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(71) Applicant *(for all designated States except US)*: **BIOMARCK PHARMACEUTICALS, LTD.** [US/US]; 7200 Falls of Neuse Road, Suite 202, Raleigh, North Carolina 27615 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **PARIKH, Indu** [US/US]; 2558 Booker Creek Road, Chapel Hill, North Carolina 27514 (US).

(74) Agents: **HULEATT, Jayme A.** et al.; Cooley Godward Kronish LLP, Attn: Patent Group, 1200 19th Street, N.W., Suite 500, Washington, District Of Columbia 20036 (US).

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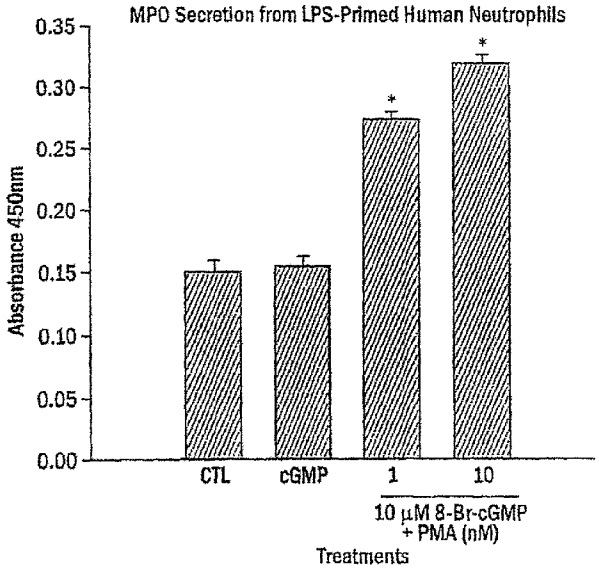
(54) **Title:** METHODS FOR ATTENUATING RELEASE OF INFLAMMATORY MEDIATORS AND PEPTIDES USEFUL THEREIN



(57) **Abstract:** The present invention includes methods of inhibiting or suppressing cellular secretory processes. More specifically the present invention relates to inhibiting or reducing the release of inflammatory mediators from inflammatory cells by inhibiting the mechanism associated with the release of inflammatory mediators from granules in inflammatory cells. In this regard, the present invention discloses an intracellular signaling mechanism that illustrates several novel intracellular targets for pharmacological intervention in disorders involving secretion of inflammatory mediators from vesicles in inflammatory cells. Peptide fragments and variants thereof of MANS peptide as disclosed in the present invention are useful in such methods.

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METHODS FOR ATTENUATING RELEASE OF INFLAMMATORY MEDIATORS AND PEPTIDES USEFUL THEREIN

Cross Reference to Related Application

[0001] The present application claims priority to U.S. Serial No.: 60/833,239 filed on July 26, 2006, which is incorporated in its entirety by reference.

Field of Invention

[0002] The present invention relates to peptides or peptide compositions and methods of their use to attenuate (or inhibit or reduce) the stimulated release of mediators of inflammation from inflammatory cells during inflammation. The present invention also relates to use of these peptides or peptide compositions to modulate an intracellular signaling mechanism regulating the secretion of inflammatory mediators from inflammatory cells.

Background of the Invention

[0003] Inflammatory leukocytes synthesize a number of inflammatory mediators that are isolated intracellularly and stored in cytoplasmic membrane-bound granules. Examples of such mediators include, but are not limited to, myeloperoxidase [MPO] in neutrophils (see, for example, Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997; 89:3503-3521), eosinophil peroxidase [EPO] and major basic protein [MBP] in eosinophils (see, for example, Gleich G J. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000; 105:651-663), lysozyme in monocytes/macrophages (see, for example, Hoff T, Spencker T, Emmendoerffer A., Goppelt-Struebe M. Effects of glucocorticoids on the TPA-induced monocytic differentiation. *J Leukoc Biol* 1992; 52:173-182; and Balboa M A, Saez Y, Balsinde J. Calcium-independent phospholipase A2 is required for lysozyme secretion in U937 promonocytes. *J Immunol* 2003; 170:5276-5280), and granzyme in natural killer (NK) cells and cytotoxic lymphocytes (see, for example, Bochan MR, Goebel WS, Brahmi Z. Stably transfected antisense granzyme B and perforin constructs inhibit human granule-mediated lytic ability. *Cell Immunol* 1995;164:234-239; Gong JH., Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia* 1994;

8:652-658; Maki G, Klingemann HG, Martinson JA, Tam YK. Factors regulating the cytotoxic activity of the human natural killer cell line, NK-92. *J Hematother Stem Cell Res* 2001; 10:369-383; and Takayama H, Trenn G, Sitkovsky MV. A novel cytotoxic T lymphocyte activation assay. *J Immunol Methods* 1987; 104:183-190). Such mediators are released at sites of injury and contribute to inflammation and tissue repair such as in the lung and elsewhere. It is known that leukocytes release these granules via an exocytotic mechanism (see, for example, Burgoyne RD, Morgan A. Secretory granule exocytosis. *Physiol Rev* 2003; 83:581-632; and Logan MR, Odemuyiwa SO, Moqbel R. Understanding exocytosis in immune and inflammatory cells: the molecular basis of mediator secretion. *J Allergy Clin Immunol* 2003; 111: 923-932), but regulatory molecules and specific pathways involved in the exocytotic process have not been fully described.

[0004] Several exogenous stimuli can provoke degranulation of leukocytes via a pathway that involves activation of protein kinase C and subsequent phosphorylation events (see, for example, Burgoyne RD, Morgan A. Secretory granule exocytosis. *Physiol Rev* 2003; 83:581-632; Logan MR, Odemuyiwa SO, Moqbel R. Understanding exocytosis in immune and inflammatory cells: the molecular basis of mediator secretion. *J Allergy Clin Immunol* 2003; 111: 923-932; Smolen JE, Sandborg RR. Ca2+-induced secretion by electropermeabilized human neutrophils: the roles of Ca2+, nucleotides and protein kinase C. *Biochim Biophys Acta* 1990; 1052:133-142; Niessen HW, Verhoeven AJ. Role of protein phosphorylation in the degranulation of electropermeabilized human neutrophils. *Biochim. Biophys. Acta* 1994; 1223:267-273; and Naucler C, Grinstein S, Sundler R., Tapper H. Signaling to localized degranulation in neutrophils adherent to immune complexes. *J Leukoc Biol* 2002; 71:701-710).

[0005] MARCKS protein (where MARCKS as used herein means "Myristoylated Alanine-Rich C Kinase Substrate"), is a ubiquitous phosphorylation target of protein kinase C (PKC), and is highly expressed in leukocytes (see, for example, Aderem AA, Albert KA, Keum MM, Wang JK, Greengard P, Cohn ZA. Stimulus-dependent myristoylation of a major substrate for protein kinase C. *Nature* 1988; 332:362-364; Thelen M, Rosen A, Nairn AC, Aderem A. Regulation by phosphorylation of reversible association of a myristoylated protein kinase C substrate with the plasma membrane. *Nature* 1991; 351:320-322; and Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC, Aderem A. MARCKS is an actin filament crosslinking protein regulated by

protein kinase C and calcium-calmodulin. *Nature* 1992; 356:618-622. MARCKS protein is mechanistically involved in a process of exocytotic secretion of mucin by goblet cells that line respiratory airways (see, for example, Li et al., *J Biol Chem* 2001; 276:40982-40990; and Singer et al., *Nat Med* 2004; 10:193-196). MARCKS is myristoylated via an amide bond at the N-terminal amino acid in the MARCKS protein's amino acid sequence at the alpha-amine position of the glycine which resides at the N-terminus (i.e., at position 1) of amino acid sequence. In airway epithelial cells, the myristoylated N-terminal region of MARCKS appears to be integral to the secretory process. By the N-terminus of the MARCKS protein is meant the MANS peptide which contains Myristoyl-GAQFSKTAAKGEAAAERPGEAAVA (SEQ ID NO: 1), which are L-amino acids. Additionally, the peptide fragments of the MANS peptide disclosed herein, also preferably are composed of L-amino acids. The mechanism appears to involve binding of MARCKS, a myristoylated protein, to membranes of intracellular granules.

[0006] An N-terminal myristoylated peptide from the N-terminus of MARCKS has been shown to block both mucin secretion and binding of MARCKS to mucin granule membranes in goblet cells (see, for example, Singer et al., *Nat Med* 2004; 10:193-196). This peptide contains 24 amino acids of the MARCKS protein beginning with the N-terminal glycine of the MARCKS protein which is myristoylated via an amide bond and is known as myristoylated alpha-N-terminal sequence (MANS); i.e., Myristoyl-GAQFSKTAAKGEAAAERPGEAAVA (SEQ ID NO: 1). Also Vergeres *et al.*, *J. Biochem.* 1998, 330; 5-11, discloses that the N-terminal glycine residue of MARCKS proteins is myristoylated via a reaction catalyzed by myristoyl CoA:protein N-myristoyl transferase (NMT).

[0007] In inflammatory diseases, such as asthma, COPD and chronic bronchitis; in genetic diseases such as cystic fibrosis; in allergic conditions (atopy, allergic inflammation); in bronchiectasis; and in a number of acute, infectious respiratory illnesses such as pneumonia, rhinitis, influenza or the common cold, arthritis or auto-immune diseases, inflammatory cells are usually found in or migrate to areas of injury or infection associated with inflammatory disease states, especially in or to respiratory passages or airways of patients suffering from such diseases. These inflammatory cells can contribute greatly to the pathology of diseases via tissue damage done by inflammatory mediators released from these cells. One example of such tissue damage or destruction via this chronic inflammation occurs in cystic fibrosis patients where mediators

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