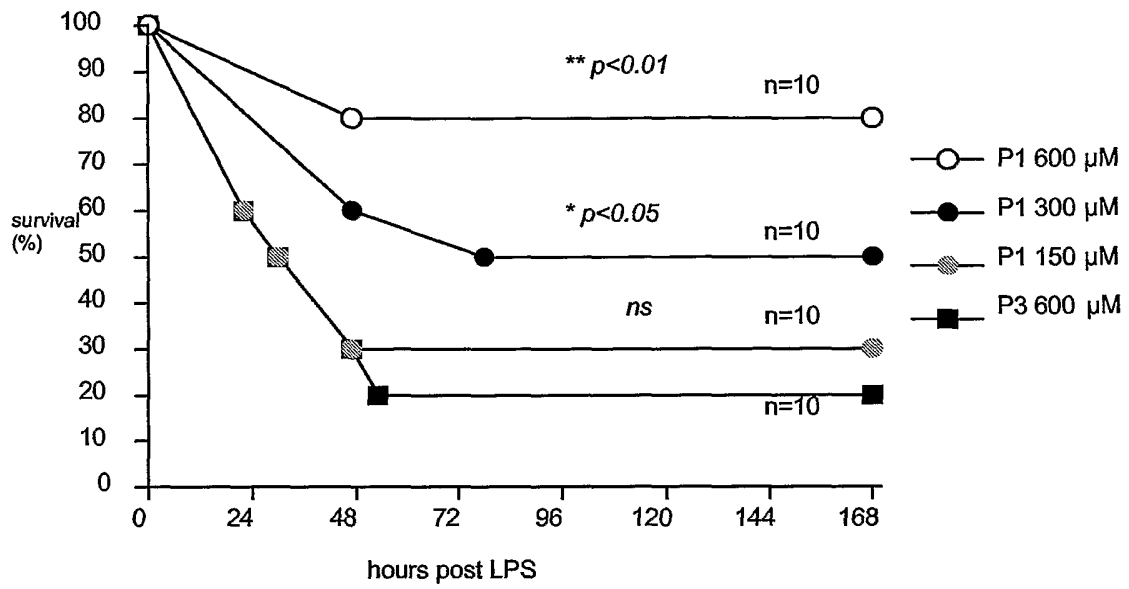


Figure 5

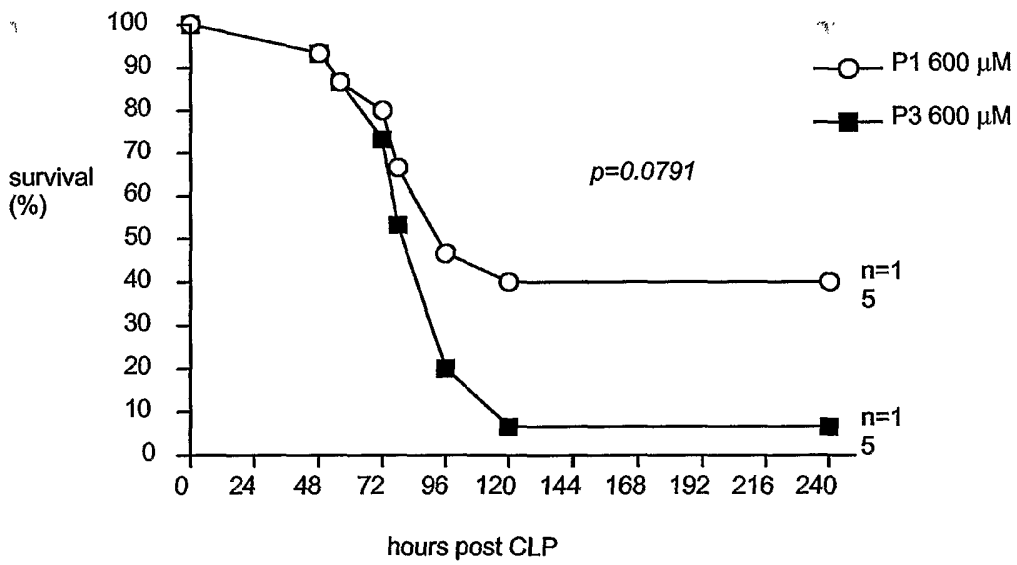
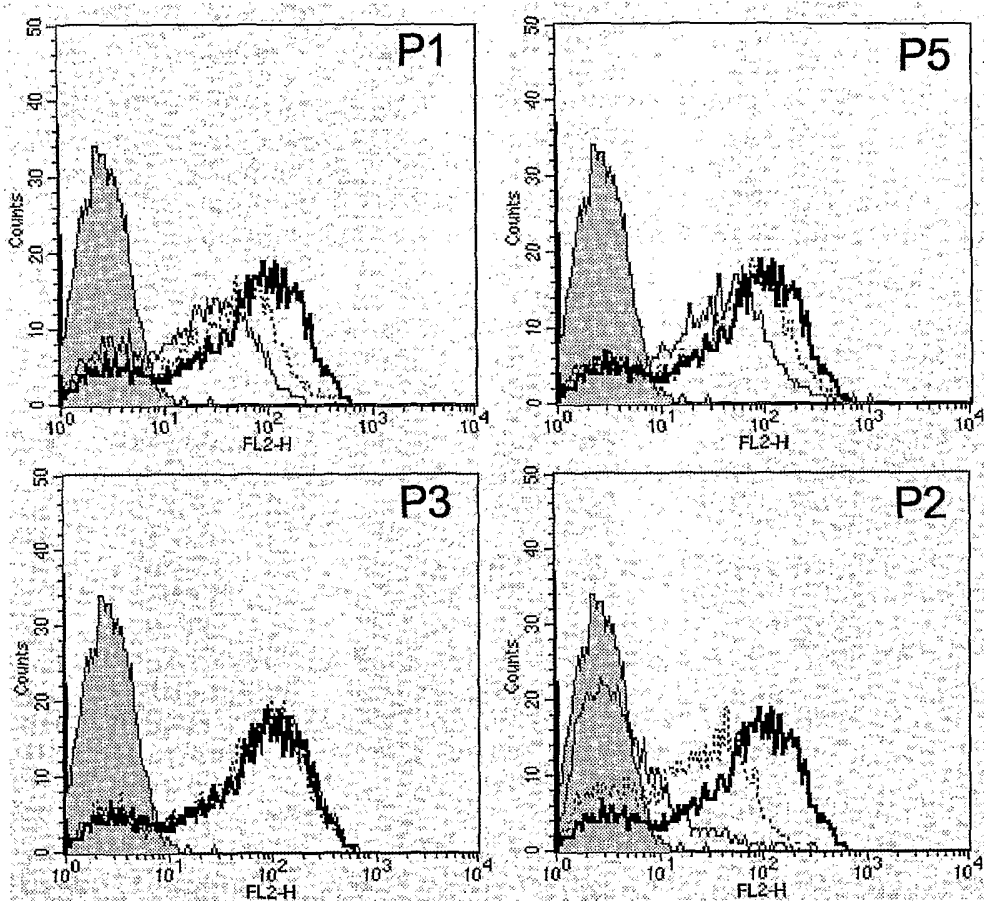


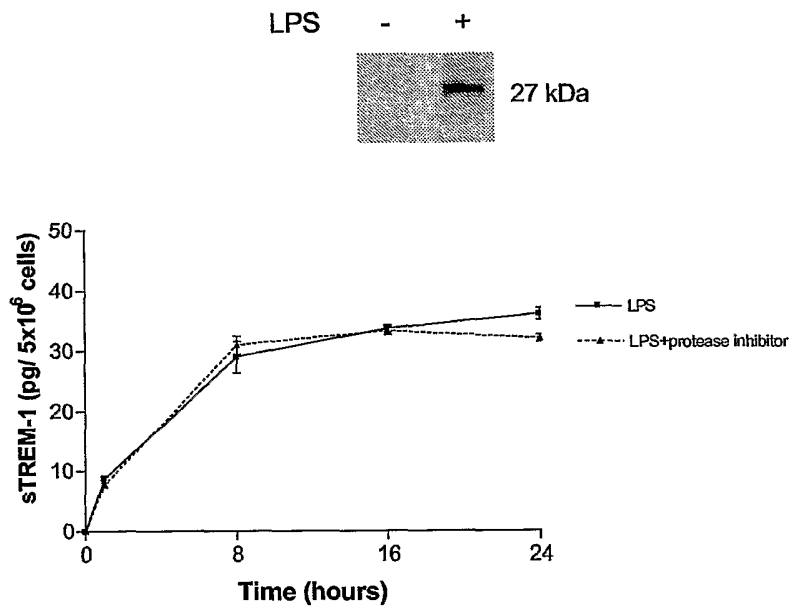
Figure 6



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Figure 7

A



B

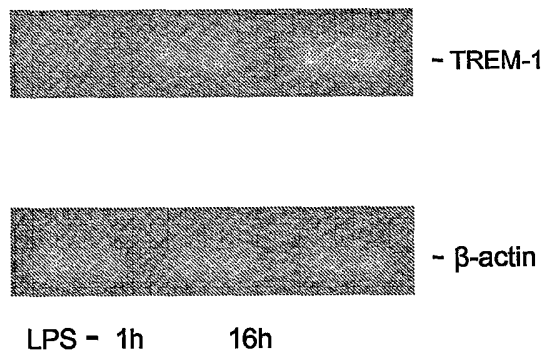


Figure 8A

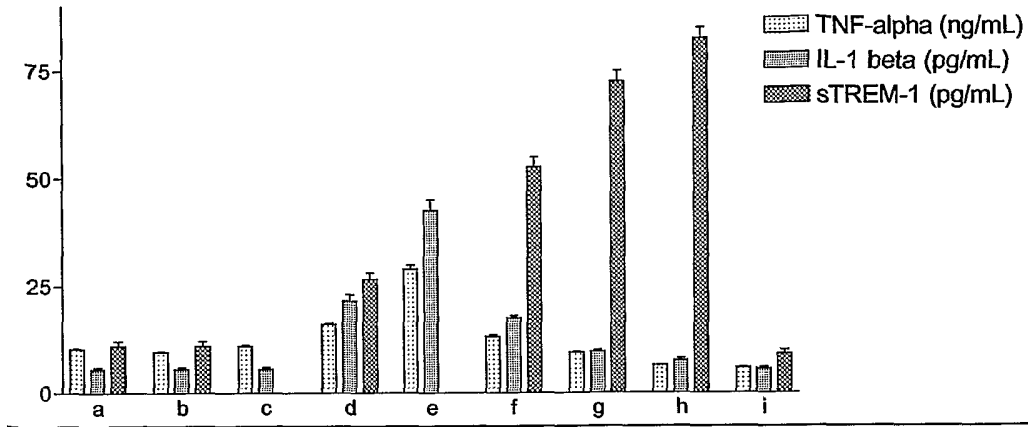


Figure 8B

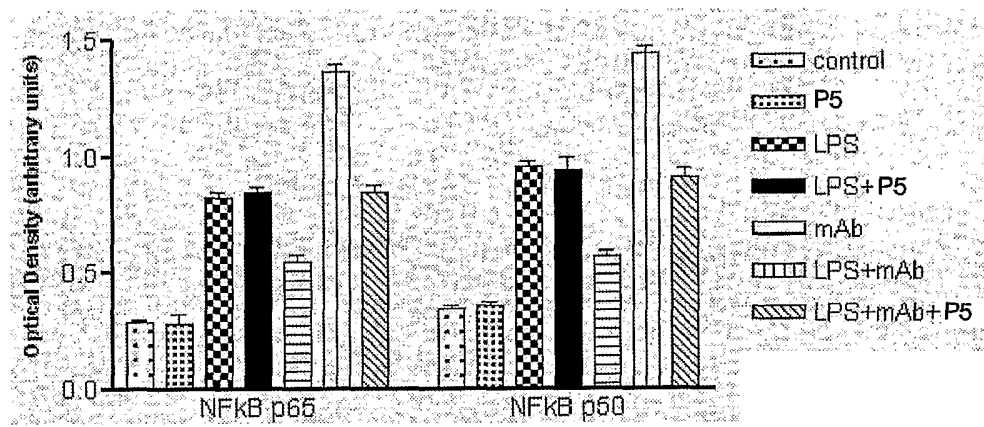


Figure 9

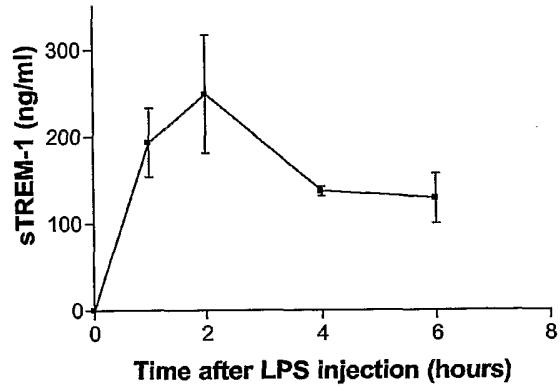


Figure 10A

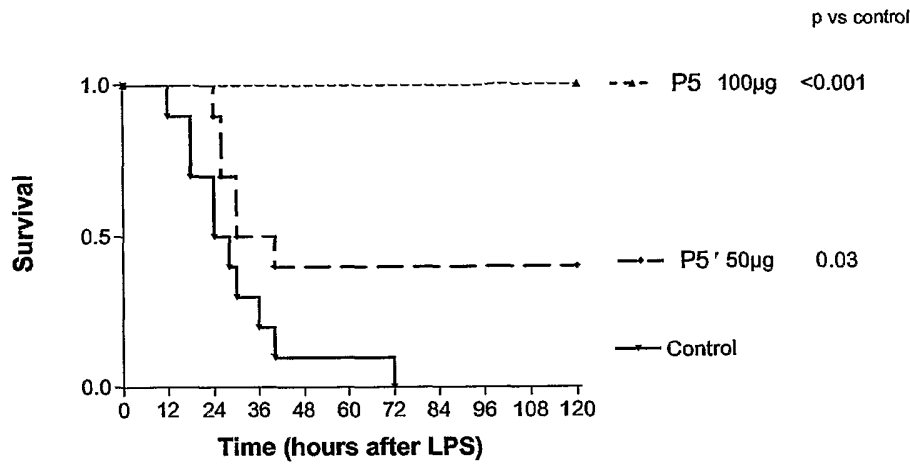


Figure 10B

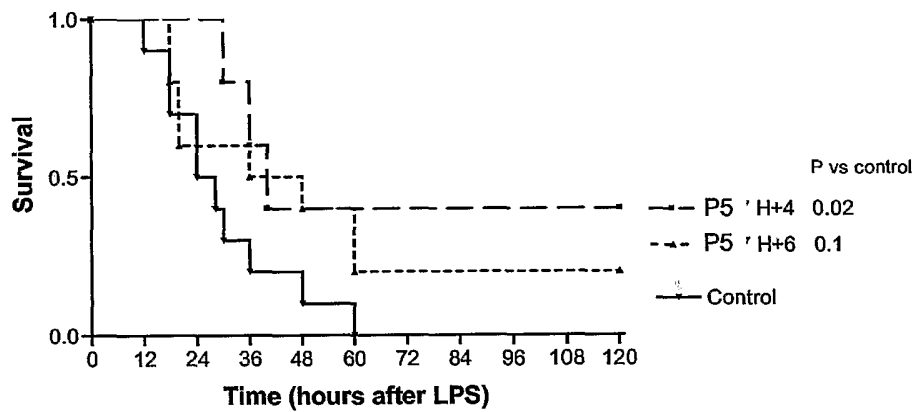
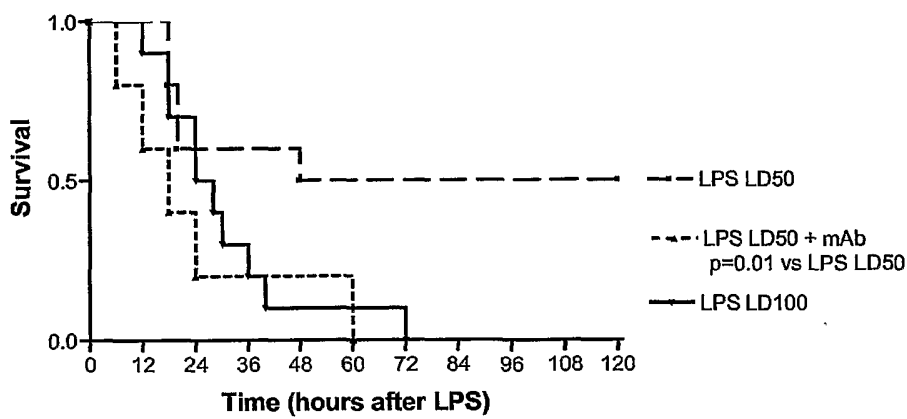


Figure 10C



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Figure 11A

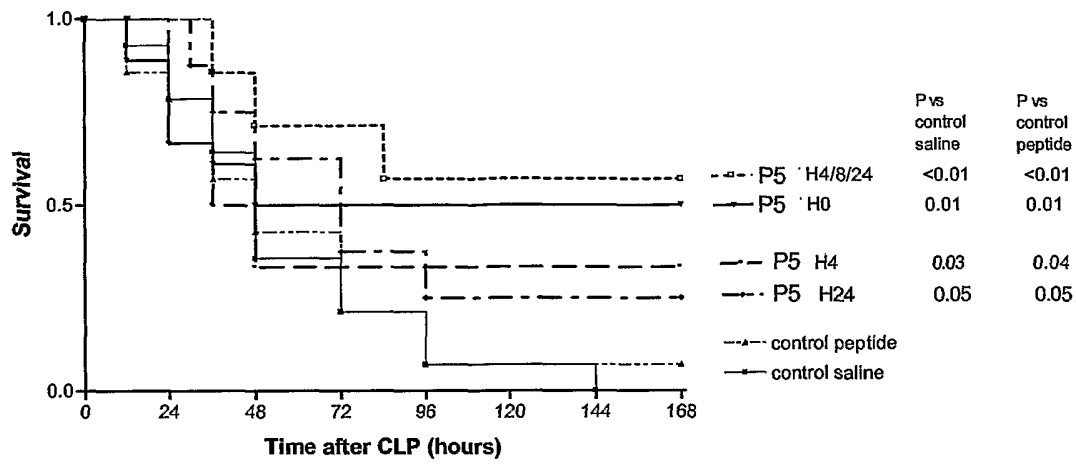
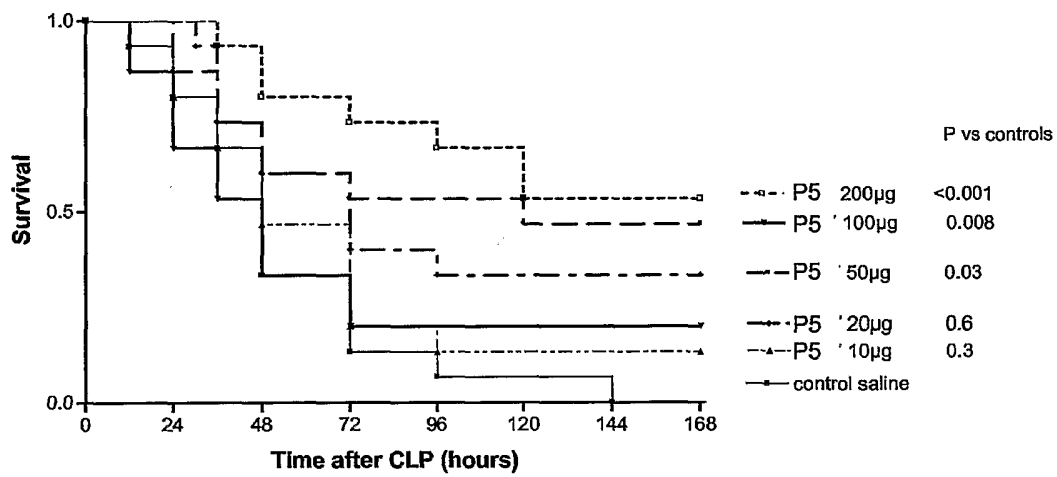


Figure 11B



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Figure 12

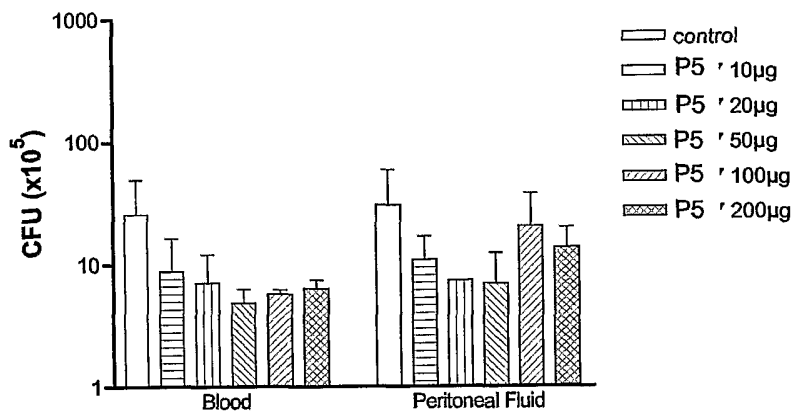


Figure 13A

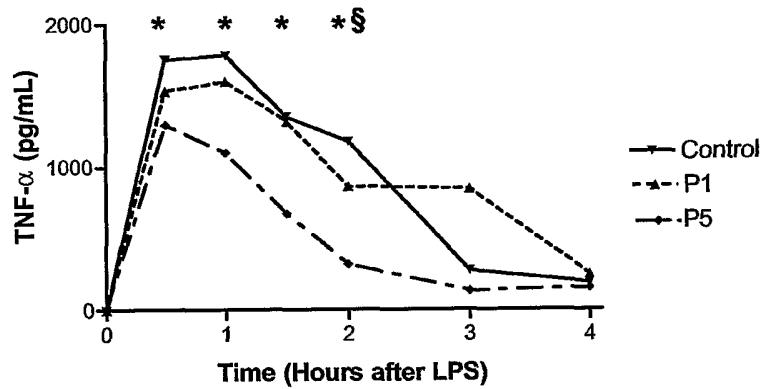


Figure 13B

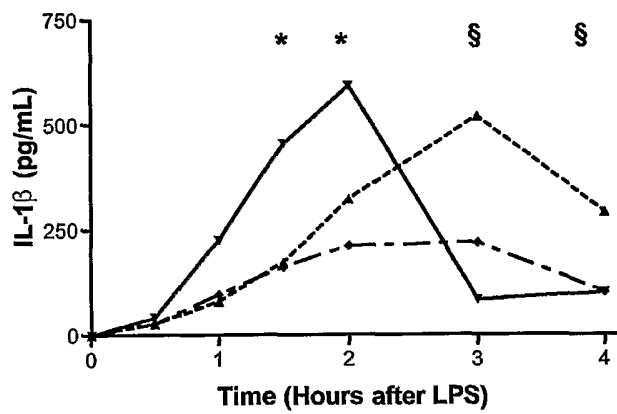


Figure 14

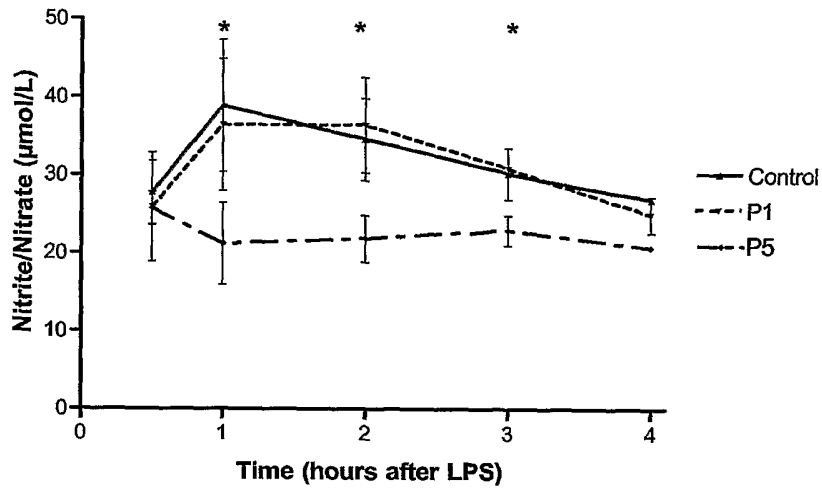


Figure 15

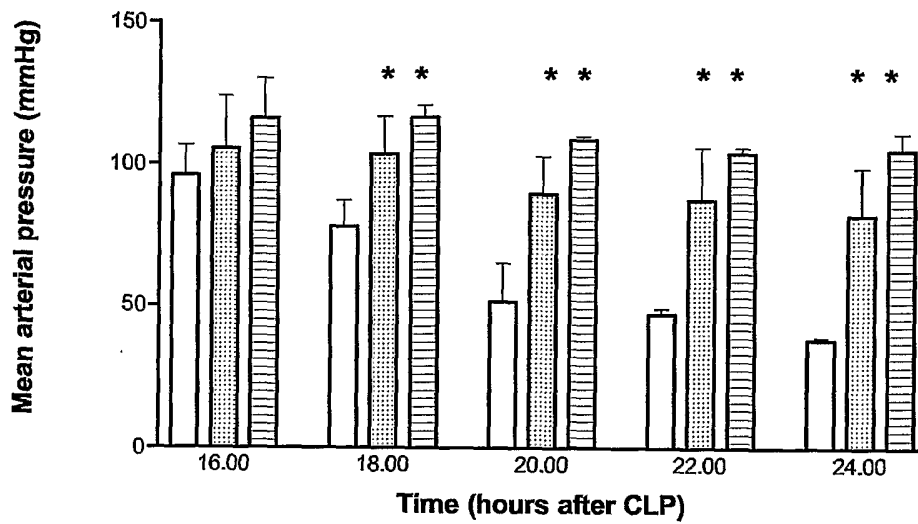


Figure 16

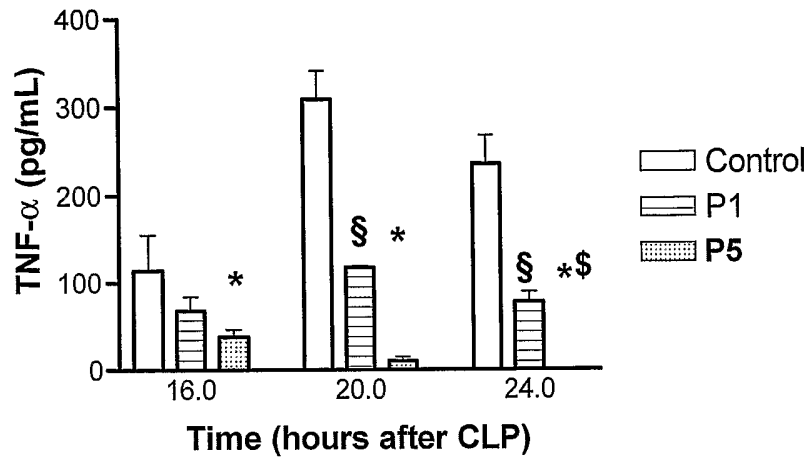
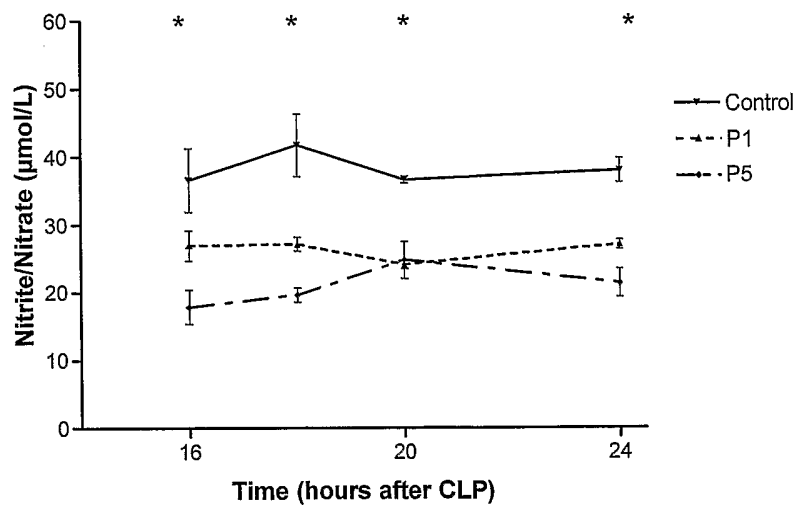


Figure 17



PCT REQUEST

Print Out (Original in Electronic Form)

VIII-5-1	Declaration: Non-prejudicial disclosure or exceptions to lack of novelty Declaration as to non-prejudicial disclosures or exceptions to lack of novelty (Rules 4.17(v) and 51 bis.1(a)(v)): Name (LAST, First)	in relation to this international application The Applicants declares that the subject matter claimed in this international application was disclosed as follows:
VIII-5-1(i)	Kind of disclosure:	abuse
VIII-5-1(i)	Date of disclosure:	22 November 2004 (22.11.2004)
VIII-5-1(i) ii)	Title of disclosure:	A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. Journal of Experimental Medicine 2004 200:1419-1426
VIII-5-1(i)	Place of disclosure:	
VIII-5-1(v)	This declaration is made for the purposes of:	AP: (BW GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW); EA: (AM AZ BY KG KZ MD RU TJ TM); EP: (AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU MC NL PL PT RO SE SI SK TR); OA: (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG); AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/052338

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/705 A61K38/17 A61P37/02 G01N33/48 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, Sequence Search, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/165875 A1 (COLONNA MARCO ET AL) 4 September 2003 (2003-09-04) cited in the application paragraphs 13-25, 157, 158, 167, 281, 304, 305, 341, 364-370, ,38-343 - pages 6,387; claims	1-31
X	WO 2004/081233 A (BIOXELL S.P.A; MARIANI, MARGHERITA; SINIGAGLIA, FRANCESCO; PANINA, PAO) 23 September 2004 (2004-09-23) page 11; claims	1-31

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document but published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
O document referring to an oral disclosure, use, exhibition or other means	*Z* document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 22 November 2005	Date of mailing of the international search report 20/12/2005
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Madruga, J
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/052338

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOUCHON A ET AL: "TREM-1 amplifies inflammation and is a crucial mediator of septic shock" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 410, no. 6832, 26 April 2001 (2001-04-26), pages 1103-1107, XP002285055 ISSN: 0028-0836 cited in the application the whole document	1-31
X	COLONNA MARCO: "TREMs in the immune system and beyond." NATURE REVIEWS IMMUNOLOGY, vol. 3, no. 6, June 2003 (2003-06), pages 445-453, XP008055962 ISSN: 1474-1733 cited in the application the whole document	1-31
X	COLONNA M ET AL: "TREM-1 (TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS): A NEW PLAYER IN ACUTE INFLAMMATORY RESPONSES" JOURNAL OF INFECTIOUS DISEASES, CHICAGO, IL, US, vol. 187, no. SUPPL 2, 15 June 2003 (2003-06-15), pages S397-S401, XP008033778 ISSN: 0022-1899 cited in the application the whole document	1-31
P,X	GIBOT SEBASTIEN ET AL: "A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 200, no. 11, 6 December 2004 (2004-12-06), pages 1419-1426, XP002355335 ISSN: 0022-1007 the whole document	1-31
A	RADAEV SERGEI ET AL: "Crystal structure of the human myeloid cell activating receptor TREM-1." STRUCTURE (CAMBRIDGE), vol. 11, no. 12, December 2003 (2003-12), pages 1527-1535, XP002355336 ISSN: 0969-2126 cited in the application the whole document	

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/052338

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>US 6 420 526 B1 (RUBEN STEVEN M ET AL) 16 July 2002 (2002-07-16) cited in the application column 50, line 1; sequences 673,689,717 columns 78,79 column 120; claims</p> <p style="text-align: center;">-----</p>	<p>1-5, 15-31</p>

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 26-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: -

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). The sequence of a native TREM-1 protein as well as the sequence of the CDR2 or CDR3 is undefined. Thus, the peptides claimed are completely undefined by structural features and are only defined in terms of the result to be achieved, namely their ability to treat sepsis. For these reasons, the search was performed taking into consideration the non-compliance in determining the extent of the search of the claims. The search was restricted to fragments of 15-30 amino acids of the human TREM-1 protein (SEQ ID NO: 1), or the murine TREM-1 protein (SEQ ID NO: 2) as well as peptides of 15-30 amino acids comprising the following sequences derived from the CDR2 or CDR3 of TREM-1: RPSKNS, QPPKE, QPP, QPPK, RPFTRP, HPPND, HPP, HPPN.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/052338

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2005/052338

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003165875 A1	04-09-2003	NONE	
WO 2004081233 A	23-09-2004	AU 2004219885 A1	23-09-2004
US 6420526 B1	16-07-2002	NONE	