RAGE FUSION PROTEINS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 USC 119(e) from U.S. Provisional Patent Application Serial No. 60/598,555 filed August 3, 2004. The disclosure of U.S. Provisional Patent Application 60/ 598,555 is hereby incorporated by reference in its entirety herein.

FIELD OF THE INVENTION

The present invention relates to regulation of the Receptor for Advanced Glycated Endproducts (RAGE). More particularly, the present invention describes fusion proteins comprising a RAGE polypeptide, methods of making such fusion proteins, and the use of such proteins for treatment of RAGE-based disorders.

BACKGROUND

Incubation of proteins or lipids with aldose sugars results in nonenzymatic glycation and oxidation of amino groups on proteins to form Amadori adducts. Over time, the adducts undergo additional rearrangements, dehydrations, and cross-linking with other proteins to form complexes known as Advanced Glycosylation End Products (AGEs). Factors which promote formation of AGEs include delayed protein turnover (*e.g.* as in amyloidoses), accumulation of macromolecules having high lysine content, and high blood glucose levels (*e.g.* as in diabetes) (Hori *et al., J. Biol. Chem.* **270**: 25752-761, (1995)). AGEs have been implicated in a variety of disorders including complications associated with diabetes and normal aging.

AGEs display specific and saturable binding to cell surface receptors on monocytes, macrophages, endothelial cells of the microvasculature, smooth muscle cells, mesengial cells, and neurons. The Receptor for Advanced Glycated Endproducts (RAGE) is a member of the immunoglobulin supergene family of molecules. The extracellular (N-terminal) domain of RAGE includes three immunoglobulin-type regions: one V (variable) type domain followed by two C-type (constant) domains (Neeper *et al., J. Biol. Chem.*, **267**:14998-15004 (1992); Schmidt *et al., Circ. (Suppl.)* **96**#194 (1997)). A single transmembrane spanning domain and a short, highly charged cytosolic tail follow the extracellular domain. The N-terminal, extracellular domain can be isolated by proteolysis of RAGE or by molecular biological approaches to generate soluble RAGE (sRAGE) comprised of the V and C domains.

RAGE is expressed on multiple cell types including leukocytes, neurons, microglial cells and vascular endothelium (e.g., Hori *et al., J. Biol. Chem.*, **270**:25752-761 (1995)).

Increased levels of RAGE are also found in aging tissues (Schleicher *et al., J. Clin. Invest.*, **99 (3)**: 457-468 (1997)), and the diabetic retina, vasculature and kidney (Schmidt *et al., Nature Med.*, 1:1002-1004 (1995)).

In addition to AGEs, other compounds can bind to and modulate RAGE. RAGE binds to multiple functionally and structurally diverse ligands including amyloid beta (Aβ), serum amyloid A (SAA), Advanced Glycation End products (AGEs), S100 (a proinflammatory member of the Calgranulin family), carboxymethyl lysine (CML), amphoterin and CD11b/CD18 (Bucciarelli *et al., Cell Mol. Life Sci.*, **59**:1117-128 (2002); Chavakis *et al., Microbes Infect.*, **6**:1219-1225 (2004); Kokkola *et al., Scand. J. Immunol.*, **61**:1-9 (2005); Schmidt *et al., J. Clin. Invest.*, **108**:949-955 (2001); Rocken *et al., Am. J. Pathol.*, **162**:1213-1220 (2003)).

Binding of ligands such as AGEs, S100/calgranulin, β-amyloid, CML (N^{ε}-Carboxymethyl lysine), and amphoterin to RAGE has been shown to modify expression of a variety of genes. These interactions may then initiate signal transduction mechanisms including p38 activation, p21ras, MAP kinases, Erk1-2 phosphorylation, and the activation of the transcriptional mediator of inflammatory signaling, NF-κB (Yeh *et al., Diabetes*, **50**:1495-1504 (2001)). For example, in many cell types, interaction between RAGE and its ligands can generate oxidative stress, which thereby results in activation of the free radical sensitive transcription factor NF-κB, and the activation of NF-κB regulated genes, such as the cytokines IL-1β and TNF-α. Furthermore, RAGE expression is upregulated via NF-κB and shows increased expression at sites of inflammation or oxidative stress (Tanaka *et al., J. Biol. Chem.*, **275**:25781-25790 (2000)). Thus, an ascending and often detrimental spiral may be fueled by a positive feedback loop initiated by ligand binding.

Activation of RAGE in different tissues and organs can lead to a number of pathophysiological consequences. RAGE has been implicated in a variety of conditions including: acute and chronic inflammation (Hofmann *et al., Cell* **97**:889-901 (1999)), the development of diabetic late complications such as increased vascular permeability (Wautier *et al., J. Clin. Invest.*, **97**:238-243 (1995)), nephropathy (Teillet *et al., J. Am. Soc. Nephrol.*, **11**:1488-1497 (2000)), arteriosclerosis (Vlassara *et. al., The Finnish Medical Society DUODECIM, Ann. Med.*, **28**:419-426 (1996)), and retinopathy (Hammes *et al., Diabetologia*, **42**:603-607 (1999)). RAGE has also been implicated in Alzheimer's disease (Yan *et al., Nature*, **382**:685-691 (1996)), and in tumor invasion and metastasis (Taguchi *et al., Nature*, **405**:354-357 (2000)).

Despite the broad expression of RAGE and its apparent pleiotropic role in multiple diverse disease models, RAGE does not appear to be essential to normal development. For example, RAGE knockout mice are without an overt abnormal phenotype, suggesting that while RAGE can play a role in disease pathology when stimulated chronically, inhibition of RAGE does not appear to contribute to any unwanted acute phenotype (Liliensiek *et al., J. Clin. Invest.*, 113:1641-50 (2004)).

Antagonizing binding of physiological ligands to RAGE may down-regulate the pathophysiological changes brought about by excessive concentrations of AGEs and other RAGE ligands. By reducing binding of endogenous ligands to RAGE, symptoms associated with RAGE-mediated disorders may be reduced. Soluble RAGE (sRAGE) is able to effectively antagonize the binding of RAGE ligands to RAGE. However, sRAGE can have a half-life when administered *in vivo* that may be too short to be therapeutically useful for one or more disorders. Thus, there is a need to develop compounds that antagonize the binding of AGEs and other physiological ligands to the RAGE receptor where the compound has a desireable pharmacokinetic profile.

SUMMARY

In a first aspect, there is provided a fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin, and a C_H3 domain of an immunoglobulin;

wherein the RAGE polypeptide comprises a RAGE ligand binding site;

wherein the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the RAGE interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising the C_H2 domain of the immunoglobulin, or a portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin; and

wherein the fusion protein comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

In a second aspect, there is provided a nucleic acid encoding a fusion protein as defined above.

In a third aspect, there is provided an expression vector that encodes for a fusion protein as defined above and/or comprises a nucleic acid as defined above.

In a fourth aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of a fusion protein as defined above in a pharmaceutical carrier.

In a fifth aspect, there is provided a method of making a fusion protein as defined above comprising the step of covalently linking the RAGE polypeptide to the polypeptide comprising the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin.

In a sixth aspect, there is provided the use of a fusion protein as defined above, in the preparation of a medicament for the treatment of a RAGE-mediated disorder in a subject.

In a seventh aspect, there is provided a host cell comprising an expression vector as defined above.

In an eighth aspect, there is provided a method of producing a fusion protein comprising culturing a host cell as defined above.

Embodiments of the present disclosure comprise RAGE fusion proteins and methods of using such proteins. The present invention may be embodied in a variety of ways. Embodiments of the present invention may comprise a fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the fusion protein comprises a RAGE ligand binding site. The fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising C_H2 domain of an immunoglobulin, or a portion of the C_H2 domain.

The present disclosure also comprises a method to make a RAGE fusion protein. In one embodiment the method comprises linking a RAGE polypeptide to a second, non-RAGE polypeptide. In one embodiment, the RAGE polypeptide comprises a RAGE ligand binding site. The method may comprise linking a RAGE polypeptide directly to a polypeptide comprising the C_H2 domain of an immunoglobulin or a portion of the C_H2 domain.

In other embodiments, the present disclosure may comprise methods and compositions for treating a RAGE-mediated disorder in a subject. The method may comprise administering a fusion protein of the present invention to the subject. The composition may comprise a RAGE fusion protein of the present invention in a pharmaceutically acceptable carrier.

There are various advantages that may be associated with particular embodiments of the present disclosure. In one embodiment, the fusion proteins of the present disclosure may be metabolically stable when administered to a subject. Also, the fusion proteins of the present disclosure may exhibit high-affinity binding for RAGE ligands. In certain embodiments, the fusion proteins of the present disclosure bind to RAGE ligands with affinities in the high nanomolar to low micromolar range. By binding with high affinity to physiological RAGE ligands, the fusion proteins of the present disclosure may be used to inhibit binding of endogenous ligands to RAGE, thereby providing a means to ameliorate RAGE-mediated diseases.

Also, the fusion proteins of the present invention may be provided in protein or nucleic acid form. In one example embodiment, the fusion protein may be administered systemically and remain in the vasculature to potentially treat vascular diseases mediated in part by RAGE. In another example embodiment, the fusion protein may be administered locally to treat diseases where RAGE ligands contribute to the pathology of the disease. Alternatively, a nucleic acid construct encoding the fusion protein may be delivered to a site by the use of an appropriate carrier such as a virus or naked DNA where transient local expression may locally inhibit the interaction between RAGE ligands and receptors. Thus, administration may be transient (*e.g.*, as where the fusion protein is administered) or more permanent in nature (*e.g.*, as where the fusion protein is administered as a recombinant DNA).

There are additional features of the disclosure which will be described hereinafter. It is to be understood that the disclosure is not limited in its application to the details set forth in the following claims, description and figures. The disclosure is capable of other embodiments and of being practiced or carried out in various ways.

BRIEF DESCRIPTION OF THE FIGURES

Various features, aspects and advantages of the present invention will become more apparent with reference to the following figures.

FIG. 1 shows various RAGE sequences in accordance with alternate embodiments of the present invention: **Panel A,** SEQ ID NO: 1, the amino acid sequence for human RAGE; and SEQ ID NO: 2, the amino acid sequence for human RAGE without the signal sequence of amino acids 1-22; **Panel B,** SEQ ID NO: 3, the amino acid sequence for human RAGE without the signal sequence of amino acids 1-23; **Panel C,** SEQ ID NO: 4, the amino acid sequence of human sRAGE; SEQ ID NO: 5, the amino acid sequence of human sRAGE without the signal sequence of amino acids 1-22, and SEQ ID NO: 6, the amino acid

sequence of human sRAGE without the signal sequence of amino acids 1-23; **Panel D**, SEQ ID NO: 7, an amino acid sequence comprising the V-domain of human RAGE; SEQ ID NO: 8, an alternate amino acid sequence comprising the V-domain of human RAGE; SEQ ID NO: 9, an N-terminal fragment of the V-domain of human RAGE; SEQ ID NO: 10, an alternate N-terminal fragment of the V-domain of human RAGE; SEQ ID NO: 11, the amino acid sequence for amino acids 124-221 of human RAGE; SEQ ID NO: 12, the amino acid sequence for amino acids 227-317 of human RAGE; SEQ ID NO: 13, the amino acid sequence for amino acids 23-123 of human RAGE; **Panel E**, SEQ ID NO: 14, the amino acid sequence for amino acids 24-123 of human RAGE; SEQ ID NO: 15, the amino acid sequence for amino acids 23-136 of human RAGE; SEQ ID NO: 16, the amino acid sequence for amino acids 24-136 of human RAGE; SEQ ID NO: 17, the amino acid sequence for amino acids 23-226 of human RAGE; SEQ ID NO: 18, the amino acid sequence for amino acids 24-226 of human RAGE; **Panel F**, SEQ ID NO: 19, the amino acid sequence for amino acids 23-251 of human RAGE; SEQ ID NO: 20, the amino acid sequence for amino acids 24-251 of human RAGE; SEQ ID NO: 21, a RAGE interdomain linker; SEQ ID NO: 22, a second RAGE interdomain linker; SEQ ID NO: 23, a third RAGE interdomain linker; SEQ ID NO: 24, a fourth RAGE interdomain linker; **Panel G**, SEQ ID NO: 25, DNA encoding human RAGE amino acids 1-118; SEQ ID NO: 26, DNA encoding human RAGE amino acids 1-123; and SEQ ID NO: 27, DNA encoding human RAGE amino acids 1-136; **Panel H**, SEQ ID NO: 28, DNA encoding human RAGE amino acids 1-230; and SEQ ID NO: 29, DNA encoding human RAGE amino acids 1-251; **Panel I**, SEQ ID NO: 38, a partial amino acid sequence for the C_H2 and C_H3 domains of human IgG; SEQ ID NO:39, DNA encoding a portion of the human C_H2 and C_H3 domains of human IgG; SEQ ID NO: 40, an amino acid sequence for the C_H2 and C_H3 domains of human IgG; **Panel J**, SEQ ID NO: 41, a DNA encoding the human C_H2 and C_H3 domains of human IgG; SEQ ID NO: 42, an amino acid sequence for the C_H2 domain of human IgG; SEQ ID NO: 43, an amino acid sequence for the C_H3 domain of human IgG; and SEQ ID NO: 44, a fifth RAGE interdomain linker.

FIG. 2 shows the DNA sequence (SEQ ID NO: 30) of a RAGE fusion protein (TTP-4000) coding region in accordance with an embodiment of the present invention. Coding sequence 1-753 highlighted in bold encodes RAGE N-terminal protein sequence whereas sequence 754-1386 encodes human IgG Fc (γ1) protein sequence.

FIG. 3 shows the DNA sequence (SEQ ID NO: 31) of an alternate RAGE fusion protein (TTP-3000) coding region in accordance with an embodiment of the present

invention. Coding sequence 1-408 highlighted in bold encodes RAGE N-terminal protein sequence, whereas sequence 409-1041 codes human IgG Fc (γ1) protein sequence.

FIG. 4 shows the amino acid sequences, SEQ ID NO: 32 (TTP-4000), SEQ ID NO: 33, and SEQ ID NO: 34, that each encode a four domain RAGE fusion protein in accordance with alternate embodiments of the present invention. RAGE sequence is highlighted with bold font.

FIG. 5 shows the amino acid sequences, SEQ ID NO: 35 (TTP-3000), SEQ ID NO: 36, and SEQ ID NO: 37, that each encode a three domain RAGE fusion protein in accordance with alternate embodiments of the present invention. RAGE sequence is highlighted with bold font.

FIG. 6, Panel A, shows a comparison of the protein domains in human RAGE and human Ig gamma-1 Fc protein, and cleavage points used to make TTP-3000 (at position 136) and TTP-4000 (at position 251) in accordance with alternate embodiments of the present invention; and **Panel B** shows the domain structure for TTP-3000 and TTP-4000 in accordance with alternate embodiments of the present invention.

FIG. 7 shows results of an *in vitro* binding assay for sRAGE, and RAGE fusion proteins TTP-4000 (TT4) and TTP-3000 (TT3), to the RAGE ligands amyloid-beta (A-beta), S100b (S100), and amphoterin (Ampho), in accordance with an embodiment of the present invention.

FIG. 8 shows results of an *in vitro* binding assay for RAGE fusion protein TTP-4000 (TT4) ("Protein") to amyloid-beta as compared to a negative control only including the immunodetection reagents ("Complex Alone"), and antagonism of such binding by a RAGE antagonist ("RAGE Ligand") in accordance with an embodiment of the present invention.

FIG. 9 shows results of an *in vitro* binding assay for RAGE fusion protein TTP-3000 (TT3) ("Protein") to amyloid-beta as compared to a negative control only including the immunodetection reagents ("Complex Alone"), and antagonism of such binding by a RAGE antagonist ("RAGE Ligand") in accordance with an embodiment of the present invention.

FIG. 10 shows results of a cell-based assay measuring the inhibition of S100b-RAGE induced production of TNF-α by RAGE fusion proteins TTP-3000 (TT3) and TTP-4000 (TT4), and sRAGE in accordance with an embodiment of the present invention.

FIG. 11 shows a pharmacokinetic profile for RAGE fusion protein TTP-4000 in accordance with an embodiment of the present invention wherein each curve represents a different animal under the same experimental conditions.

FIG. 12 shows relative levels of TNF-α release from THP-1 cells due to stimulation by RAGE fusion protein TTP-4000 and human IgG stimulation as a measure of an inflammatory response in accordance with an embodiment of the present invention

FIG. 13 shows the use of RAGE fusion protein TTP-4000 to reduce restenosis in diabetic animals in accordance with alternate embodiments of the present invention, wherein **panel A** shows that TTP-4000 RAGE-fusion protein reduced the intima/media ratio as compared to a negative control (IgG), and **panel B** shows that TTP-4000 RAGE-fusion protein reduced vascular smooth muscle cell proliferation in a dose-responsive manner.

FIG. 14 shows use of RAGE fusion protein TTP-4000 to reduce amyloid formation and cognitive dysfunction in animals with Alzheimer's Disease (AD) in accordance with alternate embodiments of the present invention wherein **panel A** shows TTP-4000 RAGE-fusion protein reduced amyloid load in the brain, and **panel** B shows TTP-4000 RAGE-fusion protein improved cognitive function.

FIG. 15 shows saturation-binding curves with TTP-4000 to various immobilized known RAGE ligands in accordance with an embodiment of the present invention.

DETAILED DESCRIPTION

For the purposes of this specification, unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification are approximations that can vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass any and all subranges subsumed therein. For example, a stated range of "1 to 10" should be considered to include any and all subranges between (and inclusive of) the minimum value of 1 and the maximum value of 10; that is, all subranges beginning with a minimum value of 1 or more, e.g. 1 to 6.1, and ending with a maximum value of 10 or less,

e.g., 5.5 to 10. Additionally, any reference referred to as being "incorporated herein" is to be understood as being incorporated in its entirety.

It is further noted that, as used in this specification, the singular forms "a," "an," and "the" include plural referents unless expressly and unequivocally limited to one referent. The term "or" is used interchangeably with the term "and/or" unless the context clearly indicates otherwise.

Also, the terms "portion" and "fragment" are used interchangeably to refer to parts of a polypeptide, nucleic acid, or other molecular construct.

As used herein, the term "upstream" refers to a residue that is N-terminal to a second residue where the molecule is a protein, or 5' to a second residue where the molecule is a nucleic acid. Also as used herein, the term "downstream" refers to a residue that is C-terminal to a second residue where the molecule is a protein, or 3' to a second residue where the molecule is a nucleic acid.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Practitioners are particularly directed to Current Protocols in Molecular Biology (Ansubel) for definitions and terms of the art. Abbreviations for amino acid residues are the standard 3-letter and/or 1-letter codes used in the art to refer to one of the 20 common L-amino acids.

A "nucleic acid" is a polynucleotide such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The term is used to include single-stranded nucleic acids, double-stranded nucleic acids, and RNA and DNA made from nucleotide or nucleoside analogues.

The term "vector" refers to a nucleic acid molecule that may be used to transport a second nucleic acid molecule into a cell. In one embodiment, the vector allows for replication of DNA sequences inserted into the vector. The vector may comprise a promoter to enhance expression of the nucleic acid molecule in at least some host cells. Vectors may replicate autonomously (extrachromasomal) or may be integrated into a host cell chromosome. In one embodiment, the vector may comprise an expression vector capable of producing a protein derived from at least part of a nucleic acid sequence inserted into the vector.

As is known in the art, conditions for hybridizing nucleic acid sequences to each other can be described as ranging from low to high stringency. Generally, highly stringent hybridization conditions refer to washing hybrids in low salt buffer at high temperatures. Hybridization may be to filter bound DNA using hybridization solutions standard in the art such as 0.5M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), at 65°C, and washing in 0.25 M

$NaHPO_4$, 3.5% SDS followed by washing 0.1 x SSC/0.1% SDS at a temperature ranging from room temperature to 68°C depending on the length of the probe (*see e.g.* Ausubel, F.M. *et al., Short Protocols in Molecular Biology*, 4th Ed., Chapter 2, John Wiley & Sons, N.Y). For example, a high stringency wash comprises washing in 6x SSC/0.05% sodium pyrophosphate at 37°C for a 14 base oligonucleotide probe, or at 48°C for a 17 base oligonucleotide probe, or at 55°C for a 20 base oligonucleotide probe, or at 60°C for a 25 base oligonucleotide probe, or at 65°C for a nucleotide probe about 250 nucleotides in length. Nucleic acid probes may be labeled with radionucleotides by end-labeling with, for example, [γ-^{32}P]ATP, or incorporation of radiolabeled nucleotides such as [α-^{32}P]dCTP by random primer labeling. Alternatively, probes may be labeled by incorporation of biotinylated or fluorescein labeled nucleotides, and the probe detected using Streptavidin or anti-fluorescein antibodies.

As used herein, "small organic molecules" are molecules of molecular weight less than 2,000 Daltons that contain at least one carbon atom.

"Polypeptide" and "protein" are used interchangeably herein to describe protein molecules that may comprise either partial or full-length proteins.

The term "fusion protein" refers to a protein or polypeptide that has an amino acid sequence derived from two or more proteins. The fusion protein may also include linking regions of amino acids between amino acid portions derived from separate proteins.

As used herein, a "non-RAGE polypeptide" is any polypeptide that is not derived from RAGE or a fragment thereof. Such non-RAGE polypeptides include immunoglobulin peptides, dimerizing polypeptides, stabilizing polypeptides, amphiphilic peptides, or polypeptides comprising amino acid sequences that provide "tags" for targeting or purification of the protein.

As used herein, "immunoglobulin peptides" may comprise an immunoglobulin heavy chain or a portion thereof. In one embodiment, the portion of the heavy chain may be the Fc fragment or a portion thereof. As used herein, the Fc fragment comprises the heavy chain hinge polypeptide, and the C_H2 and C_H3 domains of the heavy chain of an immunoglobulin, in either monomeric or dimeric form. Or, the C_H1 and Fc fragment may be used as the immunoglobulin polypeptide. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. An example of

biological activity that may be altered includes reduction of an isotype's ability to bind to some Fc receptors as for example, by modification of the hinge region.

The terms "identity" or "percent identical" refers to sequence identity between two amino acid sequences or between two nucleic acid sequences. Percent identity can be determined by aligning two sequences and refers to the number of identical residues (*i.e.*, amino acid or nucleotide) at positions shared by the compared sequences. Sequence alignment and comparison may be conducted using the algorithms standard in the art (*e.g.* Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482; Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443; Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci., USA*, 85:2444) or by computerized versions of these algorithms (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive, Madison, WI) publicly available as BLAST and FASTA. Also, ENTREZ, available through the National Institutes of Health, Bethesda MD, may be used for sequence comparison. In one embodiment, the percent identity of two sequences may be determined using GCG with a gap weight of 1, such that each amino acid gap is weighted as if it were a single amino acid mismatch between the two sequences.

As used herein, the term "conserved residues" refers to amino acids that are the same among a plurality of proteins having the same structure and/or function. A region of conserved residues may be important for protein structure or function. Thus, contiguous conserved residues as identified in a three-dimensional protein may be important for protein structure or function. To find conserved residues, or conserved regions of 3-D structure, a comparison of sequences for the same or similar proteins from different species, or of individuals of the same species, may be made.

As used herein, the term "homologue" means a polypeptide having a degree of homology with the wild-type amino acid sequence. Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percent homology between two or more sequences (e.g. Wilbur, W. J. and Lipman, D. J., 1983, *Proc. Natl. Acad. Sci. USA*, 80:726-730). For example, homologous sequences may be taken to include an amino acid sequences which in alternate embodiments are at least 75% identical, 85% identical, 90% identical, 95% identical, or 98% identical to each other.

As used herein, a polypeptide or protein "domain" comprises a region along a polypeptide or protein that comprises an independent unit. Domains may be defined in terms of structure, sequence and/or biological activity. In one embodiment, a polypeptide domain

may comprise a region of a protein that folds in a manner that is substantially independent from the rest of the protein. Domains may be identified using domain databases such as, but not limited to PFAM, PRODOM, PROSITE, BLOCKS, PRINTS, SBASE, ISREC PROFILES, SAMRT, and PROCLASS.

As used herein, "immunoglobulin domain" is a sequence of amino acids that is structurally homologous, or identical to, a domain of an immunoglobulin. The length of the sequence of amino acids of an immunoglobulin domain may be any length. In one embodiment, an immunoglobulin domain may be less than 250 amino acids. In an example embodiment, an immunoglobulin domain may be about 80-150 amino acids in length. For example, the variable region, and the C_H1, C_H2, and C_H3 regions of an IgG are each immunoglobulin domains. In another example, the variable, the C_H1, C_H2, C_H3 and C_H4 regions of an IgM are each immunoglobulin domains.

As used herein, a "RAGE immunoglobulin domain" is a sequence of amino acids from RAGE protein that is structurally homologous, or identical to, a domain of an immunoglobulin. For example, a RAGE immunoglobulin domain may comprise the RAGE V-domain, the RAGE Ig-like C2-type 1 domain ("C1 domain"), or the RAGE Ig-like C2-type 2 domain ("C2 domain").

As used herein, an "interdomain linker" comprises a polypeptide that joins two domains together. An Fc hinge region is an example of an interdomain linker in an IgG.

As used herein, "directly linked" identifies a covalent linkage between two different groups (*e.g.*, nucleic acid sequences, polypeptides, polypeptide domains) that does not have any intervening atoms between the two groups that are being linked.

As used herein, "ligand binding domain" refers to a domain of a protein responsible for binding a ligand. The term ligand binding domain includes homologues of a ligand binding domain or portions thereof. In this regard, deliberate amino acid substitutions may be made in the ligand binding site on the basis of similarity in polarity, charge, solubility, hydrophobicity, or hydrophilicity of the residues, as long as the binding specificity of the ligand binding domain is retained.

As used herein, a "ligand binding site" comprises residues in a protein that directly interact with a ligand, or residues involved in positioning the ligand in close proximity to those residues that directly interact with the ligand. The interaction of residues in the ligand binding site may be defined by the spatial proximity of the residues to a ligand in the model or structure. The term ligand binding site includes homologues of a ligand binding site, or portions thereof. In this regard, deliberate amino acid substitutions may be made in the

ligand binding site on the basis of similarity in polarity, charge, solubility, hydrophobicity, or hydrophilicity of the residues, as long as the binding specificity of the ligand binding site is retained. A ligand binding site may exist in one or more ligand binding domains of a protein or polypeptide.

As used herein, the term "interact" refers to a condition of proximity between a ligand or compound, or portions or fragments thereof, and a portion of a second molecule of interest. The interaction may be non-covalent, for example, as a result of hydrogen-bonding, van der Waals interactions, or electrostatic or hydrophobic interactions, or it may be covalent.

As used herein, a "ligand" refers to a molecule or compound or entity that interacts with a ligand binding site, including substrates or analogues or parts thereof. As described herein, the term "ligand" may refer to compounds that bind to the protein of interest. A ligand may be an agonist, an antagonist, or a modulator. Or, a ligand may not have a biological effect. Or, a ligand may block the binding of other ligands thereby inhibiting a biological effect. Ligands may include, but are not limited to, small molecule inhibitors. These small molecules may include peptides, peptidomimetics, organic compounds and the like. Ligands may also include polypeptides and/or proteins.

As used herein, a "modulator compound" refers to a molecule which changes or alters the biological activity of a molecule of interest. A modulator compound may increase or decrease activity, or change the physical or chemical characteristics, or functional or immunological properties, of the molecule of interest. For RAGE, a modulator compound may increase or decrease activity, or change the characteristics, or functional or immunological properties of the RAGE, or a portion threof A modulator compound may include natural and/or chemically synthesized or artificial peptides, modified peptides (e.g., phosphopeptides), antibodies, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, glycolipids, heterocyclic compounds, nucleosides or nucleotides or parts thereof, and small organic or inorganic molecules. A modulator compound may be an endogenous physiological compound or it may be a natural or synthetic compound. Or, the modulator compound may be a small organic molecule. The term "modulator compound" also includes a chemically modified ligand or compound, and includes isomers and racemic forms.

An "agonist" comprises a compound that binds to a receptor to form a complex that elicits a pharmacological response specific to the receptor involved.

An "antagonist" comprises a compound that binds to an agonist or to a receptor to form a complex that does not give rise to a substantial pharmacological response and can inhibit the biological response induced by an agonist.

RAGE agonists may therefore bind to RAGE and stimulate RAGE-mediated cellular processes, and RAGE antagonists may inhibit RAGE-mediated processes from being stimulated by a RAGE agonist. For example, in one embodiment, the cellular process stimulated by RAGE agonists comprises activation of TNF-α gene transcription.

The term "peptide mimetics" refers to structures that serve as substitutes for peptides in interactions between molecules (Morgan et al., 1989, *Ann. Reports Med. Chem.*, 24:243-252). Peptide mimetics may include synthetic structures that may or may not contain amino acids and/or peptide bonds but that retain the structural and functional features of a peptide, or agonist, or antagonist. Peptide mimetics also include peptoids, oligopeptoids (Simon et al., 1972, *Proc. Natl. Acad, Sci., USA*, 89:9367); and peptide libraries containing peptides of a designed length representing all possible sequences of amino acids corresponding to a peptide, or agonist or antagonist of the invention.

The term "treating" refers to improving a symptom of a disease or disorder and may comprise curing the disorder, substantially preventing the onset of the disorder, or improving the subject's condition. The term "treatment" as used herein, refers to the full spectrum of treatments for a given disorder from which the patient is suffering, including alleviation of one symptom or most of the symptoms resulting from that disorder, a cure for the particular disorder, or prevention of the onset of the disorder.

As used herein, the term "EC50" is defined as the concentration of an agent that results in 50% of a measured biological effect. For example, the EC50 of a therapeutic agent having a measurable biological effect may comprise the value at which the agent displays 50% of the biological effect.

As used herein, the term "IC50" is defined as the concentration of an agent that results in 50% inhibition of a measured effect. For example, the IC50 of an antagonist of RAGE binding may comprise the value at which the antagonist reduces ligand binding to the ligand binding site of RAGE by 50%.

As used herein, an "effective amount" means the amount of an agent that is effective for producing a desired effect in a subject. The term "therapeutically effective amount" denotes that amount of a drug or pharmaceutical agent that will elicit therapeutic response of an animal or human that is being sought. The actual dose which comprises the effective

amount may depend upon the route of administration, the size and health of the subject, the disorder being treated, and the like.

The term "pharmaceutically acceptable carrier" as used herein may refer to compounds and compositions that are suitable for use in human or animal subjects, as for example, for therapeutic compositions administered for the treatment of a RAGE-mediated disorder or disease.

The term "pharmaceutical composition" is used herein to denote a composition that may be administered to a mammalian host, *e.g.*, orally, parenterally, topically, by inhalation spray, intranasally, or rectally, in unit dosage formulations containing conventional non-toxic carriers, diluents, adjuvants, vehicles and the like.

The term "parenteral" as used herein, includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques.

RAGE Fusion Proteins

Embodiments of the present invention comprise RAGE fusion proteins, methods of making such fusion proteins, and methods of use of such fusion proteins. The present invention may be embodied in a variety of ways.

For example, embodiments of the present invention provide fusion proteins comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

A RAGE protein or polypeptide may comprise full-length human RAGE protein (e.g., SEQ ID NO: 1), or a fragment of human RAGE. As used herein, a fragment of a RAGE polypeptide is at least 5 amino acids in length, may be greater than 30 amino acids in length, but is less than the full amino acid sequence. In alternate embodiments, the RAGE polypeptide may comprise a sequence that is 70%, or 80%, or 85%, or 90% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first

residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence.

The fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide 90% identical to sRAGE, or a fragment of sRAGE. As used herein, sRAGE is the RAGE protein that does not include the transmembrane region or the cytoplasmic tail (Park *et al., Nature Med.*, 4:1025-1031 (1998)). For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, a RAGE polypeptide may comprise human sRAGE with the signal sequence removed (e.g., SEQ ID NO: 5 or SEQ ID NO: 6) (FIG. 1C) or a portion of that amino acid sequence.

In other embodiments, the RAGE protein may comprise a RAGE V domain (e.g., SEQ ID NO: 7 or SEQ ID NO: 8; FIG. 1D) (Neeper *et al.*, (1992); Schmidt et al. (1997)). Or, a sequence 90% identical to the RAGE V domain or a fragment thereof may be used.

Or, the RAGE protein may comprise a fragment of the RAGE V domain (e.g., SEQ ID NO: 9 or SEQ ID NO: 10, FIG. 1D). In one embodiment the RAGE protein may comprise a ligand binding site. In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence 90% identical thereto, or SEQ ID NO: 10, or a sequence 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

Thus, the RAGE polypeptide used in the fusion proteins of the present invention may comprise a fragment of full length RAGE. As is known in the art, RAGE comprises three immunoglobulin-like polypeptide domains, the V domain, and the C1 and C2 domains each linked to each other by an interdomain linker. Full-length RAGE also includes a transmembrane polypeptide and a cytoplasmic tail downstream (C-terminal) of the C2 domain, and linked to the C2 domain.

In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE.

For example, the RAGE polypeptide may comprise amino acids 23-116 of human RAGE (SEQ ID NO: 7) or a sequence 90% identical thereto, or amino acids 24-116 of human RAGE (SEQ ID NO: 8) or a sequence 90% identical thereto, corresponding to the V domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 124-221 of human RAGE (SEQ ID NO: 11) or a sequence 90% identical thereto, corresponding to the C1 domain of RAGE. In another embodiment, the RAGE polypeptide may comprise amino

acids 227-317 of human RAGE (SEQ ID NO: 12) or a sequence 90% identical thereto, corresponding to the C2 domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 23-123 of human RAGE (SEQ ID NO: 13) or a sequence 90% identical thereto, or amino acids 24-123 of human RAGE (SEQ ID NO: 14) or a sequence 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 23-226 of human RAGE (SEQ ID NO: 17) or a sequence 90% identical thereto, or amino acids 24-226 of human RAGE (SEQ ID NO: 18) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain and the interdomain linker linking these two domains. Or, the RAGE polypeptide may comprise amino acids 23-339 of human RAGE (SEQ ID NO: 5) or a sequence 90% identical thereto, or 24-339 of human RAGE (SEQ ID NO: 6) or a sequence 90% identical thereto, corresponding to sRAGE (i.e., encoding the V, C1, and C2 domains and interdomain linkers). Or, fragments of each of these sequences may be used.

The fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the fusion protein may comprise a polypeptide derived from an immunoglobulin. In one embodiment, the immunoglobulin polypeptide may comprise an immunoglobulin heavy chain or a portion (i.e., fragment) thereof. For example, the heavy chain fragment may comprise a polypeptide derived from the Fc fragment of an immunoglobulin, wherein the Fc fragment comprises the heavy chain hinge polypeptide, and C_H2 and C_H3 domains of the immunoglobulin heavy chain as a monomer. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1 or portions of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, in one embodiment, the RAGE fusion protein of the present invention comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus in one embodiment, the fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin,

or a fragment or portion of the C_H2 domain of an immunoglobulin. In one embodiment, the C_H2 domain, or a fragment thereof comprises SEQ ID NO: 42. In one embodiment, the RAGE polypeptide may comprise a ligand binding site. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

The RAGE polypeptide used in the fusion proteins of the present invention may comprise a RAGE immunoglobulin domain. Additionally or alternatively, the fragment of RAGE may comprise an interdomain linker. Or, the RAGE polypeptide may comprise a RAGE immunoglobulin domain linked to an upstream (i.e., closer to the N-terminus) or downstream (i.e., closer to the C-terminus) interdomain linker. In yet another embodiment, the RAGE polypeptide may comprise two (or more) RAGE immunoglobulin domains each linked to each other by an interdomain linker. The RAGE polypeptide may further comprise multiple RAGE immunoglobulin domains linked to each other by one or more interdomain linkers and having a terminal interdomain linker attached to the N-terminal RAGE immunoglobulin domain and/or the C-terminal immunoglobulin domain. Additional combinations of RAGE immunoglobulin domains and interdomain linkers are within the scope of the present invention.

In one embodiment, the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin may comprise the C_H2 and C_H3 domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiment, the polypeptide comprising the C_H2 and C_H3 domains, or a portion thereof, of a human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

As described above, the fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE polypeptide domain may comprise a fragment of full-length RAGE protein. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence 90% identical thereto corresponding to the V

domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1.

For example, in one embodiment, the fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The fusion protein may comprise a first RAGE immunoglobulin domain and a first RAGE interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the second RAGE immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the C_H2 immunoglobulin domain. In one embodiment, a four domain RAGE fusion protein may comprise SEQ ID NO: 32. In alternate embodiments, a four domain RAGE fusion protein comprises SEQ ID NO: 33 or SEQ ID NO: 34.

Alternatively, a three domain fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of a C_H2 immunoglobulin domain or a portion of a C_H2 immunoglobulin domain. In one embodiment, a three domain RAGE fusion protein may comprise SEQ ID NO: 35. In alternate embodiments, a three domain RAGE fusion protein may comprise SEQ ID NO: 36 or SEQ ID NO: 37.

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a interdomain linker comprising several amino acids (e.g., 1-3, 1-5,

or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is 70% identical, or 80% identical, or 90% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342.

Methods of Producing RAGE Fusion Proteins

The present invention also comprises a method to make a RAGE fusion protein. Thus, in one embodiment, the present invention comprises a method of making a RAGE fusion protein comprising the step of covalently linking a RAGE polypeptide linked to a second, non-RAGE polypeptide wherein the RAGE polypeptide comprises a RAGE ligand binding site. For example, the linked RAGE polypeptide and the second, non-RAGE polypeptide may be encoded by a recombinant DNA construct. The method may further comprise the step of incorporating the DNA construct into an expression vector. Also, the method may comprise the step of inserting the expression vector into a host cell.

For example, embodiments of the present invention provide fusion proteins comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin

domain. In one embodiment, the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

The fusion protein may be engineered by recombinant DNA techniques. For example, in one embodiment, the present invention may comprise an isolated nucleic acid sequence encoding a RAGE polypeptide linked to a second, non-RAGE polypeptide. In an embodiment, the RAGE polypeptide may comprise a RAGE ligand binding site.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence 70%, or 80%, or 90% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence. The fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (e.g., SEQ ID NO: 5 or SEQ ID NO: 6) (FIG. 1C) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (e.g., SEQ ID NO: 7 or SEQ ID NO: 8; FIG. 1D). Or, a sequence 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (e.g., SEQ ID NO: 9 or SEQ ID NO: 10, FIG. 1D). In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence 90% identical thereto, or SEQ ID NO: 10, or a sequence 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

In an embodiment, the nucleic acid sequence comprises SEQ ID NO: 25 to encode amino acids 1-118 of human RAGE or a fragment thereof. For example, a sequence comprising nucleotides 1- 348 of SEQ ID NO: 25 may be used to encode amino acids 1-116 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 26 to encode amino acids 1-123 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 27 to encode amino acids 1-136 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 28 to encode

amino acids 1-230 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 29 to encode amino acids 1-251 of human RAGE. Or fragments of these nucleic acid sequences may be used to encode RAGE polypeptide fragments.

The fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the fusion protein may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, the RAGE fusion protein of the present invention may comprise an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin. For example, in one embodiment, the fusion protein may be encoded by a recombinant DNA construct. Also, the method may comprise the step of incorporating the DNA construct into an expression vector. Also, the method may comprise transfecting the expression vector into a host cell.

Thus, in one embodiment, the present invention comprises a method of making a RAGE fusion protein comprising the step of covalently linking a RAGE polypeptide to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In one embodiment, the fusion protein may comprise a RAGE ligand binding site. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto

For example, in one embodiment, the present invention comprises a nucleic acid encoding a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. In one embodiment, the C_H2 domain, or a fragment thereof, comprises SEQ ID NO: 42. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1. As an example embodiment, the polypeptide comprising

the C_H2 and C_H3 domains of a human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41.

In one embodiment, the RAGE polypeptide may comprise a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin may comprise a polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion of both, or either, of these domains. As an example embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

The fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The fusion protein may comprise a first RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein.

Alternatively, a three domain fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence 90% identical thereto corresponding to the V domain of RAGE and a downstream interdomain linker. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein.

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is 70% identical, or 80% identical, or 90% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342.

The method may further comprise the step of incorporating the DNA construct into an expression vector. Thus, in a embodiment, the present invention comprises an expression vector that encodes for a fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In an embodiment, the RAGE polypeptide comprise constructs, such as those described herein, having a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, the expression vector used to transfect the cells may comprise the nucleic acid sequence SEQ ID NO:30, or a fragment thereof, or SEQ ID NO: 31, or a fragment thereof.

The method may further comprise the step of transfecting a cell with the expression vector of the present invention. Thus, in an embodiment, the present invention comprises a cell transfected with the expression vector that expressed the RAGE fusion protein of the present invention, such that the cell expresses a fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In an embodiment, the RAGE polypeptide comprise constructs, such as those described herein, having a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, the expression vector may comprise the nucleic acid sequence SEQ ID NO:30, or a fragment thereof, or SEQ ID NO: 31, or a fragment thereof.

For example, plasmids may be constructed to express RAGE-IgG Fc fusion proteins by fusing different lengths of a 5' cDNA sequence of human RAGE with a 3' cDNA sequence of human IgG1 Fc (γ1). The expression cassette sequences may be inserted into an expression vector such as pcDNA3.1 expression vector (Invitrogen, CA) using standard recombinant techniques.

Also, the method may comprise transfecting the expression vector into a host cell. In one embodiment, the recombinant may be transfected into Chinese Hamster Ovary cells and expression optimized. In alternate embodiments, the cells may produce 0.1 to 20 grams/liter, or 0.5 to 10 grams/liter, or about 1-2 grams/liter.

As is known in the art, such nucleic acid constructs may be modified by mutation, as for example, by PCR amplification of a nucleic acid template with primers comprising the mutation of interest. In this way, polypeptides comprising varying affinity for RAGE ligands may be designed. In one embodiment, the mutated sequences may be 90% or more identical to the starting DNA. As such, variants may include nucleotide sequences that hybridize under stringent conditions (i.e., equivalent to about 20-27°C below the melting temperature (TM) of the DNA duplex in 1 molar salt).

The coding sequence may be expressed by transfecting the expression vector into an appropriate host. For example, the recombinant vectors may be stably transfected into Chinese Hamster Ovary (CHO) cells, and cells expressing the fusion protein selected and cloned. In an embodiment, cells expressing the recombinant construct are selected for plasmid-encoded neomycin resistance by applying antibiotic G418. Individual clones may be selected and clones expressing high levels of recombinant protein as detected by Western Blot analysis of the cell supernatant may be expanded, and the gene product purified by affinity chromatography using Protein A columns.

Sample embodiments of recombinant nucleic acids that encode the fusion proteins of the present invention are shown in FIGS. 2-5. For example, as described above, the fusion protein produced by the recombinant DNA construct may comprise a RAGE polypeptide linked to a second, non-RAGE polypeptide. The fusion protein may comprise two domains derived from RAGE protein and two domains derived from an immunoglobulin.. An example nucleic acid construct encoding a fusion protein, TTP-4000 (TT4), having this type of structure is shown as FIG. 2 (SEQ ID NO: 30). As shown in FIG. 2, coding sequence 1-753 (highlighted in bold) encodes the RAGE N-terminal protein sequence whereas the sequence from 754-1386 encodes the IgG Fc protein sequence.

When derived from SEQ ID NO: 30, or a sequence 90% identical thereto, the fusion protein may comprise the four domain amino acid sequence of SEQ ID NO: 32, or the polypeptide with the signal sequence removed (e.g., SEQ ID NO: 33 or SEQ ID NO: 34) (FIG. 4). In FIG. 4, the RAGE amino acid sequence is highlighted with bold font. The immunoglobulin sequence is the C_H2 and C_H3 immunoglobulin domains of IgG. As shown in FIG. 6B, the first 251 amino acids of the full-length TTP-4000 RAGE fusion protein contains as the RAGE polypeptide sequence a signal sequence comprising amino acids 1-22/23, the V immunoglobulin domain (including the ligand binding site) comprising amino acids 23/24-116, an interdomain linker comprising amino acids 117 to 123, a second

immunoglobulin domain (C1) comprising amino acids 124-221, and a downstream interdomain linker comprising amino acids 222-251.

In an embodiment, the fusion protein may not necessarily comprise the second RAGE immunoglobulin domain. For example, the fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. An example nucleic acid construct encoding this type of fusion protein is shown as FIG. 3 (SEQ ID NO: 31). As shown in FIG. 3, the coding sequence from nucleotides 1 to 408 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the sequence from 409-1041 codes the IgG1 Fc (γ1) protein sequence.

When derived from SEQ ID NO: 31, or a sequence 90% identical thereto, the fusion protein may comprise the three domain amino acid sequence of SEQ ID NO: 35, or the polypeptide with the signal sequence removed (e.g., SEQ ID NO: 36 or SEQ ID NO: 37) (FIG. 5). In FIG. 5, the RAGE amino acid sequence is highlighted with bold font. As shown in FIG. 6B, the first 136 amino acids of the full-length TTP-3000 RAGE fusion protein contains as the RAGE polypeptide a signal sequence comprising amino acids 1-22/23, the V immunoglobulin domain (including the ligand binding site) comprising amino acids 23/24-116, and an interdomain linker comprising amino acids 117 to 136. The sequence from 137 to 346 includes the C_H2 and C_H3 immunoglobulin domains of IgG.

The fusion proteins of the present invention may comprise improved *in vivo* stability over RAGE polypeptides not comprising a second polypeptide. The fusion protein may be further modified to increase stability, efficacy, potency and bioavailability. Thus, the fusion proteins of the present invention may be modified by post-translational processing or by chemical modification. For example, the fusion protein may be synthetically prepared to include L-, D-, or unnatural amino acids, alpha-disubstituted amino acids, or N-alkyl amino acids. Additionally, proteins may be modified by acetylation, acylation, ADP-ribosylation, amidation, attachment of lipids such as phosphatidyinositol, formation of disulfide bonds, and the like. Furthermore, polyethylene glycol can be added to increase the biological stability of the fusion protein.

Binding of RAGE Antagonists to RAGE fusion proteins

The fusion proteins of the present invention may comprise a number of applications. For example, the fusion protein of the present invention may be used in a binding assay to identify RAGE ligands, such as RAGE agonists, antagonists, or modulators.

For example, in one embodiment, the present invention provides a method for detection of RAGE modulators comprising: (a) providing a fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide, where the RAGE polypeptide comprises a ligand binding site; (b) mixing a compound of interest and a ligand having a known binding affinity for RAGE with the fusion protein; and (c) measuring binding of the known RAGE ligand to the RAGE fusion protein in the presence of the compound of interest. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein.

The RAGE fusion proteins may also provide kits for the detection of RAGE modulators. For example, in one embodiment, a kit of the present invention may comprise (a) a compound having known binding affinity to RAGE as a positive control; (b) a RAGE fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide, wherein the RAGE polypeptide comprises a RAGE ligand binding site; and (c) instructions for use. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence 70%, 80%, or 90% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence. The fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (e.g., SEQ ID NO: 5 or SEQ ID NO: 6) (FIG. 1C) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (e.g., SEQ ID NO: 7 or SEQ ID NO: 8; FIG. 1D). Or, a sequence 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (e.g., SEQ ID NO: 9 or SEQ ID

NO: 10, FIG. 1D). In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence 90% identical thereto, or SEQ ID NO: 10, or a sequence 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

The fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the fusion protein may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, the RAGE fusion protein of the present invention may comprise an Fc sequence derived from RAGE rather than an immunoglobulin chain. In an embodiment, the fusion protein may comprise a RAGE immunoglobulin domain linked to a polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. In one embodiment, the RAGE polypeptide may comprise a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin may comprise a polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion of both, or either, of these domains. As an example embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

The fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a

human Fc polypeptide. The fusion protein may comprise a first RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein.

Alternatively, a three domain fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence 90% identical thereto corresponding to the V domain of RAGE and a downstream interdomain linker. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein.

As described herein, RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be

used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is 70% identical, or 80% identical, or 90% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342.

For example, the RAGE fusion protein may be used in a binding assay to identify potential RAGE ligands. In one example embodiment of such a binding assay, a known RAGE ligand may coated onto a solid substrate (*e.g.*, Maxisorb plates) at a concentration of about 5 micrograms per well, where each well contains a total volume of about 100 microliters (μL). The plates may be incubated at 4°C overnight to allow the ligand to absorb. Alternatively, shorter incubation periods at higher temperature (*e.g.*, room temperature) may be used. After a period of time to allow for the ligand to bind to the substrate, the assay wells may be aspirated and a blocking buffer (*e.g.*, 1% BSA in 50 mM imidizole buffer, pH 7.2) may be added to block nonspecific binding. For example, blocking buffer may be added to the plates for 1 hour at room temperature. The plates may then be aspirated and/or washed with a wash buffer. In one embodiment, a buffer comprising 20 mM Imidizole, 150 mM NaCl, 0.05% Tween-20, 5 mM $CaCl_2$ and 5mM $MgCl_2$, pH 7.2 may be used as a wash buffer. The fusion protein may then added at increasing dilutions to the assay wells. The RAGE fusion protein may then be allowed to incubate with the immobilized ligand in the assay well such that binding can attain equilibrium. In one embodiment, the RAGE fusion protein is allowed to incubate with the immobilized ligand for about one hour at 37°C. In alternate embodiments, longer incubation periods at lower temperatures may be used. After the fusion protein and immobilized ligand have been incubated, the plate may be washed to remove any unbound fusion protein. The fusion protein bound to the immobilized ligand may be detected

in a variety of ways. In one embodiment, detection employs an ELISA. Thus, in one embodiment, an immunodetection complex containing a monoclonal mouse anti-human IgG1, biotinylated goat anti-mouse IgG, and an avidin linked alkaline phosphatase may be added to the fusion protein immobilized in the assay well. The immunodetection complex may be allowed to bind to the immobilized fusion protein such that binding between the fusion protein and the immunodetection complex attains equilibrium. For example, the complex may be allowed to bind to the fusion protein for one hour at room temperature. At that point, any unbound complex may be removed by washing the assay well with wash buffer. The bound complex may be detected by adding the alkaline phosphatase substrate, *para*-nitrophenylphosphate (PNPP), and measuring conversion of PNPP to *para*-nitrophenol (PNP) as an increase in absorbance at 405 nm.

In an embodiment, RAGE ligand bind to the RAGE fusion protein with nanomolar (nM) or micromolar (μM) affinity. An experiment illustrating binding of RAGE ligands to RAGE fusion proteins of the present invention is shown in FIG. 7. Solutions of TTP-3000 (TT3) and TTP-4000 (TT4) having initial concentrations of 1.082 mg/mL, and 370 μg/mL, respectively, were prepared. As shown FIG. 7, at various dilutions, the fusion proteins TTP-3000 and TTP-4000 are able to bind to immobilized RAGE ligands Amyloid-beta (Abeta) (Amyloid Beta (1-40) from Biosource), S100b (S100), and amphoterin (Ampho), resulting in an increase in absorbance. In the absence of ligand (*i.e.*, coating with only BSA) there was no increase in absorbance.

The binding assay of the present invention may be used to quantify ligand binding to RAGE. In alternate embodiments, RAGE ligands may bind to the fusion protein of the present invention with binding affinities ranging from 0.1 to 1000 nanomolar (nM), or from 1 to 500 nM, or from 10 to 80 nM.

The fusion protein of the present invention may also be used to identify compounds having the ability to bind to RAGE. As shown in FIGS. 8 and 9, respectively, a RAGE ligand may be assayed for its ability to compete with immobilized amyloid beta for binding to TTP-4000 (TT4) or TTP-3000 (TT3) fusion proteins. Thus, it may be seen that a RAGE ligand at a final assay concentration (FAC) of 10 μM can displace binding of RAGE fusion protein to amyloid-beta at concentrations of 1:3, 1:10, 1:30, and 1:100 of the initial TTP-4000 solution (FIG. 8) or TTP-3000 (FIG. 9).

Modulation of Cellular Effectors

Embodiments of the fusion proteins of the present invention may be used to modulate a biological response mediated by RAGE. For example, the fusion proteins may be designed to modulate RAGE-induced increases in gene expression. Thus, in an embodiment, fusion proteins of the present invention may be used to modulate the function of biological enzymes. For example, the interaction between RAGE and its ligands may generate oxidative stress and activation of NF-κB, and NF-κB regulated genes, such as the cytokines IL-1β, TNF-α, and the like. In addition, several other regulatory pathways, such as those involving p21ras, MAP kinases, ERK1, and ERK2, have been shown to be activated by binding of AGEs and other ligands to RAGE.

Use of the fusion proteins of the present invention to modulate expression of the cellular effector TNF-α is shown in FIG. 10. THP-1 myeloid cells may be cultured in RPMI-1640 media supplemented with 10% FBS and induced to secrete TNF-α via stimulation of RAGE with S100b. When such stimulation occurs in the presence of a RAGE fusion protein, induction of TNF-α by S100b binding to RAGE may be inhibited. Thus, as shown in FIG. 10, addition of 10 μg TTP-3000 (TT3) or TTP-4000 (TT4) RAGE fusion protein reduces S100b induction of TNF-α by about 50% to 75%. Fusion protein TTP-4000 may be at least as effective in blocking S100b induction of TNF-α as is sRAGE (FIG. 10). Specificity of the inhibition for the RAGE sequences of TTP-4000 and TTP-3000 is shown by the experiment in which IgG alone was added to S100b stimulated cells. Addition of IgG and S100b to the assay shows the same levels of TNF-α as S100b alone.

Physiological Characteristics of RAGE Fusion Proteins

While sRAGE can have a therapeutic benefit in the modulation of RAGE-mediated diseases, human sRAGE may have limitations as a stand-alone therapeutic based on the relatively short half-life of sRAGE in plasma. For example, whereas rodent sRAGE has a half-life in normal and diabetic rats of approximately 20 hours, human sRAGE has a half-life of less than 2 hours when assessed by retention of immunoreactivity sRAGE (Renard *et al.*, *J. Pharmacol. Exp. Ther.*, 290:1458-1466 (1999)).

To generate a RAGE therapeutic that has similar binding characteristics as sRAGE, but a more stable pharmacokinetic profile, a RAGE fusion protein comprising a RAGE ligand binding site linked to one or more human immunoglobulin domains may be used. As is known in the art, the immunoglobulin domains may include the Fc portion of the immunoglobulin heavy chain.

The immunoglobulin Fc portion may confer several attributes to a fusion protein. For example, the Fc fusion protein may increase the serum half-life of such fusion proteins, often from hours to several days. The increase in pharmacokinetic stability is generally a result of the interaction of the linker between C_H2 and C_H3 regions of the Fc fragment with the FcRn receptor (Wines *et al.*, *J. Immunol.*, 164:5313-5318 (2000)).

Although fusion proteins comprising an immunoglobulin Fc polypeptide may provide the advantage of increased stability, immunoglobulin fusion proteins may elicit an inflammatory response when introduced into a host. The inflammatory response may be due, in large part, to the Fc portion of the immunoglobulin of the fusion protein. The proinflammatory response may be a desirable feature if the target is expressed on a diseased cell type that needs to be eliminated (*e.g.*, a cancer cell, an or a population of lymphocytes causing an autoimmune disease). The proinflammatory response may be a neutral feature if the target is a soluble protein, as most soluble proteins do not activate immunoglobulins. However, the proinflammatory response may be a negative feature if the target is expressed on cell types whose destruction would lead to untoward side-effects. Also, the proinflammatory response may be a negative feature if an inflammatory cascade is established at the site of a fusion protein binding to a tissue target, since many mediators of inflammation may be detrimental to surrounding tissue, and/or may cause systemic effects.

The primary proinflammatory site on immunoglobulin Fc fragments resides on the hinge region between the C_H1 and C_H2. This hinge region interacts with the FcR1-3 on various leukocytes and trigger these cells to attack the target. (Wines *et al.*, *J. Immunol.*, 164:5313-5318 (2000)).

As therapeutics for RAGE-mediated diseases, RAGE fusion proteins may not require the generation of an inflammatory response. Thus, embodiments of the RAGE fusion proteins of the present invention may comprise a fusion protein comprising a RAGE polypeptide linked to an immunoglobulin domain(s) where the Fc hinge region from the immunoglobulin is removed and replaced with a RAGE polypeptide. In this way, interaction between the RAGE fusion protein and Fc receptors on inflammatory cells may be minimized. It may be important, however, to maintain proper stacking and other three-dimensional structural interactions between the various immunoglobulin domains of the fusion protein. Thus, embodiments of the fusion proteins of the present invention may substitute the biologically inert, but structurally similar RAGE interdomain linker that separates the V and C1 domains of RAGE, or the linker that separates the C1 and C2 domains of RAGE, in lieu of the normal hinge region of the immunoglobulin heavy chain. Thus, the

RAGE polypeptide of the fusion protein may comprise an interdomain linker sequence that is naturally found downstream of a RAGE immunoglobulin domain to form a RAGE immunglobulin domain/linker fragment. In this way, the three dimensional interactions between the immunoglobulin domains contributed by either RAGE or the immunoglobulin may be maintained.

In an embodiment, a RAGE fusion protein of the present invention may comprise a substantial increase in pharmacokinetic stability as compared to sRAGE. For example, FIG. 11 shows that once the RAGE fusion protein TTP-4000 has saturated its ligands, it may retain a half-life of greater than 300 hours. This may be contrasted with the half-life for sRAGE of only a few hours in human plasma.

Thus, in an embodiment, the RAGE fusion proteins of the present invention may be used to antagonize binding of physiological ligands to RAGE as a means to treat RAGE-mediated diseases without generating an unacceptable amount of inflammation. The fusion proteins of the present invention may exhibit a substantial decrease in generating a proinflammatory response as compared to IgG. For example, as shown in FIG. 12, the RAGE fusion protein TTP-4000 does not stimulate TNF-α release from cells under conditions where human IgG stimulation of TNF-α release is detected.

Treatment of Disease with RAGE Fusion Proteins

The present invention may also comprise methods for the treatment of RAGE-mediated disorder in a human subject. In an embodiment, the method may comprise administering to a subject a fusion protein comprising a RAGE polypeptide comprising a RAGE ligand binding site linked to a second, non-RAGE polypeptide. In one embodiment, the fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does

not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence that is 70%, 80% or 90% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence. The fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (e.g., SEQ ID NO: 5 or SEQ ID NO: 6) (FIG. 1C) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (e.g., SEQ ID NO: 7 or SEQ ID NO: 8; FIG. 1D). Or, a sequence 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (e.g., SEQ ID NO: 9 or SEQ ID NO: 10, FIG. 1D). In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence 90% identical thereto, or SEQ ID NO: 10, or a sequence 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

The fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the fusion protein may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41.

For example, the RAGE polypeptide may comprise amino acids 23-116 of human RAGE (SEQ ID NO: 7) or a sequence 90% identical thereto, or amino acids 24-116 of

human RAGE (SEQ ID NO: 8) or a sequence 90% identical thereto, corresponding to the V domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 124-221 of human RAGE (SEQ ID NO: 11) or a sequence 90% identical thereto, corresponding to the C1 domain of RAGE. In another embodiment, the RAGE polypeptide may comprise amino acids 227-317 of human RAGE (SEQ ID NO: 12) or a sequence 90% identical thereto, corresponding to the C2 domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 23-123 of human RAGE (SEQ ID NO: 13) or a sequence 90% identical thereto, or amino acids 24-123 of human RAGE (SEQ ID NO: 14) or a sequence 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 23-226 of human RAGE (SEQ ID NO: 17) or a sequence 90% identical thereto, or amino acids 24-226 of human RAGE (SEQ ID NO: 18) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain and the interdomain linker linking these two domains. Or, the RAGE polypeptide may comprise amino acids 23-339 of human RAGE (SEQ ID NO: 5) or a sequence 90% identical thereto, or 24-339 of human RAGE (SEQ ID NO: 6) or a sequence 90% identical thereto, corresponding to sRAGE (i.e., encoding the V, C1, and C2 domains and interdomain linkers). Or, fragments of each of these sequences may be used.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, in one embodiment, the RAGE fusion protein of the present invention comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus in one embodiment, the fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. In one embodiment, the C_H2 domain, or a fragment thereof comprises SEQ ID NO: 42.

In one embodiment, the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin may comprise the C_H2 and C_H3 domains of a human IgG1. As an example embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

The fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The fusion protein may comprise a first RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein.

Alternatively, a three domain fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence 90% identical thereto corresponding to the V domain of RAGE and a downstream interdomain linker. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein.

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid

sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is 70% identical, or 80% identical, or 90% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342.

In an embodiment, a fusion protein of the present invention may be administered by various routes. Administration of the RAGE fusion protein of the present invention may employ intraperitoneal (IP) injection. Alternatively, the RAGE fusion protein may be administered orally, intranasally, or as an aerosol. In another embodiment, administration is intravenous (IV). The RAGE fusion protein may also be injected subcutaneously. In another embodiment, administration of the fusion protein is intra-arterial. In another embodiment, administration is sublingual. Also, administration may employ a time-release capsule. In yet another embodiment, administration may be transrectal, as by a suppository or the like. For example, subcutaneous administration may be useful to treat chronic disorders when the self-administration is desireable.

A variety of animal models have been used to validate the use of compounds that modulate RAGE as therapeutics. Examples of these models are as follows:

a) sRAGE inhibited neointimal formation in a rat model of restenosis following arterial injury in both diabetic and normal rats by inhibiting endothelial, smooth muscle and macrophage activation via RAGE (Zhou *et al., Circulation* **107**:2238-2243 (2003));

b) Inhibition of RAGE/ligand interactions, using either sRAGE or an anti-RAGE antibody, reduced amyloid plaque formation in a mouse model of systemic amyloidosis (Yan *et al., Nat. Med.*, 6:643-651 (2000)). Accompanying the reduction in amyloid plaques was a reduction in the inflammatory cytokines, interleukin-6 (IL-6) and macrophage colony stimulating factor (M-CSF) as well as reduced activation of NF-kB in the treated animals;

c) RAGE transgenic mice (RAGE overexpressers and RAGE dominant negative expressers) exhibit plaque formation and cognitive deficits in a mouse model of AD (Arancio *et al., EMBO J.*, **23**:4096-4105 (2004));

d) Treatment of diabetic rats with sRAGE reduced vascular permeability (Bonnardel-Phu *et al., Diabetes*, **48**:2052-2058 (1999));

e) Treatment with sRAGE reduced atherosclerotic lesions in diabetic apolipoprotein E-null mice and prevented the functional and morphological indices of diabetic nephropathy in db/db mice (Hudson et al., *Arch. Biochem. Biophys.*, **419**:80-88 (2003)); and

f) sRAGE attenuated the severity of inflammation in a mouse model of collagen-induced arthritis (Hofmann *et al., Genes Immunol.*, 3:123-135 (2002)), a mouse model of experimental allergic encephalomyelitis (Yan *et al., Nat. Med.* 9:28-293 (2003)) and a mouse model of inflammatory bowel disease (Hofmann *et al., Cell*, 97:889-901 (1999)).

Thus, in an embodiment, the fusion proteins of the present invention may be used to treat a symptom of diabetes and/or complications resulting from diabetes mediated by RAGE. In alternate embodiments, the symptom of diabetes or diabetic late complications may comprise diabetic nephropathy, diabetic retinopathy, a diabetic foot ulcer, a cardiovascular complication of diabetes, or diabetic neuropathy.

Originally identified as a receptor for molecules whose expression is associated with the pathology of diabetes, RAGE itself is essential to the pathophysiology of diabetic complications. *In vivo*, inhibition of RAGE interaction with its ligand(s) has been shown to be therapeutic in multiple models of diabetic complications and inflammation (Hudson et al., *Arch. Biochem. Biophys.*, **419**:80-88 (2003)). For example, a two-month treatment with anti-RAGE antibodies normalized kidney function and reduced abnormal kidney histopathology

in diabetic mice (Flyvbjerg *et al.*, *Diabetes* 53:166-172 (2004)). Furthermore, treatment with a soluble form of RAGE (sRAGE) which binds to RAGE ligands and inhibits RAGE/ligand interactions, reduced atherosclerotic lesions in diabetic apolipoprotein E-null mice and attenuated the functional and morphological pathology of diabetic nephropathy in db/db mice (Bucciarelli *et al.*, *Circulation* **106**:2827-2835 (2002)).

Also, it has been shown that nonenzymatic glycoxidation of macromolecules ultimately resulting in the formation of advanced glycation endproducts (AGEs) is enhanced at sites of inflammation, in renal failure, in the presence of hyperglycemia and other conditions associated with systemic or local oxidant stress (Dyer *et al.*, *J. Clin. Invest.*, **91**:2463-2469 (1993); Reddy *et al.*, *Biochem.*, **34**:10872-10878 (1995); Dyer *et al.*, *J. Biol. Chem.*, **266**:11654-11660 (1991); Degenhardt *et al.*, *Cell Mol. Biol.*, **44**:1139-1145 (1998)). Accumulation of AGEs in the vasculature can occur focally, as in the joint amyloid composed of AGE-β_2-microglobulin found in patients with dialysis-related amyloidosis (Miyata *et al.*, *J. Clin. Invest.*, **92**:1243-1252 (1993); Miyata *et al.*, *J. Clin. Invest.*, **98**:1088-1094 (1996)), or generally, as exemplified by the vasculature and tissues of patients with diabetes (Schmidt *et al.*, *Nature Med.*, **1**:1002-1004 (1995)). The progressive accumulation of AGEs over time in patients with diabetes suggests that endogenous clearance mechanisms are not able to function effectively at sites of AGE deposition. Such accumulated AGEs have the capacity to alter cellular properties by a number of mechanisms. Although RAGE is expressed at low levels in normal tissues and vasculature, in an environment where the receptor's ligands accumulate, it has been shown that RAGE becomes upregulated (Li *et al.*, *J. Biol. Chem.*, **272**:16498-16506 (1997); Li *et al.*, *J. Biol. Chem.*, **273**:30870-30878 (1998); Tanaka *et al.*, *J. Biol. Chem.*, **275**:25781-25790 (2000)). RAGE expression is increased in endothelium, smooth muscle cells and infiltrating mononuclear phagocytes in diabetic vasculature. Also, studies in cell culture have demonstrated that AGE-RAGE interaction causes changes in cellular properties important in vascular homeostasis.

Use of the RAGE fusion proteins in the treatment of diabetes related pathology is illustrated in FIG. 13. The RAGE fusion protein TTP-4000 was evaluated in a diabetic rat model of restenosis which involved measuring smooth muscle proliferation and intimal expansion following vascular injury. As illustrated in FIG. 13, TTP-4000 treatment may significantly reduce the intima/media (I/M) ratio (FIG. 13A; Table 1) in diabetes-associated restenosis in a dose-responsive manner. Also, TTP-4000 treatment may significantly reduce restenosis-associated vascular smooth muscle cell proliferation in a dose-responsive manner.

Table 1

Effect of TTP-4000 in Rat Model of Restenosis

	IgG (n=9)	TTP-4000 (n=9) Low dose** (0.3 mg/animal qod x 4)	TTP-4000 (n=9) High dose** (1.0 mg/animal qod x 4)
Luminal area (mm^2)	0.2 ± 0.03	0.18 ± 0.04	0.16 ± 0.02
Medial area (mm^2)	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
I/M ratio	1.71 ± 0.27	1.61 ± 0.26	1.44* ± 0.15

*P<0.05

** For both high and low dose, a loading dose of 3 mg/animal was used.

In other embodiments, the fusion proteins of the present invention may also be used to treat or reverse amyloidoses and Alzheimer's disease. RAGE is a receptor for amyloid beta (Aβ) as well as other amyloidogenic proteins including SAA and amylin (Yan *et al., Nature,* **382**:685-691 (1996); Yan *et al., Proc. Natl. Acad. Sci., USA,* **94**:5296-5301 (1997); Yan *et al., Nat. Med.,* **6**:643-651 (2000); Sousa *et al., Lab Invest.,* **80**:1101-1110 (2000)). Also, the RAGE ligands, including AGEs, S100b and Aβ proteins, are found in tissue surrounding the senile plaque in man (Luth *et al., Cereb. Cortex* **15**:211-220 (2005); Petzold *et al, Neurosci. Lett..,* **336**:167-170 (2003); Sasaki *et al., Brain Res.,* **12**:256-262 (2001; Yan *et al., Restor. Neurol Neruosci.,* **12**:167-173 (1998)). It has been shown that RAGE binds ß-sheet fibrillar material regardless of the composition of the subunits (amyloid-ß peptide, amylin, serum amyloid A, prion-derived peptide) (Yan *et al., Nature,* **382**:685-691 (1996); Yan *et al., Nat. Med.,* **6**:643-651 (2000)). In addition, deposition of amyloid has been shown to result in enhanced expression of RAGE. For example, in the brains of patients with Alzheimer's disease (AD), RAGE expression increases in neurons and glia (Yan, *et al., Nature* **382**:685-691 (1996)). Concurrent with expression of RAGE ligands, RAGE is upregulated in astrocytes and microglial cells in the hippocampus of individuals with AD but is not upregulated in individuals that do not have AD (Lue *et al., Exp. Neurol.,* **171**:29-45 (2001)). These findings suggest that cells expressing RAGE are activated via RAGE/RAGE ligand interactions in the vicinity of the senile plaque. Also, *in vitro,* Aβ-mediated activation of microglial cells can be blocked with antibodies directed against the ligand-binding domain of RAGE (Yan et al., *Proc. Natl. Acad. Sci., USA,* **94**:5296-5301 (1997)). It has also been

demonstrated that RAGE can serve as a focal point for fibril assembly (Deane *et al., Nat. Med.* 9:907-913 (2003)).

Also, *in vivo* inhibition of RAGE/ligand interactions using either sRAGE or an anti-RAGE antibody can reduce amyloid plaque formation in a mouse model of systemic amyloidosis (Yan *et al., Nat. Med.*, 6:643-651 (2000)). Double transgenic mice that over-express human RAGE and human amyloid precursor protein (APP) with the Swedish and London mutations (mutant hAPP) in neurons develop learning defects and neuropathological abnormalities earlier than their single mutant hAPP transgenic counterparts. In contrast, double transgenic mice with diminished Aβ signaling capacity due to neurons expressing a dominant negative form of RAGE on the same mutant hAPP background, show a delayed onset of neuropathological and learning abnormalities compared to their single APP transgenic counterpart (Arancio et al., *EMBO J.*, 23:4096-4105 (2004)).

In addition, inhibition of RAGE-amyloid interaction has been shown to decrease expression of cellular RAGE and cell stress markers (as well as NF-κB activation), and diminish amyloid deposition (Yan *et al., Nat. Med.*, 6:643-651 (2000)) suggesting a role for RAGE-amyloid interaction in both perturbation of cellular properties in an environment enriched for amyloid (even at early stages) as well as in amyloid accumulation.

Thus, the RAGE fusion proteins of the present invention may also be used to treat reduce amyloidosis and to reduce amyloid plaques and cognitive dysfunction associated with Alzheimer's Disease (AD). As described above, sRAGE has been shown to reduce both amyloid plaque formation in the brain and subsequent increase in inflammatory markers in an animal model of AD. FIGS. 14A and 14B show that mice that have AD, and are treated for 3 months with either TTP-4000 or mouse sRAGE had fewer amyloid beta (Aβ) plaques and less cognitive dysfunction than animals that received a vehicle or a human IgG negative control (IgG1). Like sRAGE, TTP-4000 may also reduce the inflammatory cytokines IL-1 and TNF-α (data not shown) associated with AD.

Also, fusion proteins of the present invention may be used to treat atherosclerosis and other cardiovascular disorders. Thus, it has been shown that ischemic heart disease is particularly high in patients with diabetes (Robertson, *et al., Lab Invest.*, 18:538-551 (1968); Kannel *et al, J. Am. Med. Assoc.*, 241:2035-2038 (1979); Kannel *et al., Diab. Care*, 2:120-126 (1979)). In addition, studies have shown that atherosclerosis in patients with diabetes is more accelerated and extensive than in patients not suffering from diabetes (*see e.g.* Waller *et al., Am. J. Med.*, 69:498-506 (1980); Crall *et al, Am. J. Med.* 64:221-230 (1978); Hamby *et*

al., Chest, 2:251-257 (1976); and Pyorala *et al., Diab. Metab. Rev.*, 3:463-524 (1978)). Although the reasons for accelerated atherosclerosis in the setting of diabetes are many, it has been shown that reduction of AGEs can reduce plaque formation.

For example, the RAGE fusion proteins of the present invention may also be used to treat stroke. When TTP-4000 was compared to sRAGE in a disease relevant animal model of stroke, TTP-4000 was found to provide a significantly greater reduction in infarct volume. In this model, the middle carotid artery of a mouse is ligated and then reperfused to form an infart. To assess the efficacy of RAGE fusion proteins to treat or prevent stroke, mice were treated with sRAGE or TTP-4000 or control immunoglobulin just prior to reperfusion. As can be seen in Table 2, TTP-4000 was more efficacious than sRAGE in limiting the area of infarct in these animals suggesting that TTP-4000, because of its better half-life in plasma, was able to maintain greater protection than sRAGE.

Table 2

Reduction of Infarct in Stroke

	% Reduction of Infarct**
sRAGE	15%*
TTP-4000 (300 μg)	38%*
TTP-4000 (300 μg)	21%*
TTP-4000 (300 μg)	10%*
IgG Isotype control (300 μg)	4%

*Significant to $p<0.001$; **Compared to saline

In another embodiment, the fusion proteins of the present invention may be used to treat cancer. In one embodiment, the cancer treated using the fusion proteins of the present invention comprises cancer cells that express RAGE. For example, cancers that may be treated with the RAGE fusion protein of the present invention include some lung cancers, some gliomas, some papillomas, and the like. Amphoterin is a high mobility group I nonhistone chromosomal DNA binding protein (Rauvala *et al., J. Biol. Chem.*, 262:16625-16635 (1987); Parkikinen *et al., J. Biol. Chem.* 268:19726-19738 (1993)) which has been shown to interact with RAGE. It has been shown that amphoterin promotes neurite outgrowth, as well as serving as a surface for assembly of protease complexes in the fibrinolytic system (also known to contribute to cell mobility). In addition, a local tumor growth inhibitory effect of blocking RAGE has been observed in a primary tumor model (C6 glioma), the Lewis lung metastasis model (Taguchi *et al., Nature* 405:354-360 (2000)), and

spontaneously arising papillomas in mice expressing the v-Ha-*ras* transgene (Leder *et al.*, *Proc. Natl. Acad. Sci.*, 87:9178-9182 (1990)).

In yet another embodiment, fusion proteins of the present invention may be used to treat inflammation. For example, a\in alternate embodiments, the fusion protein of the present invention is used to treat inflammation associated with autoimmunity, inflammation associated with inflammatory bowel disease, inflammation associated with rheumatoid arthritis, inflammation associated with psoriasis, inflammation associated with multiple sclerosis, inflammation associated with hypoxia, inflammation associated with stroke, inflammation associated with heart attack, inflammation associated with hemorraghic shock, inflammation associated with sepsis, inflammation associated with organ transplantation, or inflammation associated with impaired wound healing.

For example, following thrombolytic treatment, inflammatory cells such as granulocytes infiltrate the ischemic tissue and produce oxygen radicals that can destroy more cells than were killed by the hypoxia. Inhibiting the receptor on the neutrophil responsible for the neutrophils being able to infiltrate the tissue with antibodies or other protein antagonists has been shown to ameliorate the response. Since RAGE is a ligand for this neutrophil receptor, a fusion protein containing a fragment of RAGE may act as a decoy and prevent the neutrophil from trafficking to the reperfused site and thus prevent further tissue destruction. The role of RAGE in prevention of inflammation may be indicated by studies showing that sRAGE inhibited neointimal expansion in a rat model of restenosis following arterial injury in both diabetic and normal rats, presumably by inhibiting endothelial, smooth muscle cell proliferation and macrophage activation via RAGE (Zhou *et al.*, *Circulation*, 107:2238-2243 (2003)). In addition, sRAGE inhibited models of inflammation including delayed-type hypersensitivity, experimental autoimmune encephalitis and inflammatory bowel disease (Hofman *et al.*, *Cell*, 97:889-901 (1999)).

Also, in an embodiment, the fusion proteins of the present invention may be used to treat auto-immune based disorders. For example, the fusion proteins of the present invention may be used to treat kidney failure. Thus, the fusion proteins of the present invention may be used to treat systemic lupus nephritis or inflammatory lupus nephritis. For example, the S100/calgranulins have been shown to comprise a family of closely related calcium-binding polypeptides characterized by two EF-hand regions linked by a connecting peptide (Schafer *et al.*, *TIBS*, 21:134-140 (1996); Zimmer *et al.*, *Brain Res. Bull.*, 37:417-429 (1995); Rammes *et al.*, *J. Biol. Chem.*, 272:9496-9502 (1997); Lugering *et al.*, *Eur. J. Clin. Invest.*, 25:659-664 (1995)). Although they lack signal peptides, it has long been known that

S100/calgranulins gain access to the extracellular space, especially at sites of chronic immune/inflammatory responses, as in cystic fibrosis and rheumatoid arthritis. RAGE is a receptor for many members of the S100/calgranulin family, mediating their proinflammatory effects on cells such as lymphocytes and mononuclear phagocytes. Also, studies on delayed-type hypersensitivity response, colitis in IL-10 null mice, collagen-induced arthritis, and experimental autoimmune encephalitis models suggest that RAGE-ligand interaction (presumably with S-100/calgranulins) has a proximal role in the inflammatory cascade.

Thus, in various selected embodiments, the present invention may provide a method for inhibiting the interaction of an AGE with RAGE in a subject by administering to the subject a therapeutically effective amount of a fusion protein of the present invention. The subject treated using the RAGE fusion proteins of the present invention may be an animal. In an embodiment, the subject is a human. The subject may be suffering from an AGE-related disease such as diabetes, diabetic complications such as nephropathy, neuropathy, retinopathy, foot ulcer, amyloidoses, or renal failure, and inflammation. Or, the subject may be an individual with Alzheimer's disease. In an alternative embodiment, the subject may be an individual with cancer. In yet other embodiments, the subject may be suffering from systemic lupus erythmetosis or inflammatory lupus nephritis. Other diseases may be mediated by RAGE and thus, may be treated using the fusion proteins of the present invention. Thus, in additional alternative embodiments of the present invention, the fusion proteins may be used for treatment of Crohn's disease, arthritis, vasculitis, nephropathies, retinopathies, and neuropathies in human or animal subjects.

A therapeutically effective amount may comprise an amount which is capable of preventing the interaction of RAGE with an AGE or other types of endogenous RAGE ligands in a subject. Accordingly, the amount will vary with the subject being treated. Administration of the compound may be hourly, daily, weekly, monthly, yearly, or as a single event. In various alternative embodiments, the effective amount of the fusion protein may range from about 1 ng/kg body weight to about 100 mg/kg body weight, or from about 10 µg/kg body weight to about 50 mg/kg body weight, or from about 100 µg/kg body weight to about 10 mg/kg body weight. The actual effective amount may be established by dose/response assays using methods standard in the art (Johnson *et al., Diabetes*. 42: 1179, (1993)). Thus, as is known to those in the art, the effective amount may depend on bioavailability, bioactivity, and biodegradability of the compound.

Compositions

The present invention may comprise a composition comprising a fusion protein of the present invention mixed with a pharmaceutically acceptable carrier. The fusion protein may comprise a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence that is 70%, 80% or 90% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence. The fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper *et al.*, (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (e.g., SEQ ID NO: 5 or SEQ ID NO: 6) (FIG. 1C) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (e.g., SEQ ID NO: 7 or SEQ ID NO: 8; FIG. 1D). Or, a sequence 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (e.g., SEQ ID NO: 9 or SEQ ID NO: 10, FIG. 1D). In an embodiment, the ligand binding site may comprise

SEQ ID NO: 9, or a sequence 90% identical thereto, or SEQ ID NO: 10, or a sequence 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

For example, the RAGE polypeptide may comprise amino acids 23-116 of human RAGE (SEQ ID NO: 7) or a sequence 90% identical thereto, or amino acids 24-116 of human RAGE (SEQ ID NO: 8) or a sequence 90% identical thereto, corresponding to the V domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 124-221 of human RAGE (SEQ ID NO: 11) or a sequence 90% identical thereto, corresponding to the C1 domain of RAGE. In another embodiment, the RAGE polypeptide may comprise amino acids 227-317 of human RAGE (SEQ ID NO: 12) or a sequence 90% identical thereto, corresponding to the C2 domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 23-123 of human RAGE (SEQ ID NO: 13) or a sequence 90% identical thereto, or amino acids 24-123 of human RAGE (SEQ ID NO: 14) or a sequence 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 23-226 of human RAGE (SEQ ID NO: 17) or a sequence 90% identical thereto, or amino acids 24-226 of human RAGE (SEQ ID NO: 18) or a sequence 90% identical thereto, corresponding to the V-domain, the C1 domain and the interdomain linker linking these two domains. Or, the RAGE polypeptide may comprise amino acids 23-339 of human RAGE (SEQ ID NO: 5) or a sequence 90% identical thereto, or 24-339 of human RAGE (SEQ ID NO: 6) or a sequence 90% identical thereto, corresponding to sRAGE (i.e., encoding the V, C1, and C2 domains and interdomain linkers). Or, fragments of each of these sequences may be used.

The fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the fusion protein may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ε), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4), IgA1 (α1), IgA2 (α2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, in one embodiment, the RAGE fusion protein of the present invention comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus in one embodiment, the fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. In one embodiment, the C_H2 domain, or a fragment thereof comprises SEQ ID NO: 42.

In one embodiment, the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof, may comprise the C_H2 and C_H3 domains of a human IgG1. As an example embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40.

The fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The fusion protein may comprise a first RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence

90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein.

Alternatively, a three domain fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence 90% identical thereto corresponding to the V domain of RAGE and a downstream interdomain linker. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein.

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is 70% identical, or 80% identical, or 90% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For

example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342.

Pharmaceutically acceptable carriers may comprise any of the standard pharmaceutically accepted carriers known in the art. The carrier may comprise a diluent. In one embodiment, the pharmaceutical carrier may be a liquid and the fusion protein or nucleic acid construct may be in the form of a solution. In another embodiment, the pharmaceutically acceptable carrier may be a solid in the form of a powder, a lyophilized powder, or a tablet. Or, the pharmaceutical carrier may be a gel, suppository, or cream. In alternate embodiments, the carrier may comprise a liposome, a microcapsule, a polymer encapsulated cell, or a virus. Thus, the term pharmaceutically acceptable carrier encompasses, but is not limited to, any of the standard pharmaceutically accepted carriers, such as water, alcohols, phosphate buffered saline solution, sugars (e.g., sucrose or mannitol), oils or emulsions such as oil/water emulsions or a trigyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

Administration of the RAGE fusion proteins of the present invention may employ various routes. Thus, administration of the RAGE fusion protein of the present invention may employ intraperitoneal (IP) injection. Alternatively, the RAGE fusion protein may be administered orally, intranasally, or as an aerosol. In another embodiment, administration is intravenous (IV). The RAGE fusion protein may also be injected subcutaneously. In another embodiment, administration of the fusion protein is intra-arterial. In another embodiment, administration is sublingual. Also, administration may employ a time-release capsule. In yet another embodiment, administration may be transrectal, as by a suppository or the like. For example, subcutaneous administration may be useful to treat chronic disorders when the self-administration is desireable.

The pharmaceutical compositions may be in the form of a sterile injectable solution in a non-toxic parenterally acceptable solvent or vehicle. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, 3-butanediol, isotonic sodium chloride solution, or aqueous buffers, as for example, physiologically acceptable citrate, acetate, glycine, histidine, phosphate, tris or succinate buffers. The injectable solution may contain stabilizers to protect against chemical degradation and aggregate formation. Stabilizers may include antioxidants such as butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT), buffers (citrates, glycine, histidine) or surfactants (polysorbate 80, poloxamers). The solution may also contain antimicrobial preservatives,

such as benzyl alcohol and parabens. The solution may also contain surfactants to reduce aggregation, such as Polysorbate 80, poloxomer, or other surfactants known in the art. The solution may also contain other additives, such as a sugar(s) or saline, to adjust the osmotic pressure of the composition to be similar to human blood.

The pharmaceutical compositions may be in the form of a sterile lyophilized powder for injection upon reconstitution with a diluent. The diluent can be water for injection, bacteriostatic water for injection, or sterile saline. The lyophilized powder may be produced by freeze drying a solution of the fusion protein to produce the protein in dry form. As is known in the art, the lyophilized protein generally has increased stability and a longer shelf life than a liquid solution of the protein. The lyophilized powder (cake) many contain a buffer to adjust the pH, as for example physiologically acceptable citrate, acetate, glycine, histidine, phosphate, tris or succinate buffer. The lyophilized powder may also contain lyoprotectants to maintain its physical and chemical stability. The commonly used lyoprotectants are non-reducing sugars and disaccharides such as sucrose, mannitol, or trehalose. The lyophilized powder may contain stabilizers to protect against chemical degradation and aggregate formation. Stabilizers may include, but are not limited to antioxidants (BHA, BHT), buffers (citrates, glycine, histidine), or surfactants (polysorbate 80, poloxamers). The lyophilized powder may also contain antimicrobial preservatives, such as benzyl alcohol and parabens. The lyophilized powder may also contain surfactants to reduce aggregation, such as, but not limited to, Polysorbate 80 and poloxomer. The lyophilized powder may also contain additives (e.g., sugars or saline) to adjust the osmotic pressure to be similar to human blood upon reconstitution of the powder. The lyophilized powder may also contain bulking agents, such as sugars and disaccharides.

The pharmaceutical compositions for injection may also be in the form of a oleaginous suspension. This suspension may be formulated according to the known methods using suitable dispersing or wetting agents and suspending agents described above. In addition, sterile, fixed oils are conveniently employed as solvent or suspending medium. For this purpose, any bland fixed oil may be employed using synthetic mono- or diglycerides. Also, oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as a liquid paraffin. For example, fatty acids such as oleic acid find use in the preparation of injectables. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alchol. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the present invention may also be in the form of oil-in-water emulsions or aqueous suspensions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example a liquid paraffin, or a mixture thereof. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of said partial esters with ethylene oxide, for example polyoxyethylene sorbitan.

Aqueous suspensions may also contain the active compounds in admixture with excipients. Such excipients may include suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, such as a naturally-occurring phosphatide such as lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water may provide the active compound in admixture with a dispersing agent, suspending agent, and one or more preservatives. Suitable preservatives, dispersing agents, and suspending agents are described above.

The compositions may also be in the form of suppositories for rectal administration of the compounds of the invention. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will thus melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols, for example.

For topical use, creams, ointments, jellies, solutions or suspensions containing the compounds of the invention may be used. Topical applications may also include mouth washes and gargles. Suitable preservatives, antioxidants such as BHA and BHT, dispersants, surfactants, or buffers may be used.

The compounds of the present invention may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and

multilamellar vesicles. Liposomes may be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

In certain embodiments, the compounds of the present invention may be modified to further retard clearance from the circulation by metabolic enzymes. In one embodiment, the compounds may be modified by the covalent attachment of water-soluble polymers such as polyethylene glycol (PEG), copolymers of PEG and polypropylene glycol, polyvinylpyrrolidone or polyproline, carboxymethyl cellulose, dextran, polyvinyl alcohol, and the like. Such modifications also may increase the compound's solubility in aqueous solution. Polymers such as PEG may be covalently attached to one or more reactive amino residues, sulfydryl residues or carboxyl residues. Numerous activated forms of PEG have been described, including active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxsuccinimide, p-nitrophenol, imdazole or 1-hydroxy-2-nitrobenzene-3 sulfone for reaction with amino groups, multimode or halo acetyl derivatives for reaction with sulfhydryl groups, and amino hydrazine or hydrazide derivatives for reaction with carbohydrate groups.

Additional methods for preparation of protein formulations which may be used with the fusion proteins of the present invention are described in U.S. Patents No. 6,267,958, and 5,567,677.

In a further aspect of the present invention, the RAGE modulators of the invention are utilized in adjuvant therapeutic or combination therapeutic treatments with other known therapeutic agents. The following is a non-exhaustive listing of adjuvants and additional therapeutic agents which may be utilized in combination with the RAGE fusion protein modulators of the present invention:

Pharmacologic classifications of anticancer agents:

1. Alkylating agents: Cyclophosphamide, nitrosoureas, carboplatin, cisplatin, procarbazine
2. Antibiotics: Bleomycin, Daunorubicin, Doxorubicin
3. Antimetabolites: Methotrexate, Cytarabine, Fluorouracil
4. Plant alkaloids: Vinblastine, Vincristine, Etoposide, Paclitaxel,
5. Hormones: Tamoxifen, Octreotide acetate, Finasteride, Flutamide
6. Biologic response modifiers: Interferons, Interleukins,

Pharmacologic classifications of treatment for Rheumatoid Arthritis

1. Analgesics: Aspirin
2. NSAIDs (Nonsteroidal anti-inflammatory drugs): Ibuprofen, Naproxen, Diclofenac

3. DMARDs (Disease-Modifying Antirheumatic drugs): Methotrexate, gold preparations, hydroxychloroquine, sulfasalazine
4. Biologic Response Modifiers, DMARDs: Etanercept, Infliximab Glucocorticoids

Pharmacologic classifications of treatment for Diabetes Mellitus

1. Sulfonylureas: Tolbutamide, Tolazamide, Glyburide, Glipizide
2. Biguanides: Metformin
3. Miscellaneous oral agents: Acarbose, Troglitazone
4. Insulin

Pharmacologic classifications of treatment for Alzheimer's Disease

1. Cholinesterase Inhibitor: Tacrine, Donepezil
2. Antipsychotics: Haloperidol, Thioridazine
3. Antidepressants: Desipramine, Fluoxetine, Trazodone, Paroxetine
4. Anticonvulsants: Carbamazepine, Valproic acid

In one embodiment, the present invention may therefore provide a method of treating RAGE mediated diseases, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a RAGE fusion protein in combination with therapeutic agents selected from the group consisting of alkylating agents, antimetabolites, plant alkaloids, antibiotics, hormones, biologic response modifiers, analgesics, NSAIDs, DMARDs, glucocorticoids, sulfonylureas, biguanides, insulin, cholinesterase inhibitors, antipsychotics, antidepressants, and anticonvulsants. In a further embodiment, the present invention provides the pharmaceutical composition of the invention as described above, further comprising one or more therapeutic agents selected from the group consisting of alkylating agents, antimetabolites, plant alkaloids, antibiotics, hormones, biologic response modifiers, analgesics, NSAIDs, DMARDs, glucocorticoids, sulfonylureas, biguanides, insulin, cholinesterase inhibitors, antipsychotics, antidepressants, and anticonvulsants.

EXAMPLES

Features and advantages of the inventive concept covered by the present invention are further illustrated in the examples which follow.

Example 1: Production of RAGE-IgG Fc Fusion Proteins

Two plasmids were constructed to express RAGE-IgG Fc fusion proteins. Both plasmids were constructed by ligating different lengths of a 5' cDNA sequence from human RAGE with the same 3' cDNA sequence from human IgG Fc (γ1). These expression sequences (*i.e.*, ligation products) were then inserted in pcDNA3.1 expression vector

(Invitrogen, CA). The nucleic acid sequences that encode the fusion protein coding region are shown in FIGS. 2 and 3. For TTP-4000 fusion protein, the nucleic acid sequence from 1 to 753 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the nucleic acid sequence from 754 to 1386 encodes the IgG Fc protein sequence (FIG. 2). For TTP-3000, the nucleic acid sequence from 1 to 408 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the nucleic acid sequence from 409 to 1041 encodes the IgG Fc protein sequence (FIG. 3).

To produce the RAGE fusion proteins, the expression vectors comprising the nucleic acid sequences of either SEQ ID NO: 30 or SEQ ID NO: 31 were stably transfected into CHO cells. Positive transformants were selected for neomycin resistance conferred by the plasmid and cloned. High producing clones as detected by Western Blot analysis of supernatant were expanded and the gene product was purified by affinity chromatography using Protein A columns. Expression was optimized so that cells were producing recombinant TTP-4000 at levels of about 1.3 grams per liter.

The expressed polypeptides encoding the two fusion proteins are illustrated in FIGS. 4-6. For the four domain structure of TTP-4000, the first 251 amino acids (shown in bold in FIG. 4) contain a signal sequence (1-22/23), the V immunoglobulin (and ligand binding) domain (23/24-116), a second interdomain linker (117-123), a second immunoglobulin domain (C_H1) (124-221), and a second linker (222-251) of the human RAGE protein (FIGS. 4, 6B). The sequence from 252 to 461 includes the C_H2 and C_H3 immunoglobulin domains of IgG.

For the three domain structure of TTP-3000, the first 136 amino acids (shown in bold) contain a signal sequence (1-22/23), the V immunoglobulin (and ligand binding) domain (23/24-116) and an interdomain linker sequence (117-136) of the human RAGE protein (FIGS. 5, 6B). In addition, for TT3, the sequence from 137 to 346 includes the C_H2 and C_H3 immunoglobulin domains of IgG.

Example 2: Method for testing activity of a RAGE-IgG1 fusion protein

A. ***In vitro* ligand binding:**

Known RAGE ligands were coated onto the surface of Maxisorb plates at a concentration of 5 micrograms per well. Plates were incubated at 4°C overnight. Following ligand incubation, plates were aspirated and a blocking buffer of 1% BSA in 50 mM imidizole buffer (pH 7.2) was added to the plates for 1 hour at room temperature. The plates were then aspirated and/or washed with wash buffer (20 mM Imidizole, 150 mM NaCl, 0.05% Tween-20, 5 mM $CaCl_2$ and 5mM $MgCl_2$, pH 7.2). A solution of TTP-3000 (TT3) at

an initial concentration of 1.082 mg/mL and a solution of TTP-4000 (TT4) at an initial concentration of 370 μg/mL were prepared. The fusion protein was added at increasing dilutions of the initial sample. The RAGE fusion protein was allowed to incubate with the immobilized ligand at 37°C for one hour after which the plate was washed and assayed for binding of the fusion protein. Binding was detected by the addition of an immunodetection complex containing a monoclonal mouse anti-human IgG1 diluted 1:11,000 to a final assay concentration (FAC) of 21 ng/100 μL, a biotinylated goat anti-mouse IgG diluted 1:500, to a FAC of 500 ng/μL, and an avidin-linked alkaline phosphatase. The complex was incubated with the immobilized fusion protein for one hour at room temperature after which the plate was washed and the alkaline phosphatase substrate *para*-nitrophenylphosphate (PNPP) was added. Binding of the complex to the immobilized fusion protein was quantified by measuring conversion of PNPP to *para*-nitrophenol (PNP) which was measured spectrophotometrically at 405 nm.

As illustrated in FIG. 7, the fusion proteins TTP-4000 (TT4) and TTP-3000 (TT3) specifically interact with known RAGE ligands amyloid-beta (Abeta), S100b (S100), and amphoterin (Ampho). In the absence of ligand, i.e., BSA coating alone (BSA or BSA + wash) there was no increase in absorbance over levels attributable to non-specific binding of the immunodetection complex. Where amyloid beta is used as the labeled ligand it may be necessary to preincubate the amyloid beta before the assay. Preincubation may allow the amyloid beta to self-aggregate into pleated sheet form, as amyloid beta may preferentially bind to RAGE in the form of a pleated sheet.

Additional evidence for a specific interaction between RAGE fusion proteins TTP-4000 and TTP-3000 with RAGE ligands is exemplified in studies showing that a RAGE ligand is able to effectively compete with a known RAGE ligand for binding to the fusion proteins. In these studies, amyloid-beta (A-beta) was immobilized on a Maxisorb plate and fusion protein added as described above. In addition, a RAGE ligand was added to some of the wells at the same time as the fusion protein.

It was found that the RAGE ligand could block binding of TTP-4000 (TT4) by about 25% to 30% where TTP-4000 was present at 123 μg/mL (1:3 dilution, FIG. 8). When the initial solution of TTP-4000 was diluted by a factor of 10 or 30 (1:10 or 1:30), binding of the fusion protein to the immobilized ligand was completely inhibited by the RAGE ligand. Similarly, the RAGE ligand blocked binding of TTP-3000 (TT3) by about 50% where TTP-3000 was present at 360 μg/mL (1:3 dilution, FIG. 9). When the initial solution of TTP-3000

was diluted by a factor of 10 (1:10), binding of the fusion protein to the immobilized ligand was completely inhibited by the RAGE ligand. Thus, specificity of binding of the RAGE fusion protein to the RAGE ligand was dose dependent. Also, as shown in FIGS. 8 and 9, there was essentially no binding detected in the absence of fusion protein, *i.e.*, using only the immunodetection complex ("Complex alone").

B. Effect of RAGE fusion proteins in a cell based assay

Previous work has shown that the myeloid THP-1 cells may secrete TNF-α in response to RAGE ligands. In this assay, THP-1 cells were cultured in RPMI-1640 media supplemented with 10% FBS using a protocol provided by ATCC. The cells were induced to secrete TNF-α via stimulation of RAGE with 0.1 mg/ml S100b both in the absence and the presence of the fusion proteins TTP-3000 (TT3) or TTP-4000 (TT4) (10 μg), sRAGE (10 μg), and a human IgG (10 μg) (i.e., as a negative control). The amount of TNF-α secreted by the THP-1 cells was measured 24 hours after the addition of the proteins to the cell culture using a commercially available ELISA kit for TNF-α (R&D Systems, Minneapolis, MN). The results in FIG. 10 demonstrate that the fusion proteins inhibit the S100b/RAGE-induced production of TNF-α in these cells. As shown in FIG. 10, upon addition of 10 μg TTP-3000 or TTP-4000 RAGE fusion protein, induction of TNF-α by S100b (0.1 mg/ml FAC) was reduced by about 45% to 70%, respectively. Fusion protein TTP-4000 may be at least as effective in blocking S100b induction of TNF-α as is sRAGE (FIG. 10). Specificity of the inhibition for the RAGE sequences of TTP-4000 and TTP-3000 is shown by the experiment in which IgG alone was added to S100b stimulated cells. Addition of IgG and S100b to the assay shows the same levels of TNF-α as S100b alone. Specificity of the inhibition of TNF-α induction by TTP-4000 and TTP-3000 for RAGE sequences of the fusion protein is shown by an experiment in which IgG alone was added to S100b stimulated cells. It can be seen that the addition of IgG, *i.e.*, human IgG without the RAGE sequence (Sigma human IgG added at 10 μg/well), and S100b to the assay shows the same levels of TNF-α as S100b alone.

Example 3: Pharmacokinetic Profile of TTP-4000

To determine whether TTP-4000 would have a superior pharmacokinetic profile as compared to human sRAGE, rats and nonhuman primates were given an intravenous (IV) injection of TTP-4000 (5mg/kg) and then plasma was assessed for the presence of TTP-4000. In these experiments, two naïve male monkeys received a single IV bolus dose of TTP-4000 (5mg/ml/kg) in a peripheral vein followed by an approximate 1.0 milliliter (mL) saline flush. Blood samples (approximately 1.0 mL) were collected at pre-dose (i.e., prior to injection of

the TTP-4000), or at 0.083, 0.25, 0.5, 2, 4, 8, 12, 24, 48, 72, 96, 120, 168, 240, 288, and 336 hours post dose into tubes containing (lithium heparin). Following collection, the tubes were placed on wet ice (maximum 30 minutes) until centrifugation under refrigeration (at 2 to 8°C) at 1500 x g for 15 minutes. Each harvested plasma sample was then stored frozen (-70 °C ±10 ° C) until assayed for RAGE polypeptide using an ELISA at various time-points following the injection, as described in Example 6.

The kinetic profile shown in FIG. 11 reveals that once TTP-4000 has saturated its ligands as evidenced by the fairly steep slope of the alpha phase in 2 animals, it retains a terminal half-life of greater than 300 hours. This half-life is significantly greater than the half-life of human sRAGE in plasma (generally about 2 hours) and provides an opportunity for single injections for acute and semi-chronic indications. In FIG. 11 each curve represents a different animal under the same experimental conditions.

Example 4: TTP-4000 Fc Activation

Experiments were performed to measure the activation of the Fc receptor by RAGE fusion protein TTP-4000 as compared to human IgG. Fc receptor activation was measured by measuring TNF-α secretion from THP-1 cells that express the Fc receptor. In these experiments, a 96-well plate was coated with 10 µg/well TTP-4000 or human IgG. Fc stimulation results in TNF-α secretion. The amount of TNF-α was measured by an Enzyme Linked Immunoabsorbent Assay (ELISA).

Thus, in this assay, the myeloid cell line, THP-1 (ATTC # TIB-202) was maintained in RPMI-1640 media supplemented with 10% fetal bovine serum per ATCC instructions. Typically, 40,000-80,000 cells per well were induced to secrete TNF-alpha via Fc receptor stimulation by precoating the well with 10 ug/well of either heat aggregated (63^0C for 30 min) TTP-4000 or human IgG1. The amount of TNF-alpha secreted by the THP-1 cells was measured in supernatants collected from 24 hours cultures of cells in the treated wells using a commercially available TNF ELISA kit (R&D Systems, Minneapolis, MN # DTA00C) per instructions.

Results are shown in FIG. 12 where it can be seen that TTP-4000 generates less than 2 ng/well TNF and IgG generated greater than 40 ng/well.

Example 5: In vivo activity of TTP-4000

The activity of TTP-4000 was compared to sRAGE in several *in vivo* models of human disease.

A. **TTP-4000 in an animal model of restenosis**

The RAGE fusion protein TTP-4000 was evaluated in a diabetic rat model of restenosis which involved measuring smooth muscle proliferation and intimal expansion 21 days following vascular injury. In these experiments, balloon injury of left common carotid artery was performed in Zucker diabetic and nondiabetic rats using standard procedure. A loading dose (3mg/rat) of IgG, TTP-4000 or phosphate buffered saline (PBS) was administered intraperitoneally (IP) one day prior injury. A maintenance dose was delivered every other day until day 7 after injury (i.e., at day 1, 3, 5 and 7 after injury). The maintenance dose was high = 1 mg/animal for one group, or low = 0.3 mg/animal for the second group. To measure vascular smooth muscle cell (VSMC) proliferation, animals were sacrificed at 4 days and 21 days after injury.

For the measurement of cell proliferation, 4 day animals received intraperitoneal injection of bromodeoxyuridine (BrDdU) 50 mg/kg at 18, 12, and 2 hours before euthanasia. After sacrifice, the entire left and right carotid arteries were harvested. Specimens were stored in Histochoice for at least 24 hours before embedding. Assessment of VSMC proliferation was performed using mouse anti-BrdU monoclonal antibody. A fluorescence labeled goat anti-mouse secondary antibody was applied. The number of BrdU-positive nuclei per section were counted by two observers blinded to the treatment regimens.

The remaining rats were sacrificed at 21 days for morphometric analysis. Morphometric analyses were performed by an observer blinded to the study groups, using computerized digital microscopic planimetry software Image-Pro Plus on serial sections, (5 mm apart) carotid arteries stained by Van Gieson staining. All data were expressed as mean ± SD. Statistical analysis was performed with use of SPSS software. Continuous variables were compared using unpaired t tests. A values of $P \leq 0.05$ was considered to be statistically significant.

As seen in FIGS. 13A and 13B, TTP-4000 treatment significantly reduced the intima/media ratio and vascular smooth muscle cell proliferation in a dose-responsive fashion. In FIG. 13 B, the y-axis represents the number of BrdU proliferating cells.

B. **TTP4000 in an animal model of AD**

Experiments were performed to evaluate whether TTP-4000 could affect amyloid formation and cognitive dysfunction in a mouse model of AD. The experiments utilized transgenic mice expressing the human Swedish mutant amyloid precursor protein (APP) under the control of the PDGF-B chain promoter. Over time, these mice generate high levels

of the RAGE ligand, amyloid beta (Aβ). Previously, sRAGE treatment for 3 months has been shown to reduce both amyloid plaque formation in the brain and the associated increase in inflammatory markers in this model.

The APP mice (male) used in this experiment were designed by microinjection of the human APP gene (with the Swedish and London mutations) into mouse eggs under the control of the platelet-derived growth factor B (PDGF-B) chain gene promoter. The mice were generated on a C57BL/6 background and were developed by Molecular Therapeutics Inc. Animals were fed *ad libitum* and maintained by brother sister mating. The mice generated from this construct develop amyloid deposits starting at 6 months of age. Animals were aged for 6 months and then maintained for 90 days and sacrificed for amyloid quantification.

APP transgenic mice were administered vehicle or TTP4000 every other day [qod (i.p.)] for 90 days starting at 6 months of age. At the end of the experiment, animals were sacrificed and examined for Aβ plaque burden in the brain (i.e., plaque number). A 6-month control APP group was used to determine the baseline of amyloid deposits. In addition, at the end of the study, the animals were subjected to behavioral (Morris water maze) analysis. The investigators were blinded to the study compounds. Samples were given to the mice at 0.25 ml/mouse/every other day. In addition, one group of mice were given 200 ug/day of human sRAGE.

1. Amyloid Beta Deposition

For histological examination, the animals were anesthetized with an intraperitoneal injection (IP) of sodium pentobarbital (50 mg/kg). The animals were transcardially perfused with 4°C, phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed and placed in 4% paraformaldehyde over night. The brains were processed to paraffin and embedded. Ten serial 30-μm thick sections through the brain were obtained. Sections were subjected to primary antibody overnight at 4°C (Aβ peptide antibody) in order to detect the amyloid deposits in the brain of the transgenic animals (Guo *et al., J. Neurosci.*, 22:5900-5909 (2002)). Sections were washed in Tris-buffered saline (TBS) and secondary antibody was added and incubated for 1 hour at room temperature. After washing, the sections were incubated as instructed in the Vector ABC Elite kit (Vector Laboratories) and stained with diaminobenzoic acid (DAB). The reactions were stopped in water and cover-slipped after treatment with xylene. The amyloid area in each section was determined with a computer-assisted image analysis system, consisting of a Power Macintosh computer equipped with a Quick Capture frame grabber card, Hitachi CCD camera mounted on an

Olympus microscope and camera stand. NIH Image Analysis Software, v. 1.55 was used. The images were captured and the total area of amyloid was determined over the ten sections. A single operator blinded to treatment status performed all measurements. Summing the amyloid volumes of the sections and dividing by the total number of sections was done to calculate the amyloid volume.

For quantitative analysis, an enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of human total Aβ, $A\beta_{total}$ and $A\beta_{1-42}$ in the brains of APP transgenic mice (Biosource International, Camarillo, CA). $A\beta_{total}$ and $A\beta_{1-42}$ were extracted from mouse brains by guanidine hydrochloride and quantified as described by the manufacturer. This assay extracts the total Aβ peptide from the brain (both soluble and aggregated).

2. Cognitive Function

The Morris water-maze testing was performed as follows:. All mice were tested once in the Morris water maze test at the end of the experiment. Mice were trained in a 1.2 m open field water maze. The pool was filled to a depth of 30 cm with water and maintained at 25°C. The escape platform (10 cm square) was placed 1 cm below the surface of the water. During the trials, the platform was removed from the pool. The cued test was carried out in the pool surrounded with white curtains to hide any extra-maze cues. All animals underwent non-spatial pretraining (NSP) for three consecutive days. These trials are to prepare the animals for the final behavioral test to determine the retention of memory to find the platform. These trials were not recorded, but were for training purposes only. For the training and learning studies, the curtains were removed to extra maze cues (this allowed for identification of animals with swimming impairments). On day 1, the mice were placed on the hidden platform for 20 seconds (trial 1), for trials 2-3 animals were released in the water at a distance of 10 cm from the cued-platform or hidden platform (trial 4) and allowed to swim to the platform. On the second day of trails, the hidden platform was moved randomly between the center of the pool or the center of each quadrant. The animals were released into the pool, randomly facing the wall and were allowed 60 seconds to reach the platform (3 trials). In the third trial, animals were given three trials, two with a hidden platform and one with a cued platform. Two days following the NSP, animals were subjected to final behavioral trials (Morris water maze test). For these trials (3 per animal), the platform was placed in the center of one quadrant of the pool and the animals released facing the wall in a random fashion. The animal was allowed to find the platform or swim for 60 seconds (latency period,

the time it takes to find the platform). All animals were tested within 4-6 hours of dosing and were randomly selected for testing by an operator blinded to the test group.

The results are expressed as the mean ± standard deviations (SD). The significance of differences in the amyloid and behavioral studies were analyzed using a t-test. Comparisons were made between the 6-month-old APP control group and the TTP-4000 treated animals, as well as, the 9-month-old APP vehicle treated group and the TTP-4000 treated animals. Differences below 0.05 were considered significant. Percent changes in amyloid and behavior were determined by taking the summation of the data in each group and dividing by the comparison (i.e., 1, i.p./6 month control = % change).

FIGS. 14A and 14B show that mice treated for 3 months with either TTP-4000 or mouse sRAGE had fewer Aβ plaques and less cognitive dysfunction than vehicle and negative control human IgG1 (IgG1) treated animals. This data indicates that TTP-4000 is effective in reducing AD pathology in a transgenic mouse model. It was also found that like sRAGE, TTP-4000 can reduce the inflammatory cytokines IL-1 and TNF-α (data not shown).

C. **Efficacy of TTP-4000 in an animal model of stroke**

TTP-4000 was also compared to sRAGE in a disease relevant animal model of stroke. In this model, the middle carotid artery of a mouse was ligated for 1 hour followed by 23 hours of reperfusion at which point the mice were sacrificed and the area of the infarct in the brain was assessed. Mice were treated with sRAGE or TTP-4000 or control immunoglobulin just prior to reperfusion.

In these experiments, male C57BL/6 were injected with vehicle at 250 µl/mouse or TTP test articles (TTP-3000, TTP-4000 at 250 µl/mouse). Mice were injected intraperitoneally, 1 hour after the initiation of ischemia. Mice were subjected to one hour of cerebral ischemia followed by 24 hours of reperfusion. To induce ischemia, each mouse was anesthetized and body temperature was maintained at 36-37°C by external warming. The left common carotid artery (CCA) was exposed through a midline incision in the neck. A microsurgical clip was placed around the origin of the internal carotid artery (ICA). The distal end of the ECA was ligated with silk and transected. A 6-0 silk was tied loosely around the ECA stump. The fire-polished tip of a nylon suture was gently inserted into the ECA stump. The loop of the 6-0 silk was tightened around the stump and the nylon suture was advanced into and through the internal carotid artery (ICA), until it rested in the anterior cerebral artery, thereby occluding the anterior communicating and middle cerebral arteries. After the nylon suture had been in place for 1 hour, the animal was

re-anesthetized, rectal temperature was recorded and the suture was removed and the incision closed.

Infarct volume was determined by anesthetizing the animals with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and then removing the brains. The brains were then sectioned into four 2-mm sections through the infracted region and placed in 2% triphenyltetrazolium chloride (TTC) for 30 minutes. After, the sections were placed in 4% paraformaldehyde over night. The infarct area in each section was determined with a computer-assisted image analysis system, consisting of a Power Macintosh computer equipped with a Quick Capture frame grabber card, Hitachi CCD camera mounted on a camera stand. NIH Image Analysis Software, v. 1.55 was used. The images were captured and the total area of infarct was determined over the sections. A single operator blinded to treatment status performed all measurements. Summing the infarct volumes of the sections calculated the total infarct volume. The results are expressed as the mean ± standard deviation (SD). The significance of difference in the infarct volume data was analyzed using a t-test.

As illustrated by the data in Table 2, TTP-4000 was more efficacious than sRAGE in limiting the area of infarct in these animals suggesting that TTP-4000, because of its better half-life in plasma, was able to maintain greater protection in these mice.

Example 6: Detection of RAGE Fusion Protein by ELISA

Initially, 50 uL of the RAGE specific monoclonal antibody 1HB1011at a concentration of 10 ug/mL in 1X PBS pH 7.3 is coated on plates via overnight incubation. When ready for use, plates are washed three times with 300 uL of 1X Imidazole-Tween wash buffer and blocked with 1% BSA. The samples (diluted) and standard dilutions of known TTP-4000 dilutions are added at 100 uL final volume. The samples are allowed to incubate at room temperature for one hour. After incubation, the plates are plates are washed three times. A Goat Anti-human IgG1 1 (Sigma A3312) AP conjugate in 1XPBS with 1% BSA is added and allowed to incubate at room temperature for 1 hour. The plates are washed three times. Color was elucidated with paranitrophenylphosphate.

Example 7: Quantification of RAGE Ligand Binding to RAGE Fusion Protein

Figure 15 shows saturation-binding curves with TTP-4000 to various immobilized known RAGE ligands. The ligands are immobilized on a microtiter plate and incubated in the presence of increasing concentrations of fusion protein from 0 to 360 nM. The fusion protein-ligand interaction is detected using a polyclonal antibody conjugated with alkaline phosphatase that is specific for the IgG portion of the fusion chimera. Relative Kds were calculated using Graphpad Prizm software and match with established literature values of

RAGE-RAGE ligand values. HMG1B = Ampoterin, CML= Carboxymethyl Lysine, A beta = Amyloid beta 1-40.

The foregoing is considered as illustrative only of the principal of the invention. Since numerous modifications and changes will readily occur to those skilled in the art, it is not intended to limit the invention to the exact embodiments shown and described, and all suitable modifications and equivalents falling within the scope of the appended claims are deemed within the present inventive concept.

CLAIMS

1. A fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin, and a C_H3 domain of an immunoglobulin;

wherein the RAGE polypeptide comprises a RAGE ligand binding site;

wherein the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the RAGE interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising the C_H2 domain of the immunoglobulin, or a portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin; and

wherein the fusion protein comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

2. The fusion protein of claim 1, wherein the fusion protein comprises (a) the RAGE interdomain linker that separates the V and C1 domains of RAGE, or (b) the linker that separates the C1 and C2 domains of RAGE, in lieu of the hinge region of the immunoglobulin heavy chain.

3. The fusion protein of claim 1 or claim 2, wherein the RAGE polypeptide comprises a fragment of human RAGE, wherein the sequence of human RAGE is set forth in SEQ ID NO: 1.

4. The fusion protein of any one of claims 1 to 3, wherein the fusion protein comprises a first RAGE immunoglobulin domain and a first RAGE interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first RAGE interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to the C-terminal amino acid of the first RAGE interdomain linker, the N-terminal amino acid of the second RAGE interdomain linker is linked to the C-

terminal amino acid of the second RAGE immunoglobulin domain, and the C-terminal amino acid of the second RAGE interdomain linker is directly linked to the N-terminal amino acid of the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin.

5. The fusion protein of claim 4, wherein the second RAGE interdomain linker directly linked to the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin comprises the amino acid sequence set forth in SEQ ID NO: 22 or a sequence 90% identical thereto, or the amino acid sequence set forth in SEQ ID NO: 24 or a sequence 90% identical thereto.

6. The fusion protein of claim 1, wherein the RAGE polypeptide is a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin.

7. The fusion protein of claim 6, wherein the RAGE interdomain linker is directly linked to the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin and comprises the amino acid sequence set forth in SEQ ID NO: 21 or a sequence 90% identical thereto, or the amino acid sequence set forth in SEQ ID NO: 23 or a sequence 90% identical thereto.

8. The fusion protein of any one of the preceding claims, wherein the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence 90% identical thereto, or SEQ ID NO: 10 or a sequence 90% identical thereto.

9. The fusion protein of claim 1, wherein the RAGE polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

10. The fusion protein of claim 1, wherein the amino acid sequence of the RAGE polypeptide is set forth in any one of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 19 or SEQ ID NO: 20.

11. The fusion protein of claim 1, wherein the amino acid sequence of the polypeptide comprising the C_H2 domain of the immunoglobulin, or a portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin is or comprises the amino acid sequence set forth in SEQ ID NO: 38.

12. The fusion protein of any one of claims1 to 10, wherein the immunoglobulin is a human immunoglobulin selected from the group consisting of: IgA, IgD, IgE, IgG, and IgM.

13. The fusion protein of claim 12, wherein the immunoglobulin is selected from the group consisting of IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4.

14. The fusion protein of claim 13, wherein the immunoglobulin is IgG1.

15. A fusion protein according to claim 1, comprising the amino acid sequence set forth in any one of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36 or SEQ ID NO: 37.

16. A fusion protein according to claim 1, having the amino acid sequence set forth in SEQ ID NO: 37 or SEQ ID NO: 34.

17. A fusion protein according to claim 1, encodable by the nucleic acid sequence set forth in SEQ ID NO: 30 or SEQ ID NO: 31.

18. A nucleic acid encoding a fusion protein of any of the preceding claims.

19. The nucleic acid of claim 18, wherein the nucleic acid sequence encoding the RAGE polypeptide is or comprises the nucleic acid sequence set forth in any one of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29.

20. The nucleic acid of claim 19 comprising the nucleic acid sequence set forth in SEQ ID NO: 30 or SEQ ID NO: 31.

21. An expression vector that encodes for a fusion protein of any one of claims 1 to 17 and/or comprises a nucleic acid according to any one of claims 18-20.

22. A pharmaceutical composition comprising a therapeutically effective amount of a fusion protein of any one of claims 1 to 17 in a pharmaceutical carrier.

23. The pharmaceutical composition of claim 22 formulated as either an injectable solution or a sterile lyophilized powder.

24. A method of making a fusion protein according to any of claims 1 to 17 comprising the step of covalently linking the RAGE polypeptide to the polypeptide comprising the C_H2 domain of the immunoglobulin or the portion of the C_H2 domain of the immunoglobulin, and the C_H3 domain of the immunoglobulin.

25. The method of claim 24, wherein the fusion protein is obtained by removing the Fc hinge region of the immunoglobulin and replacing it with the RAGE polypeptide.

26. The method of claim 24, wherein the fusion protein is encoded by a recombinant DNA construct.

27. The method of claim 24, further comprising the step of incorporating the DNA construct into an expression vector.

28. The method of claim 27, further comprising transfecting the expression vector into a host cell.

29. The use of a fusion protein according to any of claims 1 to 17, in the preparation of a medicament for the treatment of a RAGE-mediated disorder in a subject.

30. The use of claim 29, wherein the medicament is intended for intravenous, intraperitoneal or subcutaneous administration of the RAGE fusion protein to the subject.

31. The use of claim 29 or 30, wherein the medicament is intended to treat a symptom of diabetes or a symptom of diabetic late complications.

32. The use of claim 31, wherein the symptom of diabetes or diabetic late complications comprises diabetic nephropathy, diabetic retinopathy, a diabetic foot ulcer, a cardiovascular complication or diabetic neuropathy.

33. The use of claim 29 or 30, wherein the medicament is intended to treat amyloidosis; Alzheimer's disease; cancer; kidney failure; or inflammation associated with any of autoimmunity, inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, hypoxia, stroke, heart attack, hemorrhagic shock, sepsis, organ transplantation or impaired wound healing.

34. A host cell comprising an expression vector of claim 21.

35. The host cell of claim 34, wherein the host cell is a Chinese Hamster Ovary cell.

36. A method of producing a fusion protein comprising culturing a host cell of claim 34 or claim 35.

SEQUENCE LISTING

<110> Mjalli, Adnan M. M.
Webster, Jeffrey C.
Rothlein, Robert
Tian, Ye E.

<120> RAGE Fusion Proteins and Methods of Use

<130> 57739-338003

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Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
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Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
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        195                 200                 205

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    290                 295                 300

His Ser Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser Ile Ser Ile
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        195                 200                 205

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Gly Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro
225                 230                 235                 240

Ala Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu
                245                 250                 255

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            260                 265                 270

Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro
        275                 280                 285

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                325                 330                 335

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35 40 45

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50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
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        195                 200                 205

Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
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225                 230                 235                 240

Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu Pro
                245                 250                 255

Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln Asp
            260                 265                 270

Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro Gln
        275                 280                 285

Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu Gly
    290                 295                 300

Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly Thr Leu Ala Leu
305                 310                 315                 320

Ala Leu Gly Ile Leu Gly Gly Leu Gly Thr Ala Ala Leu Leu Ile Gly
                325                 330                 335

Val Ile Leu Trp Gln Arg Arg Gln Arg Arg Gly Glu Glu Arg Lys Ala
            340                 345                 350

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    50                  55                  60

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            180                 185                 190

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            260                 265                 270

Lys Asp Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu
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Pro Glu Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr
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His Ser Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser Ile Ser Ile
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Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu
    50                  55                  60

Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser
65                  70                  75                  80

Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser
                85                  90
```

<210> 13
<211> 101
<212> PRT
<213> Homo sapiens

<400> 13

```
Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1               5                  10                  15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
            20                  25                  30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
        35                  40                  45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
    50                  55                  60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65                  70                  75                  80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
                85                  90                  95

Gln Ile Pro Gly Lys
            100
```

<210> 14
<211> 100
<212> PRT
<213> Homo sapiens

<400> 14

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85 90 95

Ile Pro Gly Lys
100

<210> 15
<211> 114
<212> PRT
<213> Homo sapiens

<400> 15

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu

50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
100 105 110

Ala Gly

<210> 16
<211> 113
<212> PRT
<213> Homo sapiens

<400> 16

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
100 105 110

Gly

<210> 17
<211> 204
<212> PRT
<213> Homo sapiens

<400> 17

```
Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1               5                  10                  15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
            20                  25                  30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
        35                  40                  45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
    50                  55                  60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65                  70                  75                  80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
                85                  90                  95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
            100                 105                 110

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
        115                 120                 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
    130                 135                 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
145                 150                 155                 160

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
                165                 170                 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu
            180                 185                 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln
```

195 200

<210> 18
<211> 203
<212> PRT
<213> Homo sapiens

<400> 18

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
100 105 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
115 120 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
130 135 140

Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
145 150 155 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
165 170 175

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
180 185 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln
195 200

<210> 19
<211> 229
<212> PRT
<213> Homo sapiens

<400> 19

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
100 105 110

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
115 120 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
130 135 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
145 150 155 160

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
165 170 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu

180 185 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp
195 200 205

Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly
210 215 220

Gly Ala Val Ala Pro
225

<210> 20
<211> 228
<212> PRT
<213> Homo sapiens

<400> 20

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
100 105 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
115 120 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
130 135 140

```
Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
145                 150                 155                 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
                165                 170                 175

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
            180                 185                 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu
        195                 200                 205

Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
    210                 215                 220

Ala Val Ala Pro
225
```

<210> 21
<211> 7
<212> PRT
<213> Homo sapiens

<400> 21

```
Val Tyr Gln Ile Pro Gly Lys
1               5
```

<210> 22
<211> 30
<212> PRT
<213> Homo sapiens

<400> 22

```
Thr Ala Pro Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu
1               5                   10                  15

Val Gln Leu Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro
            20                  25                  30
```

<210> 23
<211> 20
<212> PRT
<213> Homo sapiens

<400> 23

Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu
1 5 10 15

Leu Thr Ala Gly
20

<210> 24
<211> 5
<212> PRT
<213> Homo sapiens

<400> 24

Thr Ala Pro Ile Gln
1 5

<210> 25
<211> 354
<212> DNA
<213> Homo sapiens

<400> 25
atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctac 354

<210> 26
<211> 369
<212> DNA
<213> Homo sapiens

<400> 26
atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaag 369

<210> 27
<211> 408
<212> DNA
<213> Homo sapiens

<400> 27
atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggt 408

<210> 28
<211> 690
<212> DNA
<213> Homo sapiens

<400> 28
atggcagccg gaacagcagt tggagcctgg gcgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tcccaataag 420

gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcacttggat 480

gggaagcccc tggtgcctaa tgagaaggga gtatctgtga aggaacagac caggagacac 540

cctgagacag ggctcttcac actgcagtcg gagctaatgg tgaccccagc ccggggagga 600

gatccccgtc ccaccttctc ctgtagcttc agcccaggcc ttccccgaca ccgggccttg 660

cgcacagccc ccatccagcc ccgtgtctgg 690

<210> 29
<211> 753

<212> DNA
<213> Homo sapiens

<400> 29
atggcagccg gaacagcagt tggagcctgg gcgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tcccaataag 420

gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcacttggat 480

gggaagcccc tggtgcctaa tgagaaggga gtatctgtga aggaacagac caggagacac 540

cctgagacag ggctcttcac actgcagtcg gagctaatgg tgaccccagc ccggggagga 600

gatccccgtc ccaccttctc ctgtagcttc agcccaggcc ttccccgaca ccgggccttg 660

cgcacagccc ccatccagcc ccgtgtctgg gagcctgtgc ctctggagga ggtccaattg 720

gtggtggagc cagaaggtgg agcagtagct cct 753

<210> 30
<211> 1386
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 30
atggcagccg gaacagcagt tggagcctgg gcgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tcccaataag 420

gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcacttggat 480

gggaagcccc tggtgcctaa tgagaaggga gtatctgtga aggaacagac caggagacac 540

cctgagacag ggctcttcac actgcagtcg gagctaatgg tgaccccagc ccggggagga 600

gatccccgtc ccaccttctc ctgtagcttc agcccaggcc ttccccgaca ccgggccttg 660

cgcacagccc ccatccagcc ccgtgtctgg gagcctgtgc ctctggagga ggtccaattg 720

gtggtggagc cagaaggtgg agcagtagct cctccgtcag tcttcctctt ccccccaaaa 780

cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgcgtggt ggtggacgtg 840

agccacgaag accctgaggt caagttcaac tggtacgtgg acggcgtgga ggtgcataat 900

gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc 960

accgtcctgc accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa 1020

gccctcccag cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca 1080

caggtgtaca ccctgccccc atcccgggat gagctgacca agaaccaggt cagcctgacc 1140

tgcctggtca aaggcttcta tcccagcgac atcgccgtgg agtgggagag caatgggcag 1200

ccggagaaca actacaagac cacgcctccc gtgctggact ccgacggctc cttcttcctc 1260

tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc 1320

gtgatgcatg aggctctgca caaccactac acgcagaaga gcctctccct gtctccgggt 1380

aaatga 1386

<210> 31
<211> 1041
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 31
atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60

gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg 120

gcccccaaga aaccacccca gcggctggaa tggaaactga acacaggccg gacagaagct 180

tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240

aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300

gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360

cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtcc gtcagtcttc 420

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc 480

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 540

gtggaggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 600

gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 660

aaggtctcca acaaagccct cccagccccc atcgagaaaa ccatctccaa agccaaaggg 720

cagccccgag aaccacaggt gtacaccctg cccccatccc gggatgagct gaccaagaac 780

caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 840

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 900

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 960

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1020

tccctgtctc cgggtaaatg a 1041

<210> 32
<211> 461
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 32

Met Ala Ala Gly Thr Ala Val Gly Ala Trp Ala Leu Val Leu Ser Leu
1 5 10 15

Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu
20 25 30

Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
35 40 45

Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
50 55 60

Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
65 70 75 80

Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile
85 90 95

Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn
100 105 110

```
Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
        115                 120                 125

Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn Lys Val Gly Thr Cys
    130                 135                 140

Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His Leu Asp
145                 150                 155                 160

Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val Ser Val Lys Glu Gln
                165                 170                 175

Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu
            180                 185                 190

Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
        195                 200                 205

Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr Ala Pro
    210                 215                 220

Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu
225                 230                 235                 240

Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Pro Ser Val Phe Leu
                245                 250                 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
            260                 265                 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
        275                 280                 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
    290                 295                 300

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305                 310                 315                 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
                325                 330                 335
```

```
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
            340                 345                 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
        355                 360                 365

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
    370                 375                 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385                 390                 395                 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
                405                 410                 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
            420                 425                 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
        435                 440                 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
```

<210> 33
<211> 439
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 33

```
Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1               5                   10                  15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
            20                  25                  30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
        35                  40                  45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
    50                  55                  60
```

```
Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65                  70                  75                  80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
                85                  90                  95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
            100                 105                 110

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
        115                 120                 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
    130                 135                 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
145                 150                 155                 160

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
                165                 170                 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu
            180                 185                 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp
        195                 200                 205

Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly
    210                 215                 220

Gly Ala Val Ala Pro Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225                 230                 235                 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                245                 250                 255

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            260                 265                 270

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        275                 280                 285
```

```
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    290                 295                 300

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
305                 310                 315                 320

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
                325                 330                 335

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
            340                 345                 350

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        355                 360                 365

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
    370                 375                 380

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
385                 390                 395                 400

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                405                 410                 415

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
            420                 425                 430

Leu Ser Leu Ser Pro Gly Lys
        435
```

<210> 34
<211> 438
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 34

```
Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1               5                   10                  15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
            20                  25                  30
```

```
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
        35                  40                  45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
    50                  55                  60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65                  70                  75                  80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
                85                  90                  95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
            100                 105                 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
        115                 120                 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
    130                 135                 140

Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
145                 150                 155                 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
                165                 170                 175

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
            180                 185                 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu
        195                 200                 205

Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
    210                 215                 220

Ala Val Ala Pro Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
225                 230                 235                 240

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
                245                 250                 255
```

```
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
            260                 265                 270

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
        275                 280                 285

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
    290                 295                 300

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
305                 310                 315                 320

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
                325                 330                 335

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
            340                 345                 350

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
        355                 360                 365

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
    370                 375                 380

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
385                 390                 395                 400

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                405                 410                 415

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
            420                 425                 430

Ser Leu Ser Pro Gly Lys
        435
```

<210> 35
<211> 346
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 35

```
Met Ala Ala Gly Thr Ala Val Gly Ala Trp Val Leu Val Leu Ser Leu
1               5                  10                  15

Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu
            20                  25                  30

Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
        35                  40                  45

Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
    50                  55                  60

Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
65                  70                  75                  80

Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile
                85                  90                  95

Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn
            100                 105                 110

Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
        115                 120                 125

Ser Ala Ser Glu Leu Thr Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
    130                 135                 140

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
145                 150                 155                 160

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
                165                 170                 175

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
            180                 185                 190

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
        195                 200                 205

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
    210                 215                 220
```

```
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
225                 230                 235                 240

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
                245                 250                 255

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
            260                 265                 270

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
        275                 280                 285

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
    290                 295                 300

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
305                 310                 315                 320

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
                325                 330                 335

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            340                 345
```

<210> 36
<211> 324
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 36

```
Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1               5                   10                  15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
            20                  25                  30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
        35                  40                  45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
    50                  55                  60
```

```
Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65                  70                  75                  80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
                85                  90                  95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
            100                 105                 110

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
        115                 120                 125

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
    130                 135                 140

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
145                 150                 155                 160

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
                165                 170                 175

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
            180                 185                 190

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
        195                 200                 205

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
    210                 215                 220

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
225                 230                 235                 240

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
                245                 250                 255

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
            260                 265                 270

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
        275                 280                 285
```

```
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
    290                 295                 300

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
305                 310                 315                 320

Ser Pro Gly Lys
```

<210> 37
<211> 323
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial = synthetic construct

<400> 37

```
Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1               5                   10                  15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
            20                  25                  30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
        35                  40                  45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
    50                  55                  60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65                  70                  75                  80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
                85                  90                  95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
            100                 105                 110

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
        115                 120                 125

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
    130                 135                 140
```

```
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
145                 150                 155                 160

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
                165                 170                 175

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
            180                 185                 190

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
        195                 200                 205

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
    210                 215                 220

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
225                 230                 235                 240

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                245                 250                 255

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
            260                 265                 270

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
        275                 280                 285

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
    290                 295                 300

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
305                 310                 315                 320

Pro Gly Lys
```

<210> 38
<211> 210
<212> PRT
<213> Homo sapiens

<400> 38

```
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
1               5                  10                  15

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            20                  25                  30

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        35                  40                  45

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    50                  55                  60

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
65                  70                  75                  80

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                85                  90                  95

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            100                 105                 110

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        115                 120                 125

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    130                 135                 140

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
145                 150                 155                 160

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                165                 170                 175

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            180                 185                 190

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        195                 200                 205

Gly Lys
    210
```

<210> 39

<211> 633
<212> DNA
<213> Homo sapiens

<400> 39
ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 60

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 120

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 180

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 240

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 300

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 360

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 420

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 480

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 540

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 600

cagaagagcc tctccctgtc tccgggtaaa tga 633

<210> 40
<211> 220
<212> PRT
<213> Homo sapiens

<400> 40

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
1 5 10 15

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
35 40 45

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
50 55 60

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
65 70 75 80

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
85 90 95

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
100 105 110

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
115 120 125

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
130 135 140

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
145 150 155 160

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
165 170 175

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
180 185 190

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
195 200 205

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215 220

<210> 41
<211> 663
<212> DNA
<213> Homo sapiens

<400> 41
ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 60

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 120

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 180

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 240

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 300

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 360

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 420

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 480

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 540

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 600

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 660

tga 663

<210> 42
<211> 113
<212> PRT
<213> Homo sapiens

<400> 42

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
1 5 10 15

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
35 40 45

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
50 55 60

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
65 70 75 80

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
85 90 95

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
100 105 110

Lys

<210> 43
<211> 107
<212> PRT
<213> Homo sapiens

<400> 43

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
1 5 10 15

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 44
<211> 25
<212> PRT
<213> Homo sapiens

<400> 44

Ile Ser Ile Ile Glu Pro Gly Glu Glu Gly Pro Thr Ala Gly Ser Val
1 5 10 15

Gly Gly Ser Gly Leu Gly Thr Leu Ala
20 25