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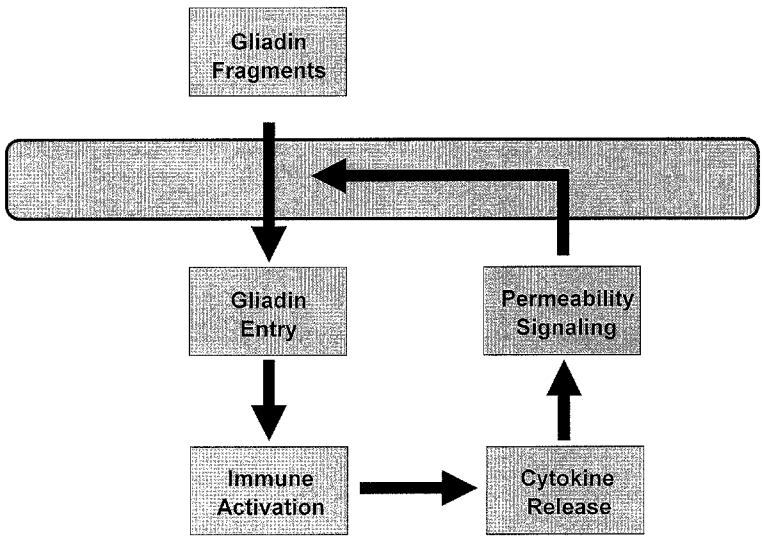
[Continued on next page]

(54) Title: INHIBITION OF GLIADIN PEPTIDES

Figure 1



(57) Abstract: Novel compounds and methods for the inhibition of biological barrier permeability and for the inhibition of peptide translocation across biological barriers are identified. Assays for determining modulators of biological barrier permeability and for peptide translocation across biological barriers are provided. Methods for treating diseases relating to aberrant biological barrier permeability and peptide translocation across biological barriers are provided. Such diseases include celiac disease, necrotizing enterocolitis, diabetes, cancer, inflammatory bowel diseases, asthma, COPD, excessive or undesirable immune response, gluten sensitivity, gluten allergy, food allergy, rheumatoid arthritis, multiple sclerosis, immune-mediated or type 1 diabetes mellitus, systemic lupus erythematosus, psoriasis, scleroderma and autoimmune thyroid diseases.

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INHIBITION OF GLIADIN PEPTIDES

PRIORITY

[0001] This application claims priority to US Provisional Application No. 61/050,915 filed May 6, 2008, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD OF THE INVENTION

[0002] This invention is related to the area of gastrointestinal inflammation. In particular, it relates to compounds and methods for the treatment of gastrointestinal inflammation.

BACKGROUND OF THE INVENTION

[0003] Environmental stimuli, such as microorganisms and gluten, can lead to increased permeability of biological barriers and initiate significant pathological events in the intestine, brain, heart, and other organs. The pathological consequences of such stimuli include the development of inflammatory diseases. Such external stimuli are presumed to exert physiological effects on biological barriers, possibly through interaction with specific cell surface receptors. However, the mechanisms used remain unclear, and specific cell surface receptors have yet to be confirmed.

[0004] Many inflammatory diseases, including those that are understood to involve increased permeability of biological barriers, are thought to be autoimmune. Such diseases include celiac disease, rheumatoid arthritis, multiple sclerosis, immune-mediated or type 1 diabetes mellitus, inflammatory bowel diseases, systemic lupus erythematosus, psoriasis, scleroderma, necrotizing enterocolitis and autoimmune thyroid diseases. Prolonged inflammation is often associated with these diseases, although the inflammation is thought to be a sequela rather than a primary pathological insult.

Biological barrier dysfunction

[0005] Biological barrier function relies upon the structural and functional integrity of tight junctions (TJ), which are one of the hallmarks of absorptive and secretory epithelia. They act as a boundary that physically separates apical and basolateral compartments of epithelial cells, and they selectively regulate the passage of materials through the epithelia by controlling access to

the space between the epithelial cells (the paracellular pathway).. To meet the many diverse physiological and pathological challenges to which epithelia are subjected, the tight junctions must be capable of rapid, physiologic, reversible, transient, energy dependent, and coordinated responses that require the presence of a complex regulatory system. Examples of epithelia containing tight junctions include, but are not limited to, the intestines (particularly the small intestine), and the blood brain barrier.

[0006] In the absence of stimuli, tight junctions are closed restricting access to the paracellular pathway. In the presence of stimuli, the tight junctions are reversibly opened. Certain bacteria have been shown to have toxins that stimulate the opening of tight junctions. *Vibrio cholerae* infected with the filamentous bacteriophage CTXΦ, produces a toxin (zonula occludens toxin, ZOT) that has been shown to cause opening of tight junctions. It has been shown that 6 His-ΔG, an N-terminal deletion of ZOT in which the first 264 amino acids have been deleted and replaced with a six histidine purification tag, retains the ability to open tight junctions.

[0007] Physiological changes in paracellular permeability, which are due to TJ regulation, can be measured as variations in transepithelial conductance. Such variations can usually be attributed to changes in paracellular permeability since the resistances of epithelial plasma membranes are relatively high. TJ represent the major barrier in the paracellular pathway, and the electrical resistance of epithelial tissues seems to depend on their integrity.

[0008] Environmental stimuli, including for example, microorganisms and gluten, can increase permeability of biological barriers as measured by a decrease in trans-epithelial electrical resistance (TEER) (*ex vivo*) or the Lactulose/mannitol test (*in vivo*). Such increases in barrier permeability are due primarily to TJ rearrangements, and they are believed to underlie many diseases including a large number of inflammatory conditions.

[0009] TJ dysfunction occurs in a variety of clinical conditions, including food allergies, infections of the gastrointestinal tract, autoimmune diseases, celiac disease and inflammatory bowel diseases. Healthy, mature gut mucosa with its intact tight junction serves as the main barrier to the passage of macromolecules. During the healthy state, small quantities of immunologically active antigens cross the gut host barrier. These antigens are absorbed across the mucosa through at least two pathways. Up to 90% of the absorbed proteins cross the intestinal barrier via the transcellular pathway, followed by lysosomal degradation that converts proteins into smaller, non-immunogenic peptides. These residual peptides are transported as

intact proteins through the paracellular pathway, which mediates a subtle, but sophisticated, regulation of intercellular tight junction that leads to antigen tolerance.

[0010] In normal bowels, the immune reaction is regulated to maintain homeostasis of the gut. When TJ integrity is compromised, in premature infants or on exposure to environmental stimuli, radiation, chemotherapy, or toxins, a deleterious immune response to environmental antigens may develop. This response can result in autoimmune diseases and food allergies that lead to inflammation.

[0011] Inflammatory bowel disease (IBD) is a phrase used to describe an inappropriate immune response that occurs in the bowels of affected individuals. Two major types of IBD have been described: Crohn's disease and ulcerative colitis (UC). Both forms of IBD show abnormal profiles of T cell mediated immunity. In the gut of Crohn's disease a strong Th1 reaction is induced; the Th2 response is upregulated in the colon of UC.

[0012] The barrier function of the intestines is impaired in IBD. For example, Crohn's disease is associated with increased permeability of the intestinal barrier even in quiescent patients. A TNF-α-induced increase in intestinal epithelial tight junction (TJ) permeability has been proposed to be an important proinflammatory mechanism contributing to intestinal inflammation in Crohn's disease and other inflammatory conditions. Increased intestinal permeability during episodes of active disease correlates with destruction or rearrangement of TJ protein complexes.

[0013] Examples of inflammatory diseases and disorders that may be treated using the instant invention include, for example, celiac disease, necrotizing enterocolitis, rheumatoid arthritis, multiple sclerosis, immune-mediated or type 1 diabetes mellitus, inflammatory bowel diseases (Crohn's disease and ulcerative colitis), systemic lupus erythematosus, psoriasis, scleroderma, and autoimmune thyroid diseases. Prolonged inflammation is often associated with these diseases, although the inflammation is thought to be a sequela rather than a primary pathological insult.

[0014] Other diseases and disorders associated with biological barrier dysfunction and which may be treated using the instant inventions include, for example, celiac disease, asthma, acute lung injury, acute respiratory distress syndrome, chronic obstructive pulmonary disease, inflammation (e.g., psoriasis and other inflammatory dermatoses), asthma, allergy, cell proliferative disorders (e.g., hyperproliferative skin disorders including skin cancer), metastasis

of cancer cells, ion transport disorders such as magnesium transport defects in the kidney, and exposure to Clostridium perfringens enterotoxin (CPE). autoimmune encephalomyelitis, optic neuritis, progressive multifocal leukoencephalopathy (PML), primary biliary cirrhosis, IgA nephropathy, Wegener's granulomatosis, multiple sclerosis, scleroderma, systemic sclerosis, Hashimoto's thyroiditis (underactive thyroid), Graves' disease (overactive thyroid), autoimmune hepatitis, autoimmune inner ear disease, bullous pemphigoid, Devic's syndrome, Goodpasture's syndrome, Lambert-Eaton myasthenic syndrome (LEMS), autoimmune lymphproliferative syndrome (ALPS), paraneoplastic syndromes, polyglandular autoimmune syndromes (PGA), alopecia areata, gastrointestinal inflammation that gives rise to increased intestinal permeability, intestinal conditions that cause protein losing enteropathy, C. difficile infection, enterocolitis, shigellosis, viral gastroenteritis, parasite infestation, bacterial overgrowth, Whipple's disease, diseases with mucosal erosion or ulcerations, gastritis, gastric cancer, collagenous colitis, and mucosal diseases without ulceration, Menetrier's disease, eosinophilic gastroenteritis, diseases marked by lymphatic obstruction, congenital intestinal lymphangiectasia, sarcoidosis lymphoma, mesenteric tuberculosis, after surgical correction of congenital heart disease, and food allergies, primarily to milk.

Inflammation

[0015] Inflammation plays a central role in the pathology of disease conditions that adversely affect a considerable proportion of the population in developed countries. This process is mediated by cytokines, a system of polypeptides that enable one cell to signal to initiate events in another cell that initiate inflammatory sequelae. Normally, the system acts as part of a defensive reaction against infectious agents, harmful environmental agents, or malignantly transformed cells. But when inflammation exceeds the requirements of its defensive role, it can initiate adverse clinical effects, such as arthritis, septic shock, inflammatory bowel disease, and a range of other human disease conditions.

[0016] Immune cells such as monocytes and macrophages secrete cytokines including tumor necrosis factor-α (TNFα) and tumor necrosis factor-β (TNFβ) in response to endotoxin or other stimuli. Cells other than monocytes or macrophages also make cytokines including TNFα. For example, human non-monocytic tumor cell lines produce TNF. $CD4^+$ and $CD8^+$ peripheral blood T lymphocytes and some cultured T and B cell lines also produce TNFα. A large body of evidence associates cytokines such as TNFα with infections, immune disorders, neoplastic pathologies, autoimmune pathologies and graft-versus host pathologies.

[0017] Small-molecule antirheumatic drugs such as methotrexate and sulfasalazine are insufficient to control inflammation in about two-thirds of arthritis patients. New biological agents developed in the last decade have proved to be effective for a majority of patients unresponsive to traditional drugs. The target for such agents is often one of the cytokine pathways--either capturing the ligand conveying the signal from one cell to another, or blocking the receptor at the surface of the effector cell, preventing transduction of the cytokine signal, thereby forestalling the inflammatory events.

[0018] A leading biological agent for treating inflammatory conditions is Enbrel™ (Etanercept), marketed by Amgen Corp. It is a chimeric molecule comprising the extracellular portion of the human TNF receptor linked as a dimer to the IgG Fc region. The compound interferes with the binding of TNF to cell-surface TNF receptors--showing the importance of modulating the TNF pathway for clinical therapy of inflammatory conditions.

[0019] Other TNFα modulating agents currently licensed in the U.S. for treating inflammatory conditions include Cimzia™ (certolizumab pegol), a pegylated antibody fragment that binds to TNFα; Remicade™ (Infliximab), a chimeric antibody that binds TNFα; and Humira™ (adalimumab), a humanized anti-TNFα antibody.

Celiac Disease

[0020] Celiac disease (CD) is a chronic autoimmune disease that is HLA-DQ2/DQ8 haplotype restricted. Glutens, the major protein fraction of wheat, and related proteins in rye and barley are the triggering agents of the disease. Ingested gluten or its derivative fractions (gliadin and subunits) elicit a harmful T cell-mediated immune response after crossing the small bowel epithelial barrier, undergoing deamidation by tissue transglutaminase (tTG) and engaging class II MHC molecules.

[0021] While the earliest events leading to CD involve innate immune responses, evidence in the literature seems to suggest that a dysfunctional cross talk between innate and adaptive immunity is also an important pathogenic element in the autoimmune process of the disease. Under physiological circumstances, the intestinal epithelium, with its intact intercellular tight junctions (tj), serves as a key barrier to the passage of macromolecules such as gluten. When the integrity of the tj system is compromised, as in CD, a paracellular leak ("leaky gut") and an inappropriate immune response to environmental antigens (i.e., gluten) may develop.

[0022] In celiac intestinal tissues and in *in vitro*, *ex vivo*, and *in vivo* animal experiments, gluten/gliadin causes a rapid increase in permeability in normal and diseased states. Animal models likewise have demonstrated the association of gluten, increased paracellular permeability and other autoimmune diseases, including type 1 diabetes (T1D).

[0023] AT-1001 is an orally administered octapeptide (Gly Gly Val Leu Val Gln Pro Gly (SEQ ID NO:1), that appears to inhibit gliadin-induced TJ disassembly and prevent the associated increase in paracellular permeability. Experiments with *ex vivo* human tissue and in mice demonstrate that AT-1001 blocks the peak of F-actin increment induced by gliadin and inhibits gliadin induced reduction in intestinal Rt (resistance).

[0024] There is a continuing need in the art for methods to treat inflammatory and autoimmune diseases as well as diseases associated with biological barrier dysfunction more effectively and to discover or identify drugs which are suitable for treating inflammatory and autoimmune diseases as well as diseases associated with biological barrier dysfunction.

SUMMARY OF THE INVENTION

[0025] One object of the present invention is to inhibit increased permeability of biological barriers in response to secreted signals.

[0026] Another object of the present invention is to provide compounds that inhibit secretion of signals that cause increased permeability of biological barriers.

[0027] In particular embodiments the present invention provides compounds that inhibit the secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to lipopolysaccharide (LPS). In other particular embodiments the present invention provides compounds that inhibit the secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to pepsin/trypsin treated gliadin (PTG).

[0028] Another object of the present invention is to provide pharmaceutical compositions that inhibit secretion of signals that cause increased permeability of biological barriers.

[0029] In particular embodiments the present invention provides pharmaceutical compositions that inhibit the secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to

lipopolysaccharide (LPS). In other particular embodiments the present invention provides pharmaceutical compositions that inhibit the secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to pepsin/trypsin treated gliadin (PTG).

[0030] Another object of the present invention is to provide methods of treating a patient showing an increased secretion of signals that cause increased permeability of biological barriers.

[0031] In particular embodiments the present invention provides methods of treating a patient showing an increased secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to lipopolysaccharide (LPS). In other particular embodiments the present invention provides methods of treating a patient showing an increased secretion of signals that cause increased permeability of biological barriers, wherein the signals are secreted in response to exposure of lymphocytes to pepsin/trypsin treated gliadin (PTG).

[0032] In certain embodiments, the invention provides a method of treating a patient with an autoimmune or inflammation-associated disease. The disease is selected from the group consisting of inflammatory bowel disease, including Crohn's disease and ulcerative colitis, necrotizing enterocolitis, type 1 diabetes, celiac disease, autoimmune hepatitis, multiple sclerosis, autism, dermatitis herpetiformis, IgA nephropathy, primary biliary chirrosis, rheumatoid arthritis, systemic lupus erythematosus, Grave's disease, Hashimoto's disease, and depression. A compound that inhibits the production, release and/or the biological effects of TNFα is administered to the patient.

[0033] Another object of the present invention is to provide methods to inhibit paracellular passage of gluten derived peptides across an epithelial barrier. Such methods comprise contacting the epithelial barrier with one or more peptide permeability inhibitors. Peptide permeability inhibitors for use in methods of the invention may comprise a peptide of any length. Such peptide permeability inhibitors may comprise a peptide from three to ten amino acids in length. In some embodiments, a peptide permeability inhibitor of the invention may comprise, consist essentially of, or consist of a peptide that comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-162. In some embodiments, a peptide permeability inhibitor of the invention may comprise, consist essentially of, or consist of a peptide that comprises, consists essentially of or consists of an

amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162. In some embodiments, the invention does not include SEQ ID NOs: 15, 24, and 25.

[0034] The present invention also provides novel methods to inhibit increased paracellular permeability associated with exposure of a biological barrier to gluten derived peptides. Such methods comprise contacting the epithelial barrier with one or more peptide permeability inhibitors. Peptide permeability inhibitors for use in methods of the invention may comprise a peptide of any length. Such peptide permeability inhibitors may comprise a peptide from three to ten amino acids in length. In some embodiments, a peptide permeability inhibitor of the invention may comprise, consist essentially of, or consist of a peptide that comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-162. In some embodiments, a peptide permeability inhibitor of the invention may comprise, consist essentially of, or consist of a peptide that comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162. In some embodiments, the invention does not include SEQ ID NOs: 15, 24, and 25.

[0035] The present invention also provides compositions, e.g., pharmaceutical compositions, comprising one or more peptide permeability inhibitors of the invention, useful to inhibit paracellular passage of gluten derived peptides across an epithelial barrier. Peptide permeability inhibitors for use in compositions of the invention may comprise a peptide of any length. In some embodiments, such peptide permeability inhibitors may comprise a peptide of between three to ten amino acids in length. Suitable peptide permeability inhibitors for use in the compositions of the invention include, but are not limited to, peptide permeability inhibitors that comprise, consist essentially of, or consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-162. In some embodiments, peptide permeability inhibitors for use in the compositions of the invention include, but are not limited to, peptide permeability inhibitors comprising peptides that comprise, consist essentially of, or consist of an amino acid sequence selected from the group consisting of of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128,

147, 150, and 160-162. In some embodiments, the invention does not include SEQ ID NOs: 15, 24, and 25.

[0036] Compositions of the invention, for example, pharmaceutical compositions, may be formulated for any type of delivery. For example, compositions of the invention may be formulated for intestinal delivery, e.g., may be delayed release compositions. Compositions of the invention may also be formulated for pulmonary delivery, oral delivery and/or transcutaneous delivery.

[0037] In one embodiment, the present invention provides a method of treating a disease in a subject in need thereof. Methods of the invention may comprise administering to the subject a pharmaceutical composition comprising one or more peptide permeability inhibitors of the invention. Methods of the invention may comprise administering to the subject a pharmaceutical composition comprising one or more peptide permeability inhibitors and one or more additional therapeutic agents. In one embodiment, the present invention provides a method of treating celiac disease in a subject in need thereof. In another embodiment, the present invention provides a method of treating necrotizing enterocolitis in a subject in need thereof. In another embodiment, the present invention provides a method of treating an excessive or undesirable immune response in a subject in need thereof. In another embodiment, the present invention provides a method of treating inflammation in a subject in need thereof. In specific embodiments, the present invention provides methods of treating inflammatory bowel disease in a subject in need thereof. Inflammatory bowel disease that can be treated using methods of the present invention may be Crohn's disease or ulcerative colitis.

[0038] In further embodiments the invention provides methods of treating an autoimmune or inflammation-associated disease in a patient in need of such treatment. The disease is selected from the group consisting of type 1 diabetes, celiac disease, autoimmune hepatitis, multiple sclerosis, autism, dermatitis herpetiformis, IgA nephropathy, primary biliary chirrosis, rheumatoid arthritis, systemic lupus erythematosus, Grave's disease, Hashimoto's disease, and depression.

[0039] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described herein, which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that any conception and specific embodiment disclosed herein may be readily utilized

as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that any description, figure, example, etc. is provided for the purpose of illustration and description only and is by no means intended to define the limits the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] **Figure 1** is a schematic representation of the events leading to Celiac disease pathology. Gliadin fragments cross the intestinal epithelium and activate immune cells to produce soluble factors including cytokines that lead to increased permeability of the intestinal epithelium.

[0041] **Figure 2** is a schematic representation of the blockade of the gliadin fragment entry, the initial step leading to Celiac disease pathology. Gliadin fragments cross the intestinal epithelium and activate immune cells to produce soluble factors including cytokines that lead to increased permeability of the intestinal epithelium.

[0042] **Figure 3** shows the effect of a peptide permeability inhibitor (SEQ ID NO:1) on permeability of a CaCO2 cell monolayer to a gliadin fragment. Apical exposure of the monolayer to the gliadin peptide PYPQPQLPY (SEQ ID NO:163) lead to an increase in permeability to that peptide, which could be blocked by apical treatment with a peptide permeability inhibitor (SEQ ID NO:1).

[0043] **Figure 4** shows the effect of a 13-mer gliadin peptide (LGQQQPFPPQQPY; SEQ ID NO:164) on permeability of a CaCO2 cell monolayer induced by a. Apical exposure of the monolayer to the gliadin peptide FITC-C6-PYPQPQLPY lead to an increase in permeability that could be blocked by treatment with a peptide permeability inhibitor (SEQ ID NO:1).

[0044] **Figure 5A** shows the effects on CaCO2 cell permeability of 72 hours treatment with peptide permeability inhibitor (SEQ ID NO:1) in combination with culture supernatants prepared from donor PBMCs (00022). After formation of tight junctions CaCO2 cells were exposed basolaterally to control supernatant (control), untreated PBMC supernatant (PBMC

sup), LPS treated PBMC supernatant (PBMC-LPS) and PTG treated PBMC supernatant (PBMC-PTG). Lucifer yellow permeability was measured after 72 hours (day3). Simultaneous apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 0 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001) but had no significant effect on permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0045] **Figure 5B** shows the effects on CaCO2 cell permeability of 72 hours exposure to culture supernatants prepared from donor PBMCs (00022) followed by addition of peptide permeability inhibitor (SEQ ID NO:1) after 48 hours treatment. After formation of tight junctions CaCO2 cells were exposed basolaterally to PBMC supernatants as described above. Peptide permeability inhibitor (SEQ ID NO:1) was added apically to the cultures after 48 hours (day 2), and lucifer yellow permeability was measured after 72 hours (day3). Apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 2 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001), and it significantly reduced permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0046] **Figure 6A** shows the effects on CaCO2 cell permeability of 72 hours treatment with peptide permeability inhibitor (SEQ ID NO:1) in combination with culture supernatants prepared from donor PBMCs (00023). After formation of tight junctions CaCO2 cells were exposed basolaterally to control supernatant (control), untreated PBMC supernatant (PBMC sup), LPS treated PBMC supernatant (PBMC-LPS) and PTG treated PBMC supernatant (PBMC-PTG). Lucifer yellow permeability was measured after 72 hours (day3). Simultaneous apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 0 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001) but had no significant effect on permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0047] **Figure 6B** shows the effects on CaCO2 cell permeability of 72 hours exposure to culture supernatants prepared from donor PBMCs (00023) followed by addition of peptide permeability inhibitor (SEQ ID NO:1) after 48 hours treatment. After formation of tight junctions CaCO2 cells were exposed basolaterally to PBMC supernatants as described above. Peptide permeability inhibitor (SEQ ID NO:1) was added apically to the cultures after 48 hours (day 2), and lucifer yellow permeability was measured after 72 hours (day3). Apical addition of

peptide permeability inhibitor (SEQ ID NO:1) on day 2 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001), and it significantly reduced permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0048] **Figure 7A** shows the effects on CaCO2 cell permeability of 72 hours treatment with peptide permeability inhibitor (SEQ ID NO:1) in combination with culture supernatants prepared from donor PBMCs (00064). After formation of tight junctions CaCO2 cells were exposed basolaterally to control supernatant (control), untreated PBMC supernatant (PBMC sup), LPS treated PBMC supernatant (PBMC-LPS) and PTG treated PBMC supernatant (PBMC-PTG). Lucifer yellow permeability was measured after 72 hours (day3). Simultaneous apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 0 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001) but had no significant effect on permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0049] **Figure 7B** shows the effects on CaCO2 cell permeability of 72 hours exposure to culture supernatants prepared from donor PBMCs (00064) followed by addition of peptide permeability inhibitor (SEQ ID NO:1) after 48 hours treatment. After formation of tight junctions CaCO2 cells were exposed basolaterally to PBMC supernatants as described above. Peptide permeability inhibitor (SEQ ID NO:1) was added apically to the cultures after 48 hours (day 2), and lucifer yellow permeability was measured after 72 hours (day3). Apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 2 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001), and it significantly reduced permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0050] **Figure 8A** shows the effects on CaCO2 cell permeability of 72 hours treatment with peptide permeability inhibitor (SEQ ID NO:1) in combination with culture supernatants prepared from donor PBMCs (00065). After formation of tight junctions CaCO2 cells were exposed basolaterally to control supernatant (control), untreated PBMC supernatant (PBMC sup), LPS treated PBMC supernatant (PBMC-LPS) and PTG treated PBMC supernatant (PBMC-PTG). Lucifer yellow permeability was measured after 72 hours (day3). Simultaneous apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 0 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001) but had no

significant effect on permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

[0051] **Figure 8B** shows the effects on CaCO2 cell permeability of 72 hours exposure to culture supernatants prepared from donor PBMCs (00065) followed by addition of peptide permeability inhibitor (SEQ ID NO:1) after 48 hours treatment. After formation of tight junctions CaCO2 cells were exposed basolaterally to PBMC supernatants as described above. Peptide permeability inhibitor (SEQ ID NO:1) was added apically to the cultures after 48 hours (day 2), and lucifer yellow permeability was measured after 72 hours (day3). Apical addition of peptide permeability inhibitor (SEQ ID NO:1) on day 2 abolished baseline permeability to Lucifer yellow (control + AT-1001; and PBMC sup + AT1001), and it significantly reduced permeability changes induced by LPS (PBMC-LPS + AT1001) or PTG treated PBMC supernatant (PBMC-PTG + AT1001).

DETAILED DESCRIPTION OF THE INVENTION

[0052] The inventors have discovered that peripheral blood mononuclear cells (PBMCs) secrete signals that increase epithelial monolayer permeability on response to stimulation with lipopolysaccharide (PLPS) and pepsin/trypsin treated gliadin (PTG). These secreted signals are present in PBMC culture supernatant, and they increase permeability of CaCO2 cell monolayers to Lucifer yellow when presented to the basolateral aspect of these cells. These permeability changes are inhibited by treatment of the cells with peptide permeability inhibitors of the invention (Figures 5A, 5B, 6A, 6B, 7A, 7B, 8A and 8B). The inventors have also discovered that specific peptides within the PTG mixture are capable of crossing epithelial cell monolayers in vitro, and that this peptide specific mechanism can be inhibited by peptide permeability inhibitors of the invention (Figures 3 and 4).

Definitions

[0053] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found, for example, in Benjamin Lewin, Genes VII, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.); The Encyclopedia of Molecular Biology, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a

Comprehensive Desk Reference, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar technical references.

[0054] As used herein, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0055] As used herein, "biological effect" refers to a biochemical and physiological effect. Biological effect includes, for example, increases or decreases in the activity of the immune system and any of its components (including, for example, complement activation), increases or decreases in receptor binding and increases or decreases in subsequent downstream effector cellular constituents (including, for example, growth factor receptor and downstream effector cellular constituents), increases or decreases in cell signaling, increases or decreases in gene expression, increases or decreased in post-translation modification of proteins (including, for example, phosphorylation), and increases or decreases in protein activity.

[0056] As used herein, "modulate" and all its forms and tenses refer to either increasing or decreasing a particular biochemical or physiological effect.

[0057] As used herein, A "component of the immune system" or an "immune cell" refers to a component or cell of the immune system that is involved in enhancing, eliciting, or maintaining an immune response. The immune system responds to various foreign particles (including, for example, viruses, bacteria, and allergens) and non-foreign particles (including, for example, native endogenous proteins). An immune response includes, for example, antibody production, chemotaxis, phagocytosis, inflammation, complement activation, production of cytotoxic molecules (including, for example, reactive oxygen species and reactive nitrogen species), cell adhesion, cell infiltration, and production and recruitment of mediators of any of the foregoing or other immune responses. A component or cell of the immune system involved in enhancing, eliciting, or maintaining an immune response includes, for example, neutrophils, complement proteins (including, for example, C1q, C1r and C1s), eosinophils, basophils, lymphocytes (including for example, T cells (including, for example, cytotoxic T cells, memory T cells, helper T cells, regulatory T cells, natural killer T cells, and γδ T cells) and B cells (including, for example, plasma B cells, memory B cells, B-1 cells, and B-2 cells)), monocytes,

macrophages, dendritic cells (DC), cell adhesion molecules (including, for example, ICAM and VCAM), myeloperoxidase, nitric oxide synthase, cyclooxygenase, and prostaglandin synthase.

[0058] As used herein, "treat" and all its forms and tenses refer to both therapeutic treatment and prophylactic or preventative treatment. Those in need of treatment include those already with the condition or disease as well as those in which the condition or disease is to be prevented.

Present Invention

[0059] The inventors have identified novel methods and compounds that inhibit increased permeability of biological barriers in response to stimuli that are known to induce secretion of pro-inflammatory cytokines. In specific embodiments the inventors have identified methods and compounds that inhibit increased permeability of biological barriers after stimulation by factors secreted by immune cells on exposure to LPS. In further specific embodiments the inventors have identified methods and compounds that inhibit increased permeability of biological barriers after stimulation by factors secreted by immune cells on exposure to PTG. Exemplary compounds of the invention that inhibit increased permeability of biological barriers are presented in Table 20.

[0060] The inventors have also identified novel methods and compounds that inhibit, reduce and/or prevent translocation of PTG-derived peptides across biological barriers. In specific embodiments the inventors have identified methods and compounds that inhibit, reduce and/or prevent translocation of the peptide comprising the amino acid sequence PYPQPQLPY (SEQ ID NO:163). Exemplary compounds of the invention that inhibit, reduce and/or prevent translocation of PTG-derived peptides across biological barriers are presented in Table 20.

[0061] Inhibitors of biological barrier permeability may be used in the practice of the present invention. Such permeability inhibitors may also be antagonists of mammalian tight junction opening. Antagonists of mammalian tight junction opening may also be used in the practice of the present invention. As used herein, permeability inhibitors prevent, inhibit or reduce the permeability of biological barriers to macromolecules including, for example, proteins, peptides and nucleic acids. For example, permeability inhibitors of the invention may comprise peptide permeability inhibitors. Examples of peptide permeability inhibitors that may be used in the practice of the present invention include, but are not limited to, peptides that comprise an amino acid sequence selected from the group consisting of: consist of an amino acid

sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

[0062] Examples of peptide permeability inhibitors include, but are not limited to, peptides that consist of an amino acid sequence selected from the group consisting of SEQ ID NOs:1-162.

[0063] When the permeability inhibitor is a peptide, any length of peptide may be used. Generally, the size of the peptide antagonist will range from about 6 to about 100, from about 6 to about 90, from about 6 to about 80, from about 6 to about 70, from about 6 to about 60, from about 6 to about 50, from about 6 to about 40, from about 6 to about 30, from about 6 to about 25, from about 6 to about 20, from about 6 to about 15, from about 6 to about 14, from about 6 to about 13, from about 6 to about 12, from about 6 to about 11, from about 6 to about 10, from about 6 to about 9, or from about 6 to about 8 amino acids in length. Peptide antagonists of the invention may be from about 8 to about 100, from about 8 to about 90, from about 8 to about 80, from about 8 to about 70, from about 8 to about 60, from about 8 to about 50, from about 8 to about 40, from about 8 to about 30, from about 8 to about 25, from about 8 to about 20, from about 8 to about 15, from about 8 to about 14, from about 8 to about 13, from about 8 to about 12, from about 8 to about 11, or from about 8 to about 10 amino acids in length. Peptide antagonists of the invention may be from about 10 to about 100, from about 10 to about 90, from about 10 to about 80, from about 10 to about 70, from about 10 to about 60, from about 10 to about 50, from about 10 to about 40, from about 10 to about 30, from about 10 to about 25, from about 10 to about 20, from about 10 to about 15, from about 10 to about 14, from about 10 to about 13, or from about 10 to about 12 amino acids in length. Peptide antagonists of the invention may be from about 12 to about 100, from about 12 to about 90, from about 12 to about 80, from about 12 to about 70, from about 12 to about 60, from about 12 to about 50, from about 12 to about 40, from about 12 to about 30, from about 12 to about 25, from about 12 to about 20, from about 12 to about 15, or from about 12 to about 14 amino acids in length. Peptide antagonists of the invention may be from about 15 to about 100, from about 15 to about 90, from about 15 to about 80, from about 15 to about 70, from about 15 to about 60, from about 15 to about 50, from about 15 to about 40, from about 15 to about 30, from about 15 to about 25, from about 15 to about 20, from about 19 to about 15, from about 15 to about 18, or from about 17 to about 15 amino acids in length.

[0064] The peptide permeability inhibitors can be chemically synthesized and purified using well-known techniques, such as described in *High Performance Liquid Chromatography of Peptides and Proteins: Separation Analysis and Conformation,* Eds. Mant *et al.*, C.R.C. Press (1991), and a peptide synthesizer, such as Symphony (Protein Technologies, Inc); or by using recombinant DNA techniques, *i.e.*, where the nucleotide sequence encoding the peptide is inserted in an appropriate expression vector, *e.g.*, an *E. coli* or yeast expression vector, expressed in the respective host cell, and purified therefrom using well-known techniques.

Compositions

[0065] Typically, compositions, such as pharmaceutical compositions, comprise one or more compounds of the invention, and optionally one or more additional active agents. Compounds of the invention may be present in an amount sufficient to inhibit the increased biological barrier permeability in a subject in need thereof. Compounds of the invention may be present in an amount sufficient to inhibit, reduce and/or prevent translocation of a gliadin-derived peptide across a biological barrier in a subject in need thereof. The amount of a compound of the invention employed in any given composition may vary according to factors such as the disease state, age, sex, and weight of the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

[0066] Generally, a pharmaceutical composition of the invention will comprise an amount of a compound of the invention in the range of about 1μg to about 1g, preferably about 1mg to about 1000mg, from about 10mg to about 100mg, from about 10mg to about 50mg, or from about 10mg to about 25mg of the compound. As used herein, "about" used to modify a numerical value means within 10% of the value.

[0067] Compositions of the invention may comprise one or more compounds of the invention at a level of from about 0.1 wt% to about 20 wt%, from about 0.1 wt% to about 18 wt%, from about 0.1 wt% to about 16 wt%, from about 0.1 wt% to about 14 wt%, from about 0.1 wt% to about 12 wt%, from about 0.1 wt% to about 10 wt%, from about 0.1 wt% to about 8 wt%, from about 0.1 wt% to about 6 wt%, from about 0.1 wt% to about 4 wt%, from about 0.1 wt% to about 2 wt%, from about 0.1 wt% to about 1 wt%, from about 0.1 wt% to about 0.9 wt%, from about 0.1 wt% to about 0.8 wt%, from about 0.1 wt% to about 0.7 wt%, from about

0.1 wt% to about 0.6 wt%, from about 0.1 wt% to about 0.5 wt%, from about 0.1 wt% to about 0.4 wt%, from about 0.1 wt% to about 0.3 wt%, or from about 0.1 wt% to about 0.2 wt% of the total weight of the composition. As used herein, "about" used to modify a numerical value means within 10% of the value. Compositions of the invention may comprise one or more compounds of the invention at a level of about 0.1 wt%, about 0.2 wt%, about 0.3 wt%, about 0.4 wt%, about 0.5 wt%, about 0.6 wt%, about 0.7 wt%, about 0.8 wt%, or about 0.9 wt% based on the total weight of the composition.

[0068] Compositions of the invention may comprise one or more compounds of the invention at a level of from about 1 wt% to about 20 wt%, from about 1 wt% to about 18 wt%, from about 1 wt% to about 16 wt%, from about 1 wt% to about 14 wt%, from about 1 wt% to about 12 wt%, from about 1 wt% to about 10 wt%, from about 1 wt% to about 9 wt%, from about 1 wt% to about 8 wt%, from about 1 wt% to about 7 wt%, from about 1 wt% to about 6 wt%, from about 1 wt% to about 5 wt%, from about 1 wt% to about 4 wt%, from about 1 wt% to about 3 wt%, or from about 1 wt% to about 2 wt% of the total weight of the composition. As used herein, "about" used to modify a numerical value means within 10% of the value. Compositions of the invention may comprise one or more compounds of the invention at a level of about 1 wt%, about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 7 wt%, about 8 wt%, or about 9 wt% based on the total weight of the composition.

[0069] Compositions of the invention, for example, pharmaceutical compositions comprising one or more compounds of the invention and one or more additional active agents, may be formulated for pulmonary delivery (e.g., may be pulmonary dosage forms). Typically such compositions may be provided as pharmaceutical aerosols, e.g., solution aerosols or powder aerosols. Those of skill in the art are aware of many different methods and devices for the formation of pharmaceutical aerosols, for example, those disclosed by Sciarra and Sciarra, *Aerosols*, in *Remington: The Science and Practice of Pharmacy*, 20th Ed., Chapter 50, Gennaro *et al*. Eds., Lippincott, Williams and Wilkins Publishing Co., (2000).

[0070] In one embodiment, the dosage forms are in the form of a powder aerosol (i.e, comprise particles). These are particularly suitable for use in inhalation delivery systems. Powders may comprise particles of any size suitable for administration to the lung.

[0071] Powder formulations may optionally contain at least one particulate pharmaceutically acceptable carrier known to those of skill in the art. Examples of suitable pharmaceutical carriers include, but are not limited to, saccharides, including monosaccharides, disaccharides,

polysaccharides and sugar alcohols such as arabinose, glucose, fructose, ribose, mannose, sucrose, trehalose, lactose, maltose, starches, dextran, mannitol or sorbitol. In one embodiment, a powder formulation may comprise lactose as a carrier.

[0072] Powder formulations may be contained in any container known to those in the art. Containers may be capsules of, for example, gelatin or plastic, or in blisters (e.g. of aluminum or plastic), for use in a dry powder inhalation device. In some embodiments, the total weight of the formulation in the container may be from about 5 mg to about 50 mg. In other embodiments, powder formulations may be contained in a reservoir in a multi-dose dry powder inhalation device adapted to deliver a suitable amount per actuation.

[0073] Powder formulations typically comprise small particles. Suitable particles can be prepared using any means known in the art, for example, by grinding in an airjet mill, ball mill or vibrator mill, sieving, microprecipitation, spray-drying, lyophilisation or controlled crystallisation. Typically, particles will be about 10 microns or less in diameter. Particles for use in the compositions of the invention may have a diameter of from about 0.1 microns to about 10 microns, from about 0.1 microns to about 9 microns, from about 0.1 microns to about 8 microns, from about 0.1 microns to about 7 microns, from about 0.1 microns to about 6 microns, from about 0.1 microns to about 5 microns, from about 0.1 microns to about 4 microns, from about 0.1 microns to about 3 microns, from about 0.1 microns to about 2 microns, from about 0.1 microns to about 1 micron, from about 0.1 microns to about 0.5 microns, from about 1 micron to about 10 microns, from about 1 micron to about 9 microns, from about 1 micron to about 8 microns, from about 1 micron to about 7 microns, from about 1 micron to about 6 microns, from about 1 micron to about 5 microns, from about 1 micron to about 4 microns, from about 1 micron to about 3 microns, from about 1 micron to about 2 microns, from about 2 microns to about 10 microns, from about 2 microns to about 9 microns, from about 2 microns to about 8 microns, from about 2 microns to about 7 microns, from about 2 microns to about 6 microns, from about 2 microns to about 5 microns, from about 2 microns to about 4 microns, or from about 2 microns to about 3 microns. As used herein, "about" used to modify a numerical value means within 10% of the value. In some embodiments, particles for use in the invention may be about 1 micron, about 2 microns, about 3 microns, about 4 microns, about 5 microns, about 6 microns, about 7 microns, about 8 microns, about 9 microns, or about 10 microns in diameter.

[0074] In one embodiment, the dosage forms are in the form of a solution aerosol (i.e., comprise droplets). Typically, droplets will be about 10 microns or less in diameter. Droplets for use in the compositions of the invention may have a diameter of from about 0.1 microns to about 10 microns, from about 0.1 microns to about 9 microns, from about 0.1 microns to about 8 microns, from about 0.1 microns to about 7 microns, from about 0.1 microns to about 6 microns, from about 0.1 microns to about 5 microns, from about 0.1 microns to about 4 microns, from about 0.1 microns to about 3 microns, from about 0.1 microns to about 2 microns, from about 0.1 microns to about 1 micron, from about 0.1 microns to about 0.5 microns, from about 1 micron to about 10 microns, from about 1 micron to about 9 microns, from about 1 micron to about 8 microns, from about 1 micron to about 7 microns, from about 1 micron to about 6 microns, from about 1 micron to about 5 microns, from about 1 micron to about 4 microns, from about 1 micron to about 3 microns, from about 1 micron to about 2 microns, from about 2 microns to about 10 microns, from about 2 microns to about 9 microns, from about 2 microns to about 8 microns, from about 2 microns to about 7 microns, from about 2 microns to about 6 microns, from about 2 microns to about 5 microns, from about 2 microns to about 4 microns, or from about 2 microns to about 3 microns. As used herein, "about" used to modify a numerical value means within 10% of the value. In some embodiments, particles and/or droplets for use in the invention may be about 1 micron, about 2 microns, about 3 microns, about 4 microns, about 5 microns, about 6 microns, about 7 microns, about 8 microns, about 9 microns, or about 10 microns in diameter.

[0075] The compositions of the invention may be formulated for enteric delivery, for example, may comprise one or more coatings including, for example, a delayed release coating containing one or more enteric agents. A delayed release coating is typically substantially stable in gastric fluid and substantially unstable (e.g., dissolves rapidly or is physically unstable) in intestinal fluid, thus providing for substantial release of the compounds of the invention and/or additional active agent from the composition in the duodenum or the jejunum.

[0076] The term “stable in gastric fluid” refers to a composition that releases 30% or less by weight of the total compound of the invention and/or additional active agent in the composition in gastric fluid with a pH of 5 or less, or simulated gastric fluid with a pH of 5 or less, in approximately sixty minutes. Examples of simulated gastric fluid and simulated intestinal fluid include, but are not limited to, those disclosed in the 2005 Pharmacopeia 23NF/28USP in Test Solutions at page 2858 and/or other simulated gastric fluids and simulated intestinal fluids

known to those of skill in the art, for example, simulated gastric fluid and/or intestinal fluid prepared without enzymes.

[0077] Compositions of the of the invention may release from about 0% to about 30%, from about 0% to about 25%, from about 0% to about 20%, from about 0% to about 15%, from about 0% to about 10%, from about 5% to about 30%, from about 5% to about 25%, from about 5% to about 20%, from about 5% to about 15%, from about 5% to about 10% by weight of the total compound of the invention and/or additional active agent in the composition in gastric fluid with a pH of 5 or less, or simulated gastric fluid with a pH of 5 or less, in approximately sixty minutes. As used herein, "about" used to modify a numerical value means within 10% of the value. Compositions of the invention may release about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% by weight of the total compound of the invention in the composition in gastric fluid with a pH of 5 or less, or simulated gastric fluid with a pH of 5 or less, in approximately sixty minutes.

[0078] The term “unstable in intestinal fluid” refers to a composition that releases 70% or more by weight of the total amount of the compound of the invention and/or additional active agent in the composition in intestinal fluid or simulated intestinal fluid in approximately sixty minutes. The term “unstable in near neutral to alkaline environments” refers to a composition that releases 70% or more by weight of the total amount of the compound of the invention and/or additional active agent in the composition in intestinal fluid with a pH of 5 or greater, or simulated intestinal fluid with a pH of 5 or greater, in approximately ninety minutes. For example, a composition that is unstable in near neutral or alkaline environments may release 70% or more by weight of a compound of the invention and/or additional active agent in a fluid having a pH greater than about 5 (e.g., a fluid having a pH of from about 5 to about 14, from about 6 to about 14, from about 7 to about 14, from about 8 to about 14, from about 9 to about 14, from about 10 to about 14, or from about 11 to about 14) in from about 5 minutes to about 90 minutes, from about 10 minutes to about 90 minutes, from about 15 minutes to about 90 minutes, from about 20 minutes to about 90 minutes, from about 25 minutes to about 90 minutes, from about 30 minutes to about 90 minutes, from about 5 minutes to about 60 minutes, from about 10 minutes to about 60 minutes, from about 15 minutes to about 60 minutes, from about 20 minutes to about 60 minutes, from about 25 minutes to about 60 minutes, or from about 30 minutes to about 60 minutes. As used herein, "about" used to modify a numerical value means within 10% of the value.

[0079] Compositions of the invention may be formulated for transcutaneous delivery (e.g., may be transcutaneous dosage forms). Typically such compositions may be provided as topical solutions and/or gels. Those of skill in the art are aware of many different methods and devices for the formation of topical medications, for example, those disclosed by Block, *Medicated Topicals*, in *Remington: The Science and Practice of Pharmacy*, 20th Ed., Chapter 44, Gennaro *et al*. Eds., Lippincott, Williams and Wilkins Publishing Co. (2000).

[0080] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0081] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not adsorb.

[0082] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome.

[0083] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment,

polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose. Other controlled release systems are well known in the art.

[0084] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0085] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a

local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0086] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0087] The amount of the compound of the invention that will be effective in the treatment, inhibition and/or prevention of a disease or disorder associated with increased biological barrier permeability can be determined by standard clinical techniques. The amount of the compound of the invention that will be effective in the treatment, inhibition and/or prevention of a disease or disorder associated with translocation of one or more gliadin-derived peptides across a biological barrier can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0088] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Additional Active Agents

[0089] In addition to one or more compounds of the invention, compositions of the invention may further comprise one or more additional active agents, e.g., therapeutic agents, immunogenic agents and/or imaging agents.

[0090] Additional therapeutic agents that can be used in the compositions of the invention include agents that act on any organ of the body, such as heart, brain, intestine, or kidneys. Suitable additional therapeutic agents include, but are not limited to, glucose metabolism agents (e.g., insulin), antibiotics, antineoplastics, antihypertensives, antiepileptics, central nervous system agents, anti-inflammatory agents and immune system suppressants.

[0091] Additional therapeutic agents that can be used in the compositions of the invention include immunosuppressive agents. Such immunosuppressants used in the method and composition of the invention can be any agent which tends to attenuate the activity of the humoral or cellular immune systems. In particular, in one aspect the invention comprises compositions wherein the immunosuppressant is selected from the group consisting of cyclosporin A, FK506, prednisone, methylprednisolone, cyclophosphamide, thalidomide, azathioprine, and daclizumab, physalin B, physalin F, physalin G, seco-steroids purified from Physalis angulata L., 15-deoxyspergualin (DSG, 15-dos), MMF, rapamycin and its derivatives, CCI-779, FR 900520, FR 900523, NK86-1086, depsidomycin, kanglemycin-C, spergualin, prodigiosin25-c, cammunomicin, demethomycin, tetranactin, tranilast, stevastelins, myriocin, gliooxin, FR 651814, SDZ214-104, bredinin, WS9482, mycophenolic acid, mimoribine, misoprostol, OKT3, anti-IL-2 receptor antibodies, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685), paclitaxel, altretamine, busulfan, chlorambucil, ifosfamide, mechlorethamine, melphalan, thiotepa, cladribine, fluorouracil, floxuridine, gemcitabine, thioguanine, pentostatin, methotrexate, 6-mercaptopurine, cytarabine, carmustine, lomustine, streptozotocin, carboplatin, cisplatin, oxaliplatin, iproplatin, tetraplatin, lobaplatin, JM216, JM335, fludarabine, aminoglutethimide, flutamide, goserelin, leuprolide, megestrol acetate, cyproterone acetate, tamoxifen, anastrozole, bicalutamide, dexamethasone, diethylstilbestrol, bleomycin, dactinomycin, daunorubicin, doxirubicin, idarubicin, mitoxantrone, losoxantrone, mitomycin-c, plicamycin, paclitaxel, docetaxel, topotecan, irinotecan, 9-amino camptothecan, 9-nitro camptothecan, GS-211, etoposide, teniposide, vinblastine, vincristine, vinorelbine, procarbazine, asparaginase, pegaspargase, octreotide, estramustine, and hydroxyurea, and combinations thereof. In one more particular aspect, the immunosuppressant is cyclosporin A.

[0092] Furthermore, the additional therapeutic agent can be selected from the group consisting of a chemotherapeutic, a gene therapy vector, a growth factor, a contrast agent, an angiogenesis factor, a radionuclide, an anti-infection agent, an anti-tumor compound, a receptor-bound agent, a hormone, a steroid, a protein, a complexing agent, a polymer, a thrombin inhibitor, an antithrombogenic agent, a tissue plasminogen activator, a thrombolytic agent, a fibrinolytic agent, a vasospasm inhibitor, a calcium channel blocker, a nitrate, a nitric oxide promoter, a vasodilator, an antihypertensive agent, an antimicrobial agent, an antibiotic, a glycoprotein IIb/IIIa inhibitor, an inhibitor of surface glycoprotein receptors, an antiplatelet agent, an antimitotic, a microtubule inhibitor, a retinoid, an antisecretory agent, an actin inhibitor, a remodeling inhibitor, an antisense nucleotide, an agent for molecular genetic intervention, an antimetabolite, an antiproliferative agent, an anti-cancer agent, a dexamethasone derivative, an anti-inflammatory steroid, a non-steroidal anti-inflammatory agent, an immunosuppressive agent, a PDGF antagonist, a growth hormone antagonist, a growth factor antibody, an anti-growth factor antibody, a growth factor antagonist, a dopamine agonist, a radiotherapeutic agent, an iodine-containing compound, a barium-containing compound, a heavy metal functioning as a radiopaque agent, a peptide, a protein, an enzyme, an extracellular matrix component, a cellular component, an angiotensin converting enzyme inhibitor, a 21-aminosteroid, a free radical scavenger, an iron chelator, an antioxidant, a sex hormone, an antipolymerase, an antiviral agent, an IgG2 Kappa antibody against Pseudomonas aeruginosa exotoxin A and reactive with A431 epidermoid carcinoma cells, monoclonal antibody against the noradrenergic enzyme dopamine beta-hydroxylase conjugated to saporin or other antibody targeted therapy agents, gene therapy agents, a prodrug, a photodynamic therapy agent, and an agent for treating benign prostatic hyperplasia (BHP), a ^{14}C-, ^{3}H-, ^{131}I-, ^{32}P- or ^{36}S-radiolabelled form or other radiolabelled form of any of the foregoing, and combinations thereof.

[0093] More particularly, the additional therapeutic agent can be selected from the group consisting of parathyroid hormone, heparin, human growth hormone, covalent heparin, hirudin, hirulog, argatroban, D-phenylalanyl-L-poly-L-arginyl chloromethyl ketone, urokinase, streptokinase, nitric oxide, triclopidine, aspirin, colchicine, dimethyl sulfoxide, cytochalasin, deoxyribonucleic acid, methotrexate, tamoxifen citrate, dexamethasone, dexamethasone sodium phosphate, dexamethasone acetate, cyclosporin, trapidal, angiopeptin, angiogenin, dopamine, ^{60}Co, ^{192}Ir , ^{32}P , ^{111}In , ^{90}Y, ^{99m}Tc, pergolide mesylate, bromocriptine mesylate, gold, tantalum, platinum, tungsten, captopril, enalapril, ascorbic acid, α-tocopherol, superoxide dismutase, deferoxamine, estrogen, azidothymidine (AZT), acyclovir, famciclovir, rimantadine

hydrochloride, ganciclovir sodium, 5-aminolevulinic acid, meta-tetrahydroxyphenylchlorin, hexadecafluoro zinc phthalocyanine, tetramethyl hematoporphyrin, and rhodamine 123, and combinations thereof.

[0094] Compositions of the invention may comprise one or more immunogenic agents, for example, antigens. Examples of antigens that can be used in the compositions of the invention (e.g., immunogenic and/or vaccine compositions) include peptides, proteins, microorganisms (e.g., attenuated and/or recombinant microorganisms), cells (e.g., cancer cells and/or recombinant cells) and viruses (e.g., attenuated and/or recombinant viruses). Examples of peptide antigens include the B subunit of the heat-labile enterotoxin of enterotoxigenic *E. coli*, the B subunit of cholera toxin, capsular antigens of enteric pathogens, fimbriae or pili of enteric pathogens, HIV surface antigens, cancer antigens (e.g., cancer cells comprising antigens, isolated antigens, etc.), dust allergens, and acari allergens. Other immunogenic compounds as are known in the art can also be used.

[0095] Examples of attenuated microorganisms and viruses that can be used in the compositions of the invention (e.g., vaccine compositions) include those of enterotoxigenic *Escherichia coli*, enteropathogenic *Escherichia coli, Vibrio cholerae, Shigella flexneri, Salmonella typhi* and rotavirus (Fasano et al, In: Le Vaccinazioni in Pediatria, Eds. Vierucci et al, CSH, Milan, pages 109-121 (1991); Guandalini et al, In: Management of Digestive and Liver Disorders in Infants and Children, Elsevior, Eds. Butz et al, Amsterdam, Chapter 25 (1993); Levine et al, Sem. Ped. Infect. Dis., 5.243-250 (1994); and Kaper et al, Clin. Micrbiol. Rev., 8:48-86 (1995), each of which is incorporated by reference herein in its entirety).

[0096] Any antigen capable of inducing a protective immune response may be used in the vaccine compositions of the invention. Examples of suitable antigens include, but are not limited to, measles virus antigens, mumps virus antigens, rubella virus antigens, *Corynebacterium diphtheriae* antigens, *Bordetella pertussis* antigens, *Clostridium tetani* antigens, *Bacillus anthracis* antigens, *Haemophilus influenzae* antigens, smallpox virus antigens, and influenza virus antigens.

[0097] Compositions of the invention may further comprise one or more protease inhibitors. Any protease inhibitor can be used, including, but not limited to, a proteinase, peptidase, endopeptidase, or exopeptidase inhibitor. A cocktail of inhibitors can also be used. Alternatively, the protease inhibitors can be selected from the group consisting of bestatin, L-trans-3-carboxyoxiran-2-carbonyl-L-leucylagmatine, ethylenediaminetetra-acetic acid (EDTA),

phenylmethylsulfonylfluoride (PMSF), aprotinin, amyloid protein precursor (APP), amyloid beta precursor protein, α1-proteinase inhibitor, collagen VI, bovine pancreatic trypsin inhibitor (BPTI), 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), antipain, benzamidine, chymostatin, ε-aminocaproate, N-ethylmaleimide, leupeptin, pepstatin A, phosphoramidon, and combinations thereof. Novel protease inhibitors can also be used. Indeed, protease inhibitors can be specifically designed or selected to decrease the proteolysis of the tight junction agonist and/or the therapeutic agent.

[0098] Compositions of the invention may also comprise one or more pharmaceutically acceptable excipients. Suitable excipients include, but are not limited to, buffers, buffer salts, bulking agents, salts, surface active agents, acids, bases, sugars, binders, and the like.

Methods of Treatment

[0099] Compounds and pharmaceutical compositions of the invention can be used for treating, ameliorating, and/or preventing a disease. Any disease may be treated using the compositions of the invention by selection of an appropriate active agent, e.g., therapeutic and/or immunogenic agent. In one embodiment, the present invention provides a method of treating diabetes response in a subject (e.g., a mammal such as a human) by administering a composition comprising one or more compounds of the invention together with one or more insulins and/or derivatives thereof. In another embodiment, the invention provides a method of suppressing an excessive or undesirable immune response in a subject (e.g., a mammal such as a human) by administering a composition comprising one or more compounds of the invention together with one or more immune-suppressive drugs that may include, for example, cyclosporin A.

[00100] Examples of diseases that can be treated using the compositions of the invention include, but are not limited to, cancer, autoimmune diseases, vascular disease, bacterial infections, gastritis, gastric cancer, collagenous colitis, inflammatory bowel disease, necrotizing enterocolitis, osteoporosis, systemic lupus erythematosus, food allergy, asthma, celiac disease and irritable bowel syndrome. For example, to treat inflammatory bowel disease, a composition comprising one or more compounds of the invention may be administered to the subject (e.g., a mammal such as a human) in need thereof.

[0100] In another example, to treat cancer of the colon or rectal area, a composition comprising a therapeutically effective amount of Erbitux® (Cetuximab) together with a GM-

CSF and/or IL-16 inhibiting amount of one or more compounds of the invention may be administered to the subject (e.g., a mammal such as a human) in need thereof. In another example, to treat breast cancer, a composition comprising a therapeutically effective amount of Herceptin® (Trastuzumab) together with a GM-CSF and/or IL-16 inhibiting amount of one or more compounds of the invention may be administered to the subject (e.g., a mammal such as a human) in need thereof. In another example, to treat various types of cancer, a composition comprising a therapeutically effective amount of Avastin® (Bevacizumab) together with a GM-CSF and/or IL-16 inhibiting amount of one or more compounds of the invention may be administered to the subject (e.g., a mammal such as a human) in need thereof. Another example involves treatment of osteoporosis by administration of a composition comprising one or more compounds of the invention together with a therapeutically effective amount of Fosamax® (Alendronate) to the subject in need thereof. Another example involves treatment of transplant rejection by administration of a composition comprising one or compounds of the invention together with a therapeutically effective amount of Cyclosporin A to the subject in need thereof. Another example involves treatment of anemia by administration of a composition comprising one or more compounds of the invention together with a therapeutically effective amount of erythropoietin to the subject in need thereof. Another example involves treatment of hemophilia by administration of a composition comprising one or more compounds of the invention together with a therapeutically effective amount of Factor VIII to the subject in need thereof.

[0101] In some embodiments, compositions of the invention (e.g., pharmaceutical compositions) may be given repeatedly over a protracted period, i.e., may be chronically administered. Typically, compositions may be administered one or more times each day in an amount suitable to prevent, reduce the likelihood of an attack of, or reduce the severity of an attack of the underlying disease condition (e.g., diabetes, cancer, transplant rejection, etc). Such compositions may be administered chronically, for example, one or more times daily over a plurality of days.

[0102] In some embodiments, compositions of the invention (e.g., pharmaceutical compositions) may be used to treat acute attacks of the underlying disease (e.g., diabetes, cancer, transplant rejection, etc). Typically, embodiments of this type will require administration of the compositions of the invention to a subject undergoing an attack in an amount suitable to reduce the severity of the attack. One or more administrations may be used.

[0103] In some embodiments, compounds of the invention may be used in the manufacture of compositions and pharmaceutical compositions for use in the methods described above.

[0104] While the invention has been described with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various modifications may be made without departing from the spirit and scope of the invention. The scope of the appended claims is not to be limited to the specific embodiments described.

Methods of Screening

[0105] Screening for inhibitors of gliadin-derived peptide translocation across biological barriers can be accomplished by a variety of techniques. Likewise, screening for inhibitors of PTG-induced factors that increase biological barrier permeability can be accomplished by a variety of techniques. Gliadin-derived peptide binding to test compounds (inhibitor candidates) can be directly measured, or inhibition of binding of gliadin-derived peptides to a cell preparation can be measured. Gliadin-derived peptides can be labeled to facilitate measurement of binding. Assays may be in cell-free systems or in cell-based systems. Any binding assay format can be used, including formats where the receptor is attached to a solid support, either directly or indirectly.

[0106] Test compounds which can be tested are any compounds. The compounds may be tested as single compounds or in combinations of compounds. The compounds may be structurally identified or of unknown structure. The compounds may be novel or previously known. The compounds may be natural products or synthetic.

[0107] According to one embodiment of the invention the test compounds are fragments of gliadin. Gliadin is a family of proteins which are produced by wheat and other grains. Examples of gliadins are gliadin alpha, gamma, and omega. Gliadins are the aqueous alcohol-soluble storage proteins in the seed. There is great heterogeneity even within a single class of gliadins. At least six, seven, eight, nine, ten, eleven, fifteen, twenty, thirty, thirty-five, fifty, or seventy-five amino acid residues may be used in fragments of gliadin as test compounds. Fragments include any molecule which is less than full length. Fragments may be, *e.g.,* synthesized or the result of proteolytic degradation. The following tables provide the sequences of a representative number of gliadins.

Table 1 Amino acid sequence of alpha-gliadin from *Triticum aestivum* (NCBI accession no. CAB76964, (SEQ ID NO:165))

```
  1 mvrvpvpqlq pqnpsqqqpq eqvplvqqqq fpgqqqpfpp qqpypqpqpf
 51 psqqpylqlq pfpqpqlpyp qpqlpypqpq lpypqpqpfr pqqpypqsqp
101 qysqpqqpis qqqqqqqqqq qqkqqqqqqq qilqqilqqq lipcrdvvlq
151 qhsiaygssq vlqqstyqlv qqlccqqlwq ipeqsrcqai hnvvhaiilh
201 qqqqqqqqqq qqplsqvsfq qpqqqypsgq gsfqpsqqnp qaqgsvqpqq
251 lpqfeeirnl aletlpamcn vyippyctia pvgifgtnyr
```

Table 2 Amino acid sequence of alpha-gliadin precursor from *Triticum turgidum* subsp. durum (NCBI accession no. CAI35909, (SEQ ID NO:166))

```
  1 mktflilall aivattatta vrvpvpqlqr qnpsqqqpqe qvplvqqqqf
 51 lgqqqpfppq qpypqpqpfp sqqpylqlqp fpqpqlpysq pqpfrpqqpy
101 pqpqprysqp qqpisqqqqq qhqqhqqhhq eqqilqqilq qqlipcmdvv
151 lqqhniahrr sqvlqqstyq llqelccqhl wqipeqsqcq aihnvvhaii
201 phqqqkqqqq pssqfsfqqp lqqyplgqgs frpsqqnpqa qgsvqpqqlp
251 qfeeirnlal qtlpamcnvy ippyctiapf gifgtn
```

Table 3 Amino acid sequence of alpha/beta-gliadin precursor from *Triticum aestivum* (NCBI accession no. AAA34280, (SEQ ID NO:167))

```
  1 mktflilvll aivattatta vrfpvpqlqp qnpsqqqpqe qvplvqqqqf
 51 lgqqqpfppq qpypqpqpfp sqlpylqlqp fpqpqlpysq pqpfrpqqpy
101 pqpqpqysqp qqpisqqqqq qqqqqqqqqq qqqilqqilq qqlipcmdvv
151 lqqhniahgr sqvlqqstyq llqelccqhl wqipeqsqcq aihnvvhaii
201 lhqqqkqqqq pssqvsfqqp lqqyplgqgs frpsqqnpqa qgsvqpqqlp
251 qfeeirnlal qtlpamcnvy ippyctiapf gifgtn
```

Table 4 Amino acid sequence of Gamma-gliadin precursor from *Triticum aestivum* (NCBI accession no. P21292, (SEQ ID NO:168))

```
  1 mktlliltil amattiatan mqvdpsgqvq wpqqqpfpqp qqpfcqqpqr
 51 tipqphqtfh hqpqqtfpqp qqtyphqpqq qfpqtqqpqq pfpqpqqtfp
101 qqpqlpfpqq pqqpfpqpqq pqqpfpqsqq pqqpfpqpqq qfpqpqqpqq
151 sfpqqqqpai qsflqqqmnp cknfllqqcn hvslvsslvs iilprsdcqv
201 mqqqccqqla qipqqlqcaa ihsvahsiim qqeqqqgvpi lrplfqlaqg
251 lgiiqpqqpa qlegirslvl ktlptmcnvy vppdcstinv pyanidagig
301 gq
```

Table 5 Amino acid sequence of Gamma-gliadin B precursor from *Triticum aestivum* (NCBI accession no. P06659, (SEQ ID NO:169))

```
  1 mktlliltil amaitiatan mqadpsgqvq wpqqqpflqp hqpfsqqpqq
 51 ifpqpqqtfp hqpqqqfpqp qqpqqqflqp rqpfpqqpqq pypqqpqqpf
101 pqtqqpqqpf pqskqpqqpf pqpqqpqqsf pqqqpsliqq slqqqlnpck
151 nfllqqckpv slvsslwsii lppsdcqvmr qqccqqlaqi pqqlqcaaih
201 svvhsiimqq eqqeqlqgvq ilvplsqqqq vgqgilvqgq giiqpqqpaq
251 levirslvlq tlptmcnvyv ppycstirap fasivasigg q
```

Table 6 Amino acid sequence of Gamma-gliadin (Gliadin B-III) from *Triticum aestivum* (NCBI accession no. P04730, (SEQ ID NO:170))

```
  1 pqqpfplqpq qsflwqsqqp flqqpqqpsp qpqqvvqiis patpttipsa
 51 gkptsapfpq qqqqhqqlaq qqipvvqpsi lqqlnpckvf lqqqcspvam
101 pqrlarsqml qqsschvmqq qccqqlpqip qqsryqaira iiysiilqeq
151 qqvqqsiqsq qqqpqqlgqc vsqpqqqsqq qlgqqpqqqq laqgtflqph
201 qiaqlevmts ialrilptmc svnvplyrtt tsvpfgvgtg vgay
```

Table 7 Amino acid sequence of Gamma-gliadin precursor from *Triticum aestivum* (NCBI accession no. P08453, (SEQ ID NO:171))

```
  1 mktlliltil amaitigtan iqvdpsgqvq wlqqqlvpql qqplsqqpqq
 51 tfpqpqqtfp hqpqqqvpqp qqpqqpflqp qqpfpqqpqq pfpqtqqpqq
101 pfpqqpqqpf pqtqqpqqpf pqqpqqpfpq tqqpqqpfpq lqqpqqpfpq
151 pqqqlpqpqq pqqsfpqqqr pfiqpslqqq lnpcknillq qskpaslvss
201 lwsiiwpqsd cqvmrqqccq qlaqipqqlq caaihsvvhs iimqqqqqqq
251 qqqgidiflp lsqheqvgqg slvqgqgiiq pqqpaqleai rslvlqtlps
301 mcnvyvppec simrapfasi vagiggq
```

Table 8 Amino acid sequence of Gamma-gliadin B-I precursor from *Triticum aestivum* (NCBI accession no. P04729, (SEQ ID NO:172))

```
  1 mktflvfali avvatsaiaq metscisgle rpwqqqplpp qqsfsqqppf
 51 sqqqqqplpq qpsfsqqqpp fsqqqpilsq qppfsqqqqp vlpqqspfsq
101 qqqlvlppqq qqqqlvqqqi pivqpsvlqq lnpckvflqq qcspvampqr
151 larsqmwqqs schvmqqqcc qqlqqipeqs ryeairaiiy siilqeqqqg
201 fvqpqqqqpq qsgqgvsqsq qqsqqqlgqc sfqqpqqqlg qqpqqqqqqq
251 vlqgtflqph qiahleavts ialrtlptmc svnvplysat tsvpfgvgtg
301 vgay
```

Table 9 Amino acid sequence of Gamma-gliadin precursor from *Triticum aestivum* (NCBI accession no. P08079, (SEQ ID NO:173))

```
  1 mktlliltil amaitigtan mqvdpssqvq wpqqqpvpqp hqpfsqqpqq
 51 tfpqpqqtfp hqpqqqfpqp qqpqqqflqp qqpfpqqpqq pypqqpqqpf
101 pqtqqpqqlf pqsqqpqqqf sqpqqqfpqp qqpqqsfpqq qppfiqpslq
151 qqvnpcknfl lqqckpvslv sslwsmiwpq sdcqvmrqqc cqqlaqipqq
201 lqcaaihtii hsiimqqeqq eqqqgmhill plyqqqqvgq gtlvqgqgii
251 q
```

Table 10 Amino acid sequence of Alpha/beta-gliadin MM1 precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P18573, (SEQ ID NO:174))

```
  1 mktflilall aivattaria vrvpvpqlqp qnpsqqqpqe qvplvqqqqf
 51 pgqqqpfppq qpypqpqpfp sqqpylqlqp fpqpqlpypq pqlpypqpql
101 pypqpqpfrp qqpypqsqpq ysqpqqpisq qqqqqqqqqq qkqqqqqqqq
151 ilqqilqqql ipcrdvvlqq hsiaygssqv lqqstyqlvq qlccqqlwqi
201 peqsrcqaih nvvhaiilhq qqqqqqqqqq qplsqvsfqq pqqqypsgqg
251 sfqpsqqnpq aqgsvqpqql pqfeeirnla letlpamcnv yippyctiap
301 vgifgtn
```

Table 11 Amino acid sequence of Alpha/beta-gliadin clone PTO-A10 (Prolamin) from *Triticum aestivum* (NCBI accession no. P04728, (SEQ ID NO:175))

```
  1 pqpqpqysqp qqpisqqqqq qqqqqqqqqq eqqilqqilq qqlipcmdvv
 51 lqqhniahgr sqvlqqstyq llqelccqhl wqipeqsqcq aihnvvhaii
101 lhqqqqkqqq qpssqfsfqq plqqyplgqg sfrpsqqnpq aqgsvqpqql
151 pqfeirnlal qtlpamcnvy ippyctiapf gifgtn
```

Table 12 Amino acid sequence of Alpha/beta-gliadin clone PW8142 precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04727, (SEQ ID NO:176))

```
  1 mktflilalv attattavrv pvpqlqpknp sqqqpqeqvp lvqqqqfpgq
 51 qqqfppqqpy pqpqpfpsqq pylqlqpfpq pqpflpqlpy pqpqsfppqq
101 pypqqrpkyl qpqqpisqqq aqqqqqqqqq qqqqqqqqil qqilqqqlip
151 crdvvlqqhn iahassqvlq qstyqllqql ccqqllqipe qsrcqaihnv
201 vhaiimhqqe qqqqlqqqqq qqlqqqqqqq qqqqqpssqv sfqqpqqqyp
251 ssqqgsfqpsq qnpqaqqgsvq pqqlpqfaei rnlalqtlpa mcnvyipphc
301 sttiapfgif gtn
```

Table 13 Amino acid sequence of Alpha/beta-gliadin clone PW1215 precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04726, (SEQ ID NO:177))

```
  1 mktflilall aivattatta vrvpvpqpqp qnpsqpqpqg qvplvqqqqf
 51 pgqqqqfppq qpypqpqpfp sqqpylqlqp fpqpqpfppq lpypqpppfs
101 pqqpypqpqp qypqpqqpis qqqaqqqqqq qqqqqqqqqq qqilqqilqq
151 qlipcrdvvl qqhniahars qvlqqstyqp lqqlccqqlw qipeqsrcqa
201 ihnvvhaiil hqqqrqqqps sqvslqqpqq qypsgqgffq psqqnpqaqg
251 svqpqqlpqf eeirnlalqt lprmcnvyip pycsttiapf gifgtn
```

Table 14 Amino acid sequence of Alpha/beta-gliadin A-IV precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04724, (SEQ ID NO:178))

```
  1 mktflilalr aivattatia vrvpvpqlqp qnpsqqqpqk qvplvqqqqf
 51 pgqqqpfppq qpypqqqpfp sqqpymqlqp fpqpqlpypq pqlpypqpqp
101 frpqqsypqp qpqysqpqqp isqqqqqqqq qqqqqqqilq qilqqqlipc
151 rdvvlqqhsi ahgssqvlqq styqlvqqfc cqqlwqipeq srcqaihnvv
201 haiilhqqqq qqqqqqqqqq qplsqvcfqq sqqqypsgqg sfqpsqqnpq
251 aqgsvqpqql pqfeeirnla letlpamcnv yippyctiap vgifgtn
```

Table 15 Amino acid sequence of Alpha/beta-gliadin A-III precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04723, (SEQ ID NO:179))

```
  1 mktflilall aivattatsa vrvpvpqlqp qnpsqqqpqe qvplmqqqqq
 51 fpgqqeqfpp qqpyphqqpf psqqpypqpq pfppqlpypq tqpfppqqpy
101 pqpqpqypqp qqpisqqqaq qqqqqqqtlq qilqqqlipc rdvvlqqhni
151 ahassqvlqq ssyqqlqqlc cqqlfqipeq srcqaihnvv haiilhhhqq
201 qqqqpssqvs yqqpqeqyps gqvsfqssqq npqaqgsvqp qqlpqfqeir
251 nlalqtlpam cnvyippycs ttiapfgifg tn
```

Table 16 Amino acid sequence of Alpha/beta-gliadin A-II precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04722, (SEQ ID NO:180))

```
  1 mktfpilall aivattatta vrvpvpqlql qnpsqqqpqe qvplvqeqqf
 51 qgqqqpfppq qpypqpqpfp sqqpylqlqp fpqpqlpypq pqpfrpqqpy
101 pqpqpqysqp qqpisqqqqq qqqqqqqqqq ilqqilqqql ipcrdvvlqq
151 hniahgssqv lqestyqlvq qlccqqlwqi peqsrcqaih nvvhaiilhq
201 qhhhhqqqqq qqqqqplsqv sfqqpqqqyp sgqgffqpsq qnpqaqgsfq
251 pqqlpqfeei rnlalqtlpa mcnvyippyc tiapfgifgt n
```

Table 17 Amino acid sequence of Alpha/beta-gliadin A-I precursor (Prolamin) from *Triticum aestivum* (NCBI accession no. P04721, (SEQ ID NO:181))

```
  1 mktflilall aivattatta vrvpvpqlqp qnpsqqqpqe qvplvqqqqf
 51 lgqqqpfppq qpypqpqpfp sqqpylqlqp flqpqlpysq pqpfrpqqpy
101 pqpqpqysqp qqpisqqqqq qqqqqqqqqq qqqqiiqqil qqqlipcmdv
151 vlqqhnivhg ksqvlqqsty qllqelccqh lwqipeqsqc qaihnvvhai
201 ilhqqqkqqq qpssqvsfqq plqqyplgqg sfrpsqqnpq aqgsvqpqql
251 pqfeeirnla rk
```

Table 18 Amino acid sequence of gamma gliadin from *Triticum aestivum* (NCBI accession no. AAQ63860, (SEQ ID NO:182))

```
  1 mniqvdpssq vpwpqqqpfp qphqpfsqqp qqtfpqpqqt fphqpqqqfs
 51 qpqqpqqqfi qpqqpfpqqp qqtypqrpqq pfpqtqqpqq pfpqsqqpqq
101 pfpqpqqqfp qpqqpqqsfp qqqpsliqqs lqqqlnpckn fllqqckpvs
151 lvsslwsmil prsdcqvmrq qccqqlaqip qqlqcaaihs ivhsiimqqe
201 qqeqrqgvqi lvplsqqqqv gqgtlvqgqg iiqpqqpaql evirslvlqt
251 latmcnvyvp pycstirapf asivagiggq yr
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Table 19 Amino acid sequence of Omega-gliadin from *Triticum monococcum* (NCBI accession no. P02865, (SEQ ID NO:183))

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  1 arqlnpsdqe lqspqqlypq qpypqqpy
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[0108] Inhibitors of gliadin-derived peptide translocation across biological barriers are useful for treating diseases characterized by inflammation, including autoimmune diseases and particularly including celiac disease. Inhibitors of PTG-induced factors that increase biological barrier permeability are useful for treating diseases characterized by inflammation, including autoimmune diseases and particularly including celiac disease.

[0109] Activity of inhibitors of gliadin-derived peptide translocation and/or inhibitors of PTG-induced permeability can be measured by any means known in the art. Signaling events which can be determined include decrease in TEER, increase in LY permeability, increase in cytokine release, microglial recruitment, tyrosine kinase phosphorylation and chemotaxis, and increase in MMP-2 and MMP-9 gelatinolytic activity in cell-conditioned media.

[0110] The invention provides methods of identifying agents, compounds or lead compounds for agents active in inhibiting PTG-induced alterations in biological barrier permeability and/or peptide translocation. Generally, screening methods of the invention

involve assaying for compounds which modulate the interaction of one or more gliadin fragments with one or more cells (e.g., epithelial cells, immune cells). A wide variety of assays for binding agents is provided including labeled in vitro protein-ligand binding assays, cell based assays, immunoassays, etc. A wide variety of formats may be used, including co-immunoprecipitation, 2-hybrid transactivation, fluorescent polarization, NMR, fluorescent resonance energy transfer (FRET), transcriptional activation, etc. For example, a wide variety of NMR-based methods are available to rapidly screen libraries of small compounds for binding to protein targets (Hajduk, P. J., et al. Quarterly Reviews of Biophysics, 1999. 32 (3): 211-40). In some embodiments, methods of the invention may be automated (e.g., high throughput screening) and may be used to screen chemical libraries for lead compounds. Identified compounds may be used to treat diseases involving increased biological barrier permeability including, for example, celiac disease, inflammatory bowel diseases and autoimmune diseases. Compounds identified by the methods of the invention may be further optimized to modulate biological barrier modulation, for example, may be derivatized. Multiple iterations of screening and derivatization may be employed to optimize the modulation of biological barrier permeability.

[0111] In vitro ligand binding assays employ a mixture of components including one or more gliadin-derived peptides or fragments and one or more gliadin binding components. Gliadin-derived peptides or fragments may be provided as fusion proteins (e.g., with purification tags such as 6-His). Assay mixtures typically further comprise a compound to be tested for inhibitory activity. Compounds to be tested may be of any kind known to those skilled in the art, for example, may be organic compounds, peptides, proteins, nucleic acids, lipids, carbohydrates and mixtures thereof. A variety of other reagents may also be included in the mixture including, but not limited to, salts, buffers, neutral proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc.

[0112] In general, assay mixtures may be incubated under conditions in which, but for the presence of the compound to be tested, gliadin-derived peptides or fragments specifically bind the gliadin binding components with a reference binding affinity. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. Incubation periods are likewise selected for optimal binding. In some embodiments, incubation periods may be minimized to facilitate rapid, high-throughput screening.

[0113] After incubation, the effect of the compound to be tested on the gliadin binding may be detected by any convenient way. For example, the gliadin-derived peptide or fragment or the gliadin binding component may be immobilized, and the other labeled; then in a solid-phase format, any of a variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g. through optical or electron density, radiative emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, etc.

[0114] A difference in the binding affinity of the gliadin-derived peptide or fragment and the gliadin binding component in the absence of the compound to be tested as compared with the binding affinity in the presence of the compound to be tested indicates that the compound modulates the binding of the gliadin-derived peptide or fragment and the gliadin binding component. A difference, as used herein, is statistically significant and preferably represents at least a 50%, 60%, 70%, 80%, or 90% difference.

[0115] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

[0116] Measurement Of Trans Epithelial Electric Resistance (TEER) And Epithelial Flux Of A Fluorescent Marker Lucifer Yellow

[0117] CaCo2 cells form monolayers that exhibit tight junctions between adjacent cells. Agonists of tight junctions can be identified by their ability to enhance the flux of compounds (e.g. ions, Lucifer Yellow) through a cell monolayer that comprises tight junctions; or by their ability to reduce TEER across a cell monolayer that comprises tight junctions. Treatment of CaCo2 monolayers with peptide tight junction agonist compounds leads to enhancement of Lucifer Yellow permeability through CaCo2 monolayers compared to vehicle alone. Treatment of CaCo2 monolayers with peptide tight junction agonist compounds leads to a decrease in TEER across CaCo2 monolayers compared to vehicle alone.

[0118] Tight junction agonists and agonists of the C1orf43 and CCDC78 proteins can be identified using the following method, and this method may be easily modified to identify antagonists and inhibitors of the C1orf43 and CCDC78 proteins:

[0119] Determination of TEER and Lucifer Yellow flux

[0120] Prepare Modified Hank's Balanced Salt Solution (MHBSS) by obtaining 1L bottle of HBSS removing 10ml of HBSS and replacing it with 10ml HEPES buffer pH 7.0. Adjust pH to 7.4 ±0.1 using concentrated NaOH (10N).

[0121] Remove CaCo-2 cells from incubator, grown on 12-well, 3.0 μM, polycarbonate Transwell® filters (Corning) and record passage#, date cells seeded and age in days.

[0122] Aspirate cell culture medium from both the apical (AP) and basolateral (BL) compartments, replacing with 0.5 ml and 1.5 ml of MHBSS, respectively. Incubate cells at 37°C for 30 minutes.

[0123] Using the MilliCell®-ERS instrument (Millipore), measure and record the transepithelial electrical resistance (TEER) across each filter and record.

[0124] Aspirate solution from the apical compartment of each filter (n=3 per condition) and replace with 0.5ml of control and test solutions containing Lucifer Yellow and test compound if appropriate.

[0125] Place all plates into incubator set at 37°C (± 0.2), 50 RPM (±5) for a total of 180 minutes.

[0126] At t = 30, 60, 120 and 180 minutes, measure and record the transepithelial electrical resistance (TEER) across each filter using the MilliCell-ERS instrument.

[0127] At t = 60, 120 and 180 minutes remove 100μl from each basolateral compartment and place it in a 96-well plate for Lucifer Yellow analysis, replace with 100μl of MHBSS.

[0128] Make a Lucifer Yellow standard curve with the following dilutions (7500μM, 3750μM, 750μM, 375μM, 75μM, 37.5μM, 7.5μM, 3.75μM, 0.75μM) and pipette 100μL of each into a 96-well plate except for the first three standards mentioned above which require a 1:10 dilutions prior to transferring to the 96-well plate.

[0129] Harvest the remaining start solutions and what is left in each apical compartment into 1.5ml vials. Freeze at -20°C for future analysis.

[0130] Analyze each 96-well plate in a Tecan Spectra Fluor Plus using Magellan at 485 and 535nm.

[0131] Materials:

[0132] Cells: CaCo-2 cells passage 40-60 grown on Transwell® plates for 21-28 days

[0133] Culture Medium: DMEM supplemented with 10% fetal bovine serum, 1% NEAA, 1% Penn/Strep

[0134] Buffers: Hank's Balanced Salt Solution (HBSS) without calcium and magnesium

[0135] Flasks: 100 X 20 mm Tissue culture dish Falcon.

[0136] Plates: 12 well polycarbonate Transwell® filters; 0.3uM pore size

EXAMPLE 2

[0137] Identification of Cytokines Upregulated on Treatment of THP-1 cells by PT-Gliadin (PTG)

[0138] The monocytic cell line THP-1 was used to characterize the profile of cytokines whose expression was upregulated on exposure to protease treated gliadin (PTG). THP-1 cells were diluted to 5x 10^5 cells/ml in RPMI medium supplemented with 10% heat inactivated fetal bovine serum.

[0139] 5x 10^5 (1ml) cells were plated in each well of a 12 well plate, and cells were incubated at 37°C overnight. Test compounds (PTG 1mg/ml; LPS 1μg/ml) were added to the cultures, and incubation was continued a further 18 hours at 37°C.

[0140] Culture supernatants were harvested, and cytokines/chemokines were measured in each sample using a nitrocellulose membrane based proteomic profiler assay (R&D Systems). Assays were performed in triplicate. The cytokines screened in this assay included C5a, CD40 ligand, G-CSF, GM-CSF, GRO-α/CXCL1, I-309/CCL1, ICAM-1, IFNγ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-16, IL-17, IL-17E, IL-23, IL-27, IL-32α, IP-10/CXCL10, I-TAC/CXCL11, MCP-1/CCL2, MIF, MIP-1α/CCL3, MIP-1β/CCL3, RANTES/CCL5, SDF-1/CXCL12, Serpin-E1/PAI-1, TNFα, and TREM-1.

[0141] After 6 hours of PTG exposure THP-1 cells demonstrated increased expression of the cytokines IL-8, MIP-1α, MIP-1β, TNF-α and Gro-α. After 24 hours of exposure to PTG increased expression of RANTES and MIF were also observed.

EXAMPLE 3

[0142] Identification of Cytokines Upregulated on Treatment of PBMCs by PT-Gliadin (PTG)

[0143] Peripheral blood mononuclear cells were isolated from donated human blood samples using methods known in the art, and these PBMCs were used to characterize the profile of cytokines whose expression was upregulated on exposure to protease treated gliadin (PTG). PBMCs were suspended in RPMI medium supplemented with 5% heat inactivated human AB serum, and 2x 10^5 cells were plated in each well of a 96 well plate. Cells were incubated at 37°C with PTG (1mg/ml) or LPS (1μg/ml) in the presence or absence of test compounds being examined for the ability to suppress cytokine production. Supernatant samples were harvested following treatment, and cytokines were assayed by ELISA (R&D Systems).

[0144] Expression of IL-6, IL-8, MIP-1α, and Gro-α were induced by treatment with LPS and PTG. Expression of these cytokines was not reduced by treatment with peptide GGVLVQPG (SEQ ID NO:1).

[0145] Increased expression of GM-CSF and IL-16 was induced by exposure to LPS and PTG. This increased expression of these cytokines was inhibited by treatment with peptide GGVLVQPG (SEQ ID NO:1).

[0146] Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims. All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

Table 20 Peptide permeability inhibitors

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
1	Gly-Gly-Val-Leu-Val-Gln-Pro-Gly	-	+
2	Ala-Gly-Val-Leu-Val-Gln-Pro-Gly	-	+
3	Gly-Ala-Val-Leu-Val-Gln-Pro-Gly	-	+
4	Gly-Gly-Ala-Leu-Val-Gln-Pro-Gly	-	+
5	Gly-Gly-Val-Ala-Val-Gln-Pro-Gly	-	+
6	Gly-Gly-Val-Leu-Ala-Gln-Pro-Gly	-	-
7	Gly-Gly-Val-Leu-Val-Ala-Pro-Gly	-	-
8	Gly-Gly-Val-Leu-Val-Gln-Ala-Gly	-	-
9	Gly-Gly-Val-Leu-Val-Gln-Pro-Ala	-	-
10	Gly-Asp-Val-Leu-Val-Gln-Pro-Gly	+	+
11	Gly-Glu-Val-Leu-Val-Gln-Pro-Gly	+	+
12	Gly-Gln-Val-Leu-Val-Gln-Pro-Gly	+	+
13	Gly-Phe-Val-Leu-Val-Gln-Pro-Gly	+	+
14	Gly-His-Val-Leu-Val-Gln-Pro-Gly	+	+
15	Gly-Arg-Val-Leu-Val-Gln-Pro-Gly	+	+
16	Gly-Lys-Val-Leu-Val-Gln-Pro-Gly	+	+
17	Gly-Ile-Val-Leu-Val-Gln-Pro-Gly	+	+
18	Gly-Trp-Val-Leu-Val-Gln-Pro-Gly	-	-
19	Gly-Pro-Val-Leu-Val-Gln-Pro-Gly	+	+
20	Gly-Val-Val-Leu-Val-Gln-Pro-Gly	+	+
21	Gly-Leu-Val-Leu-Val-Gln-Pro-Gly	+	+
22	Gly-Asn-Val-Leu-Val-Gln-Pro-Gly	+	+
23	Gly-Thr-Val-Leu-Val-Gln-Pro-Gly	+	+
24	Gly-Gly-Gly-Leu-Val-Gln-Pro-Gly	-	-
25	Gly-Gly-Leu-Leu-Val-Gln-Pro-Gly	-	-
26	Gly-Gly-Ile-Leu-Val-Gln-Pro-Gly	-	-
27	Gly-Gly-Phe-Leu-Val-Gln-Pro-Gly	+	+
28	Gly-Gly-Arg-Leu-Val-Gln-Pro-Gly	-	-
29	Gly-Gly-Asp-Leu-Val-Gln-Pro-Gly	-	-
30	Gly-Gly-Gln-Leu-Val-Gln-Pro-Gly	-	-
31	Gly-Gly-His-Leu-Val-Gln-Pro-Gly	-	-

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
32	Gly-Gly-Met-Leu-Val-Gln-Pro-Gly	+	+
33	Gly-Gly-Ser-Leu-Val-Gln-Pro-Gly	-	-
34	Gly-Gly-Thr-Leu-Val-Gln-Pro-Gly	+	+
35	Gly-Gly-Pro-Leu-Val-Gln-Pro-Gly	-	-
36	Gly-Gly-Val-Gly-Val-Gln-Pro-Gly	+	+
37	Gly-Gly-Val-Val-Val-Gln-Pro-Gly	-	-
38	Gly-Gly-Val-Ile-Val-Gln-Pro-Gly	-	-
39	Gly-Gly-Val-Phe-Val-Gln-Pro-Gly	-	-
40	Gly-Gly-Val-Arg-Val-Gln-Pro-Gly	-	-
41	Gly-Gly-Val-Asp-Val-Gln-Pro-Gly	-	-
42	Gly-Gly-Val-Gln-Val-Gln-Pro-Gly	-	-
43	Gly-Gly-Val-His-Val-Gln-Pro-Gly	-	-
44	Gly-Gly-Val-Met-Val-Gln-Pro-Gly	-	-
45	Gly-Gly-Val-Ser-Val-Gln-Pro-Gly	-	-
46	Gly-Gly-Val-Thr-Val-Gln-Pro-Gly	-	-
47	Gly-Gly-Val-Pro-Val-Gln-Pro-Gly	-	-
48	D-Ala-Gly-Val-Leu-Val-Gln-Pro-Gly	+	+
49	Asp-Gly-Val-Leu-Val-Gln-Pro-Gly	+	+
50	Glu-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
51	Gln-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
52	Phe-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
53	His-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
54	Arg-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
55	Lys-Gly-Val-Leu-Val-Gln-Pro-Gly	+	+
56	Ile-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
57	Trp-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
58	Pro-Gly-Val-Leu-Val-Gln-Pro-Gly	+	+
59	Val-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
60	Leu-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
61	Thr-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
62	Asn-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
63	D-Phe-Gly-Val-Leu-Val-Gln-Pro-Gly	-	-

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
64	Cha-Gly-Val-Leu-Lav-Gln-Pro-Gly	NT	NT
65	Met(O)2-Gly-Val-Leu-Val-Gln-Pro-Gly	NT	NT
66	Gly-Val-Leu-Val-Gln-Pro-Gly	-	-
67	Val-Leu-Val-Gln-Pro-Gly	+	+
68	Leu-Val-Gln-Pro-Gly	+	+
69	Val-Gln-Pro-Gly	+	+
70	Gln-Pro-Gly	+	+
71	Gly-Gly-Val-Leu-Val-Gln-Pro	-	+
72	Gly-Gly-Val-Leu-Val-Gln	+	+
73	Gly-Gly-Val-Leu-Val	+	+
74	Gly-Gly-Val-Leu	+	+
75	Gly-Gly-Val	+	+
76	Gly-Gly-D-Val-Leu-Val-Gln-Pro-Gly	+	+
77	Gly-Gly-Val-D-Leu-Val-Gln-Pro-Gly	+	+
78	Gly-Gly-Val-Leu-D-Val-Gln-Pro-Gly	-	-
79	Gly-Gly-Val-Leu-Val-D-Gln-Pro-Gly	+	+
80	Gly-Gly-Val-Leu-Val-Gln-D-Pro-Gly	+	+
81	Gly-D-Pro-D-Gln-D-Val-D-Leu-D-Val-Gly-Gly	+	+
82	Gly-D-Pro-D-Gln-D-Val-D-Leu-Val-Gly-Gly	+	+
83	Gly-D-Pro-D-Gln-D-Val-Leu-D-Val-Gly-Gly	+	+
84	Gly-D-Pro-D-Gln-Val-D-Leu-D-Val-Gly-Gly	+	+
85	Gly-D-Pro-Gln-D-Val-D-Leu-D-Val-Gly-Gly	+	+
86	Gly-Pro-D-Gln-D-Val-D-Leu-D-Val-Gly-Gly	-	-
87	Gly-Pro-Gln-Val-Leu-Val-Gly-Gly	+	+
88	Gly-D-Pro-Gln-Val-Leu-Val-Gly-Gly	+	+
89	Gly-Pro-D-Gln-Val-Leu-Val-Gly-Gly	-	-
90	Gly-Pro-Gln-D-Val-Leu-Val-Gly-Gly	-	-
91	Gly-Pro-Gln-Val-D-Leu-Val-Gly-Gly	+	+

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
92	Gly-Pro-Gln-Val-Leu-D-Val-Gly-Gly	+	+
93	Gly-Gly-D-Val-D-Leu-D-Val-D-Gln-D-Pro-Gly		
94	Gly-Gly-D-Val-D-Leu-D-Val-D-Gln-Pro-Gly	+	-
95	Gly-Gly-D-Val-D-Leu-D-Val-Gln-D-Pro-Gly	-	-
96	Gly-Gly-D-Val-D-Leu-Val-D-Gln-D-Pro-Gly	-	-
97	Gly-Gly-D-Val-Leu-D-Val-D-Gln-D-Pro-Gly	-	-
98	Gly-Gly-Val-D-Leu-D-Val-D-Gln-D-Pro-Gly	+	+
99	Gly-D-Phe-Val-Leu-Val-Gln-Pro-Gly	+	+
100	Ala-Pro-Gly	+	+
101	Gln-Ala-Gly	+	+
102	Gln-Pro-Ala	+	+
103	(d)Gln-Pro-Gly	+	+
104	Gln-(d)Pro-Gly	+	+
105	(d)Gln-(d)Pro-Gly	-	-
106	Gly-Pro-Gln	+	+
107	Gly-(d)Pro-Gln	-	-
108	Gly-Pro-(d)Gln	-	-
109	Gly-(d)Pro-(d)Gln	-	-
110	Ala-Pro-Gly	+	+
111	His-Pro-Gly	+	+
112	Asp-Pro-Gly	-	-
113	Arg-Pro-Gly	+	+
114	Phe-Pro-Gly	+	+
115	Gly-Pro-Gly	+	+
116	Glu-Pro-Gly	+	+
117	Lys-Pro-Gly	+	+
118	Leu-Pro-Gly	+	+
119	Met-Pro-Gly	+	+

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
120	Asn-Pro-Gly	+	+
121	Ser-Pro-Gly	+	+
122	Tyr-Pro-Gly	+	+
123	Thr-Pro-Gly	-	+
124	Ile-Pro-Gly	+	+
125	Trp-Pro-Gly	+	+
126	Pro-Pro-Gly	-	-
127	Val-Pro-Gly	-	+
128	Glp-Pro-Gly	+	+
129	Glp-Val-Gly	-	-
130	Glp-Gln-Gly	-	-
131	Glp-Ser-Gly	-	-
132	Glp-Lys-Gly	-	-
133	Glp-Phe-Gly	-	-
134	Glp-Glu-Gly	-	-
135	Glp-Thr-Gly	-	-
136	Glp-Ile-Gly	-	-
137	Glp-Tyr-Gly	-	-
138	Glp-His-Gly	-	-
139	Glp-Asn-Gly	-	-
140	Glp-Arg-Gly	-	-
141	Glp-Gly-Gly	-	-
142	Glp-Trp-Gly	-	-
143	Glp-Asp-Gly	-	-
144	Glp-Met-Gly	-	-
145	Glp-Leu-Gly	-	-
146	Glp-Pro-Gln	-	-
147	Glp-Pro-Asn	+	-
148	Glp-Pro-Gln	-	-
149	Glp-Pro-Ser	-	-
150	Glp-Pro-Pro	+	-
151	Glp-Pro-Trp	-	-

SEQ ID NO:	Sequence	Prevented TEER Reduction	Reduced LY Permeability
152	Glp-Pro-Asp	-	-
153	Glp-Pro-His	-	-
154	Glp-Pro-Leu	-	-
155	Glp-Pro-Arg	-	-
156	Glp-Pro-Val	-	-
157	Glp-Pro-Lys	-	-
158	Glp-Pro-Glu	-	-
159	Glp-Pro-Phe	-	-
160	Glp-Pro-Ile	+	-
161	Glp-Pro-Met	+	-
162	Glp-Pro-Tyr	+	-

Met(O)2 = Methioninedioxide, Cha = cyclohexyl-Ala

What is claimed is:

1. (Original) A peptide permeability inhibitor consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs:1-162, wherein said peptide permeability inhibitor inhibits translocation of a gliadin-derived peptide across a biological barrier.

2. (Original) The peptide of claim 1, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

3. (Original) The peptide of claim 1, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

4. (Original) A method of inhibiting gliadin-derived peptide translocation across a biological barrier comprising contacting said barrier with a peptide permeability inhibitor consisting of an amino acid sequence selected from the group consisting of SEQ IDF NOs:1-162.

5. (Original) The method of claim 4, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

6. (Original) The method of claim 4, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

7. (Original) A composition for inhibiting gliadin-derived peptide translocation across a biological barrier, wherein said composition comprises a peptide permeability inhibitor consisting of an amino acid sequence selected from the group consisting of SEQ IDF NOs:1-162.

8. (Original) The composition of claim 7, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

9. (Original) The composition of claim 7, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

10. (Original) A method for inhibiting gliadin-derived peptide translocation across a biological barrier comprising administering to a subject in need thereof the composition of claim 7 in an amount sufficient to inhibit said gliadin-derived peptide translocation.

11. (Original) The method of claim 10, wherein the composition is administered in conjunction with an additional therapeutic agent.

12. (Original) The method of claim 11, wherein the composition and the additional therapeutic agent are administered simultaneously.

13. (Original) The method of claim 11, wherein the composition and the additional therapeutic agent are not administered simultaneously.

14. (Original) The method of claim 11, wherein the composition further comprises the additional therapeutic agent.

15. (Original) The method of claim 11, wherein the additional therapeutic agent is selected from the group consisting of aminosalicylates, corticosteroids, immunomodulators, antibiotics, cytokines, chemokines and biologic therapeutics.

16. (Original) A peptide consisting of an amino acid sequence selected from the group consisting of SEQ IDF NOs:1-162, wherein said peptide inhibits a gliadin-induced increase in biological barrier permeability.

17. (Original) The peptide of claim 16, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

18. (Original) The peptide of claim 16, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

19. (Original) A method of inhibiting a gliadin-induced increase in biological barrier permeability comprising contacting said barrier with a peptide permeability inhibitor consisting of an amino acid sequence selected from the group consisting of SEQ IDF NOs:1-162.

20. (Original) The method of claim 19, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

21. (Original) The method of claim 19, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

22. (Original) A composition for inhibiting a gliadin-induced increase in biological barrier permeability, wherein said composition comprises a peptide permeability inhibitor consisting of an amino acid sequence selected from the group consisting of SEQ IDF NOs:1-162.

23. (Original) The composition of claim 22, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

24. (Original) The composition of claim 22, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

25. (Original) A method for inhibiting a gliadin-induced increase in biological barrier permeability comprising administering to a subject in need thereof the composition of claim 22 in an amount sufficient to inhibit said increase in biological barrier permeability.

26. (Original) The method of claim 25, wherein the composition is administered in conjunction with an additional therapeutic agent.

27. (Original) The method of claim 26, wherein the composition and the additional therapeutic agent are administered simultaneously.

28. (Original) The method of claim 26, wherein the composition and the additional therapeutic agent are not administered simultaneously.

29. (Original) The method of claim 26, wherein the composition further comprises the additional therapeutic agent.

30. (Original) The method of claim 26, wherein the additional therapeutic agent is selected from the group consisting of aminosalicylates, corticosteroids, immunomodulators, antibiotics, cytokines, chemokines and biologic therapeutics.

31. (Original) A method of treating a patient with celiac disease, comprising administering to the patient a composition comprising a peptide that inhibits translocation of a gliadin-derived peptide across a biological barrier.

32. (Original) The method of claim 31, wherein said composition comprises peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs:1-162.

33. (Original) The method of claim 32, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

34. (Original) The method of claim 32, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55,

58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

35. (Original) The method of claim 31, wherein said composition further comprises an additional therapeutic agent selected from the group consisting of aminosalicylates, corticosteroids, immunomodulators, antibiotics, cytokines, chemokines and biologic therapeutics.

36. (Original) A method of treating a patient with diabetes, comprising administering to the patient a composition comprising a peptide that inhibits a gliadin-induced increase in biological barrier permeability.

37. (Original) The method of claim 36, wherein said composition comprises peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs:1-162.

38. (Original) The method of claim 37, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

39. (Original) The method of claim 37, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

40. (Original) The method of claim 36, wherein said composition further comprises an additional therapeutic agent selected from the group consisting of aminosalicylates, corticosteroids, immunomodulators, antibiotics, cytokines, chemokines and biologic therapeutics.

41. (Original) A method of treating a patient with dermatitis herpetiformis, comprising administering to the patient a composition comprising a peptide that inhibits translocation of a gliadin-derived peptide across a biological barrier.

42. (Original) The method of claim 41, wherein said composition comprises peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs:1-162.

43. (Original) The method of claim 42, wherein the peptide does not consist of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 24 and 25.

44. (Original) The method of claim 42, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 10-17, 19-23, 27, 32, 34, 36, 48, 49, 55, 58, 67-77, 79-85, 87, 88, 91, 92, 94, 98-104, 106, 110, 111, 113-125, 127, 128, 147, 150, and 160-162.

45. (Original) the method of claim 41, wherein said composition further comprises an additional therapeutic agent selected from the group consisting of aminosalicylates, corticosteroids, immunomodulators, antibiotics, cytokines, chemokines and biologic therapeutics.

Figure 1

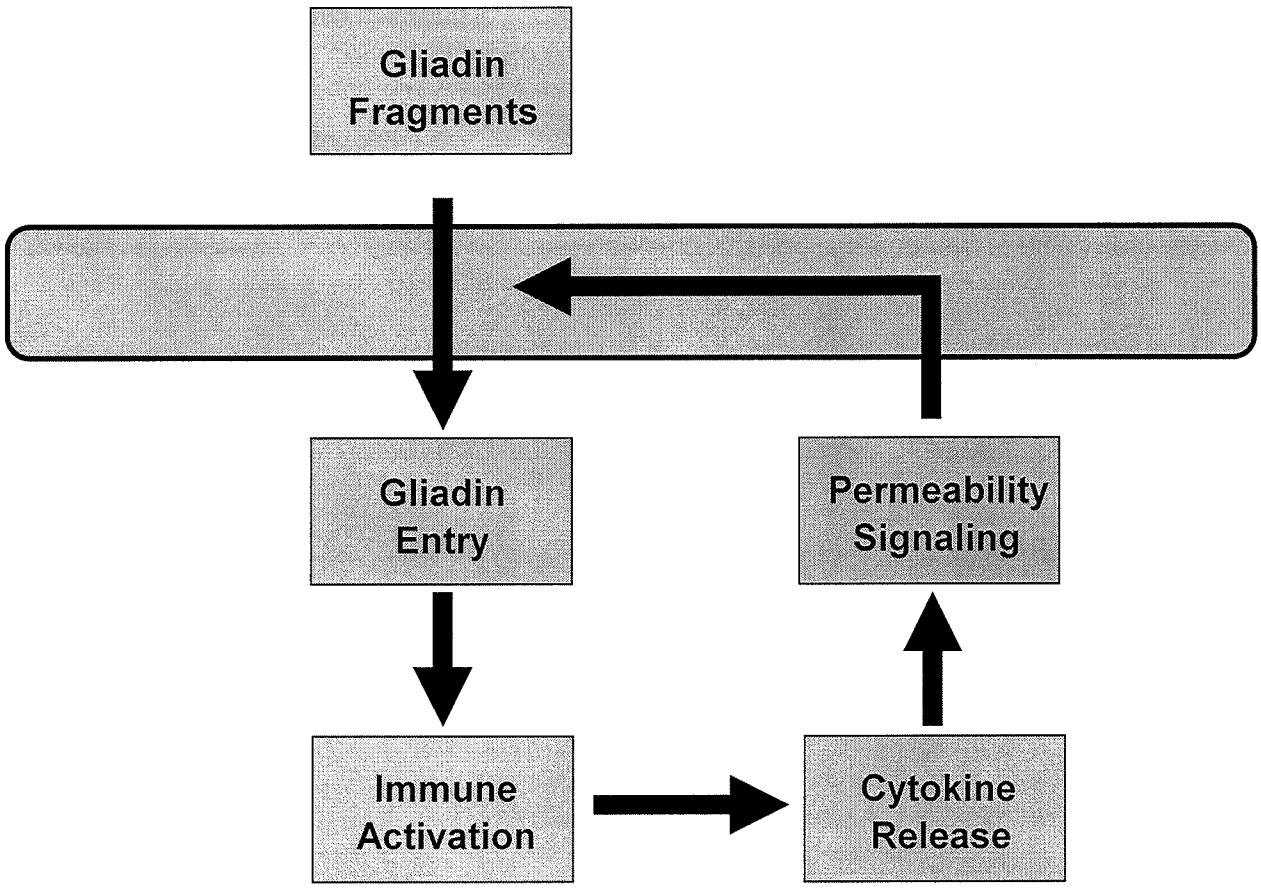
Gliadin Fragments
Gliadin Entry
Permeability Signaling
Immune Activation
Cytokine Release

Figure 2

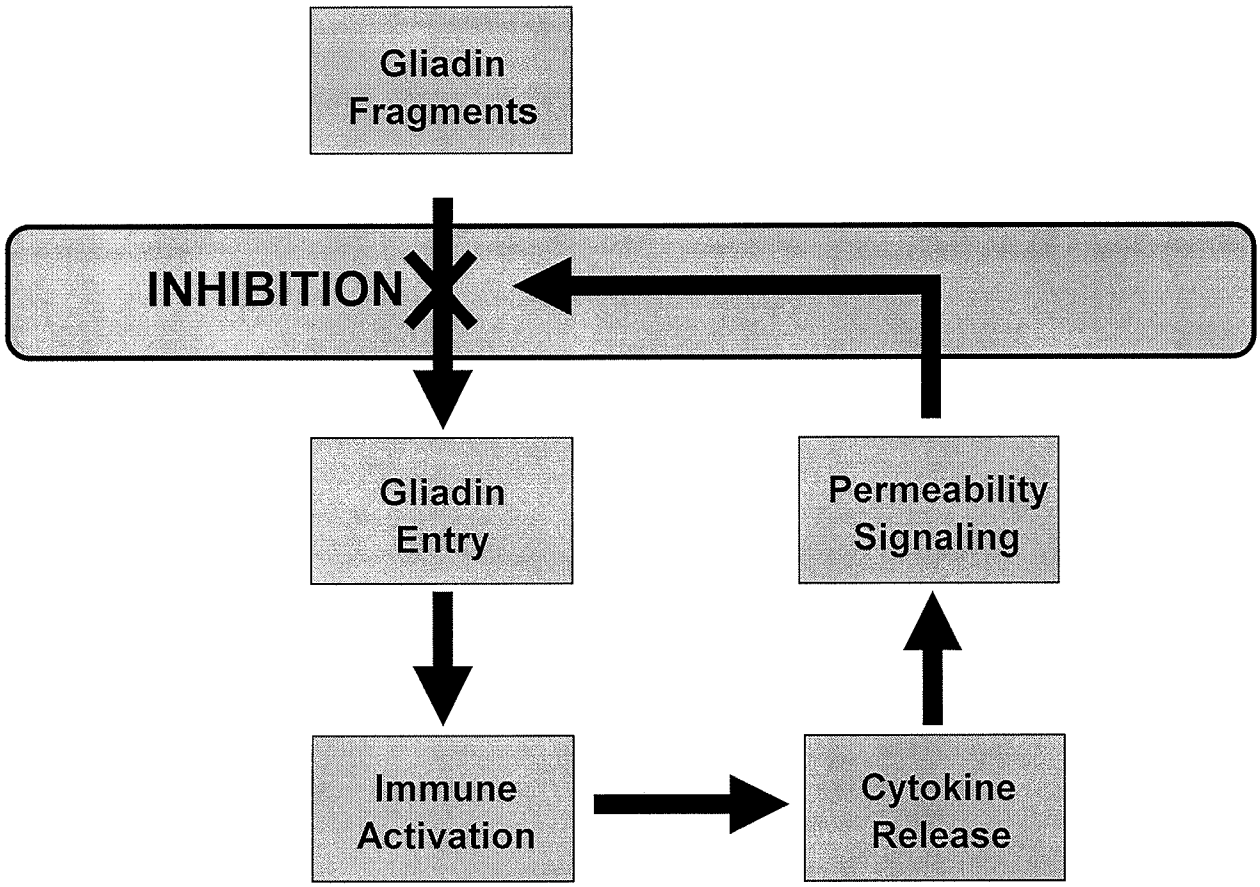
Gliadin Fragments
INHIBITION
Gliadin Entry
Permeability Signaling
Immune Activation
Cytokine Release

Figure 3

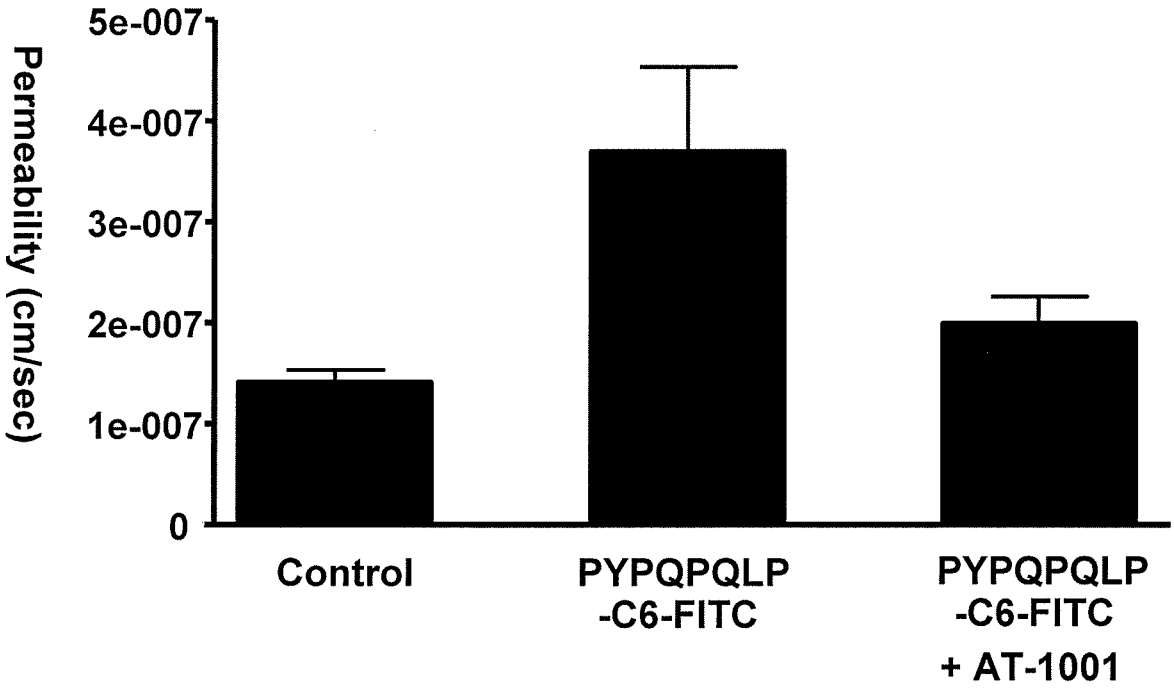
Permeability (cm/sec)
5e-007
4e-007
3e-007
2e-007
1e-007
0
Control
PYPQPQLP -C6-FITC
PYPQPQLP -C6-FITC + AT-1001

Figure 4

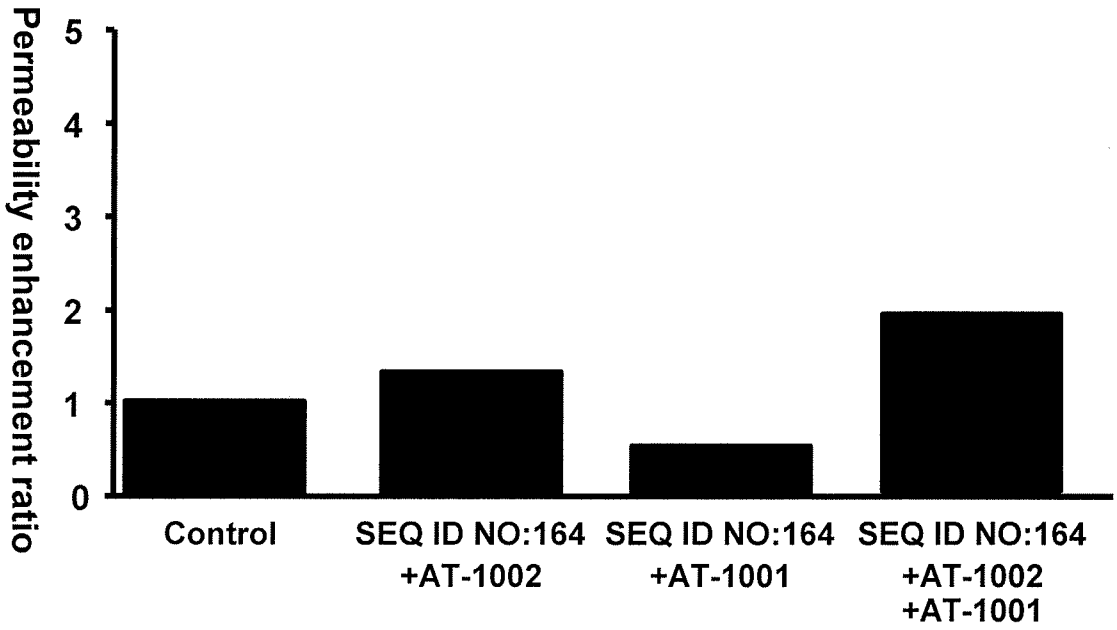
Permeability enhancement ratio
5
4
3
2
1
0
Control
SEQ ID NO:164 +AT-1002
SEQ ID NO:164 +AT-1001
SEQ ID NO:164 +AT-1002 +AT-1001

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Figure 5A

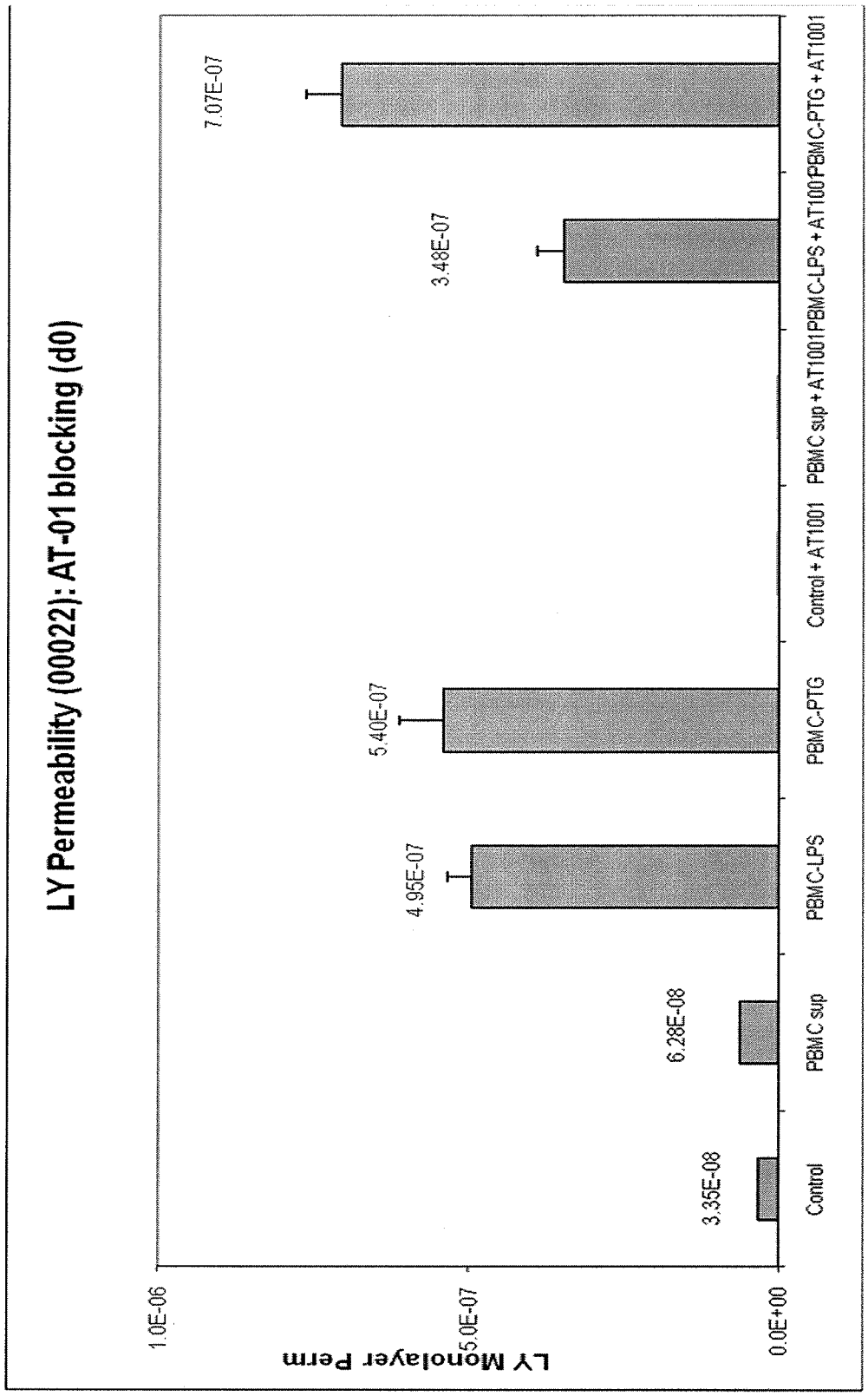

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Figure 5B

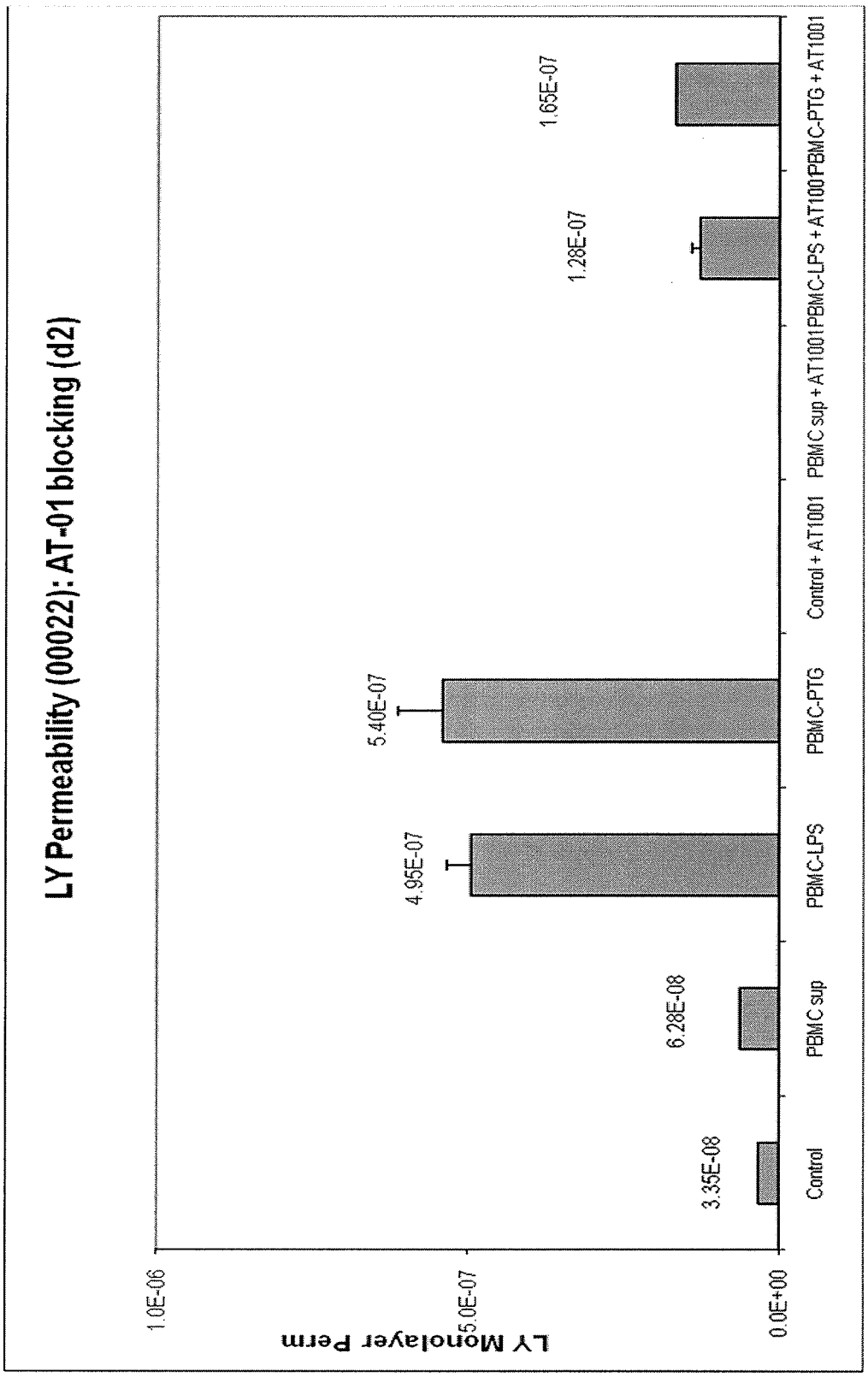

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Figure 6A

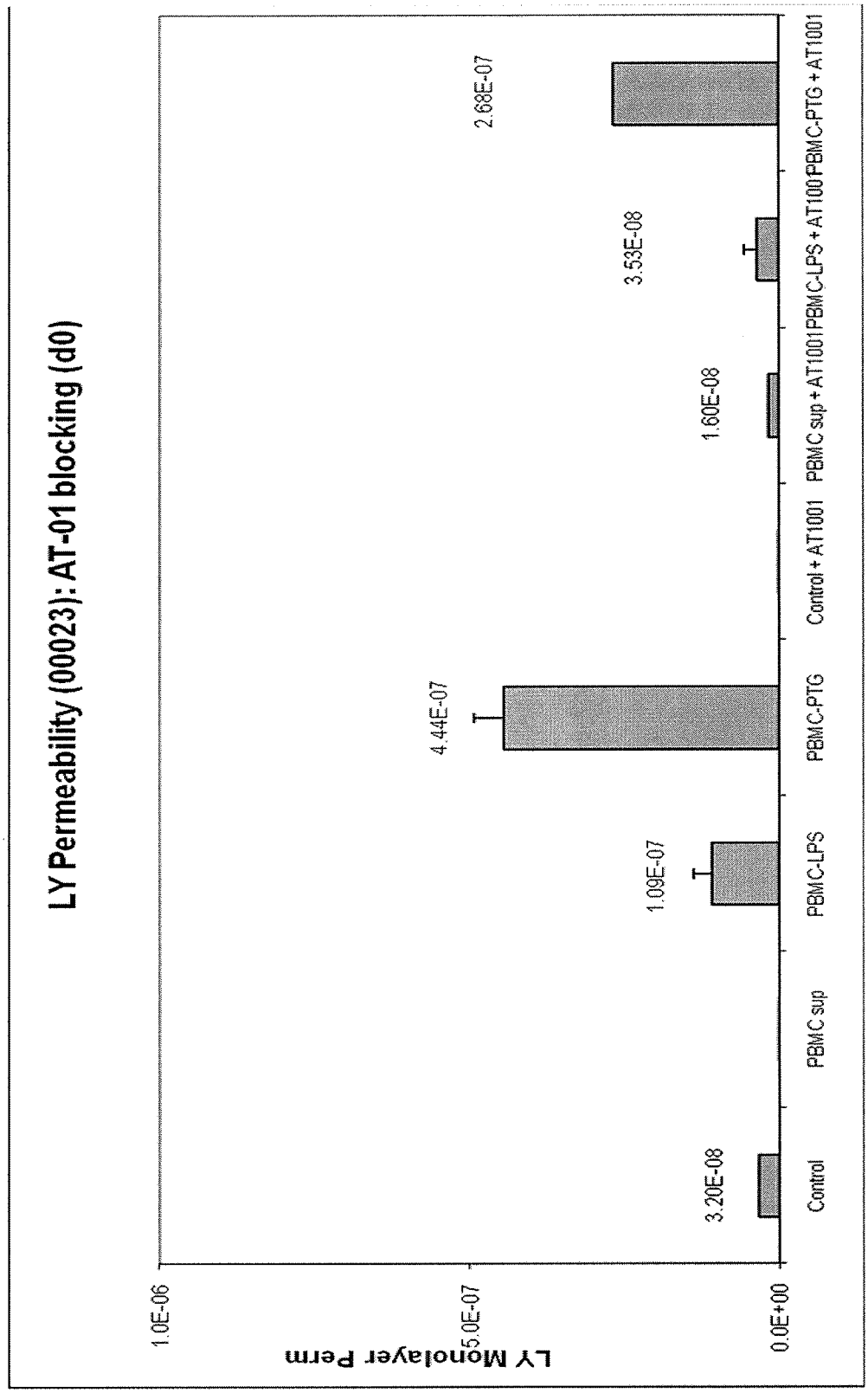

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Figure 6B

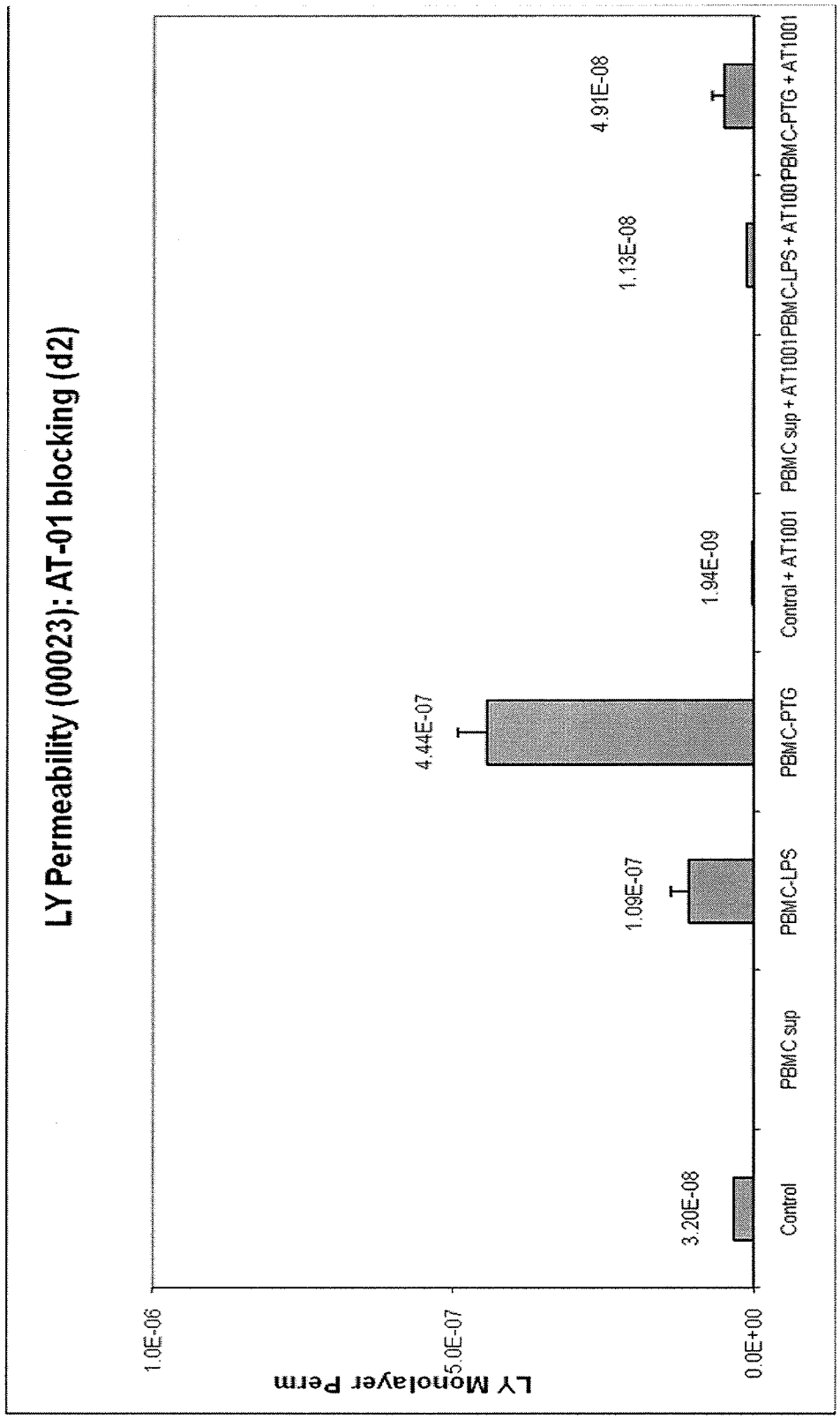

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Figure 7A

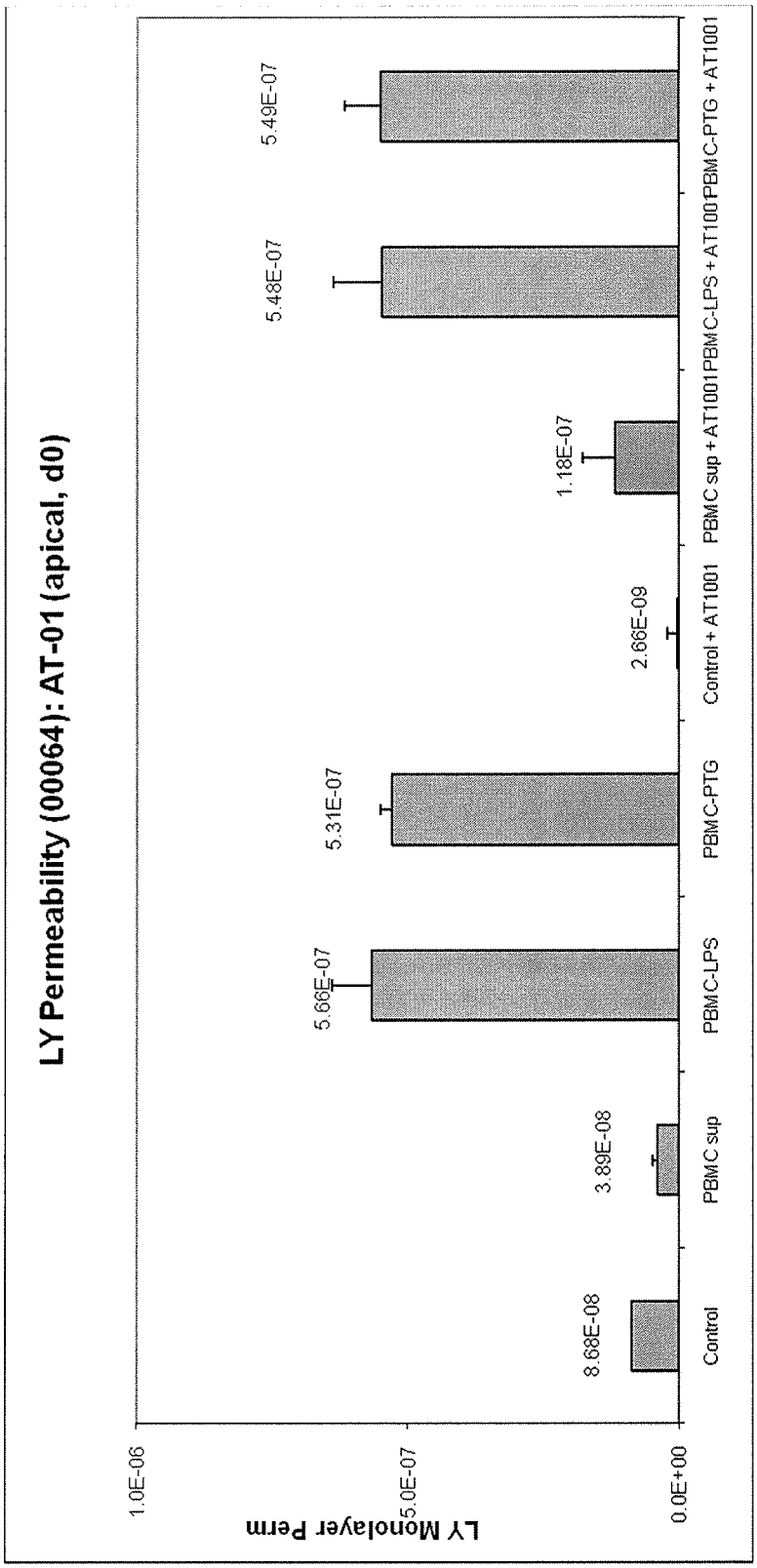

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Figure 7B

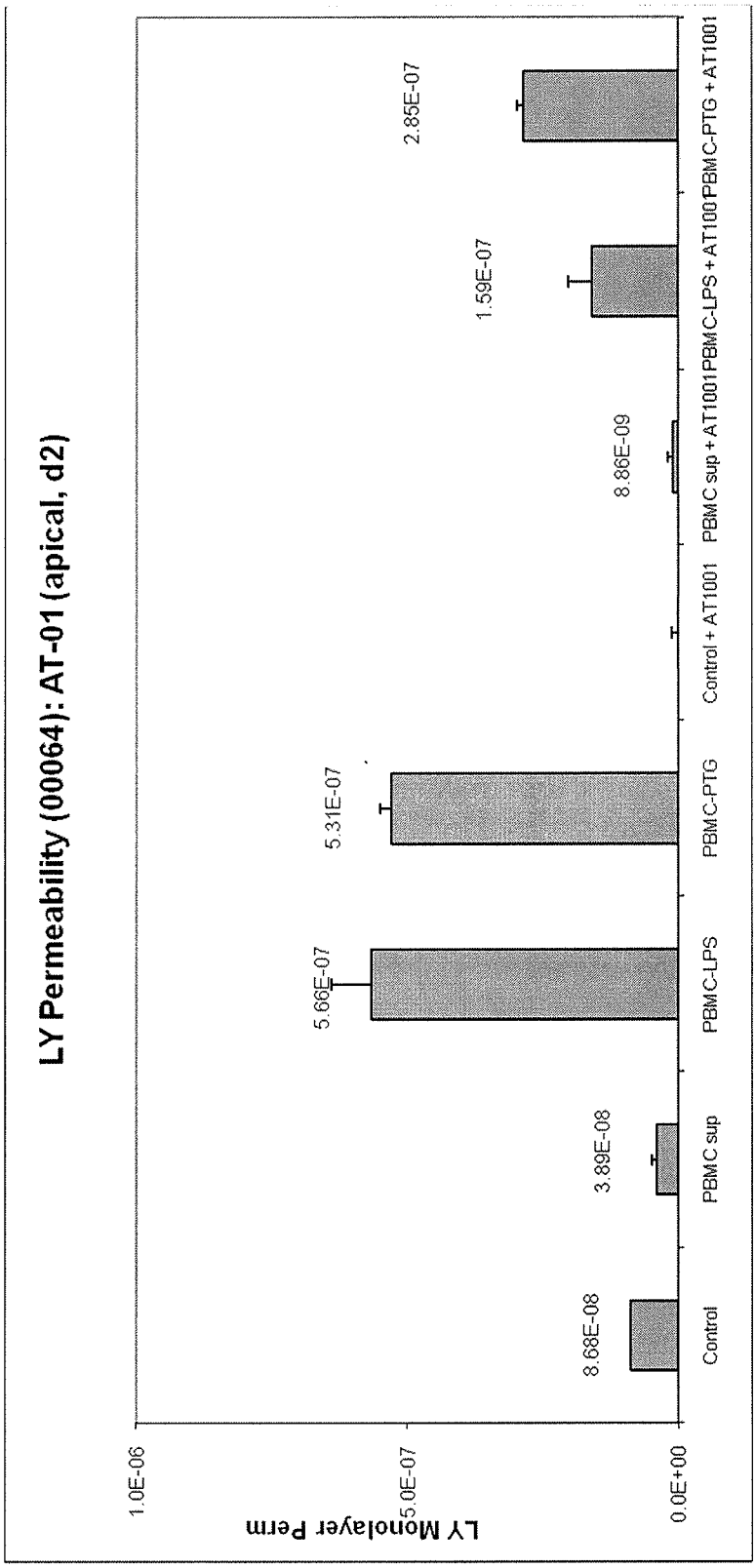

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Figure 8A

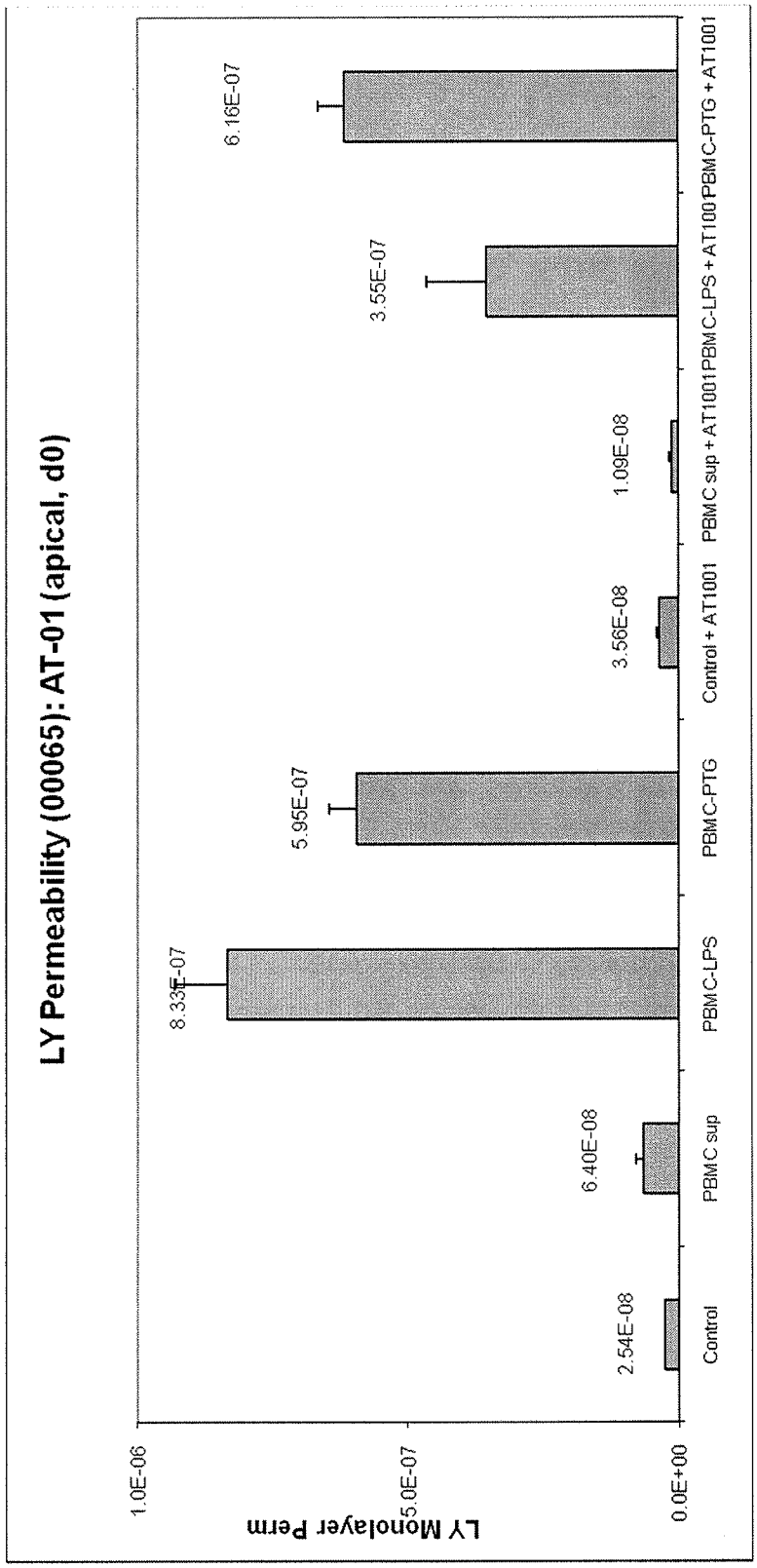

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Figure 8B