(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 February 2010 (11.02.2010)

PCT

(10) International Publication Number
WO 2010/017152 A2

(51) International Patent Classification:
A61K 9/12 (2006.01) *A61K 39/395* (2006.01)
C07H 21/04 (2006.01) *A61K 38/00* (2006.01)
A61K 31/7088 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/US2009/052624

(22) International Filing Date:
4 August 2009 (04.08.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/086,017 4 August 2008 (04.08.2008) US

(71) Applicant *(for all designated States except US)*: **IDERA PHARMACEUTICALS, INC.** [US/US]; 167 Sidney Street, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KANDIMALLA, Ekambar, R.** [US/US]; 6 Candlewood Lane, Southboro, MA 01172 (US). **PUTTA, Mallikarjuna** [IN/US]; 824 Arboretum Way, Burlington, MA 01803 (US). **BHAGAT, Lakshmi** [IN/US]; 1550 Worcester Street, Suite 605, Framingham, MA 01702 (US). **WANG, Daqing** [CN/US]; 7 Selfridge Road, Bedford, MA 01730 (US). **YU, Dong** [CN/US]; 25 Indian Pond Road, Westboro, MA 01581 (US). **AGRAWAL, Sudhir** [US/US]; 61 Lamplighter Drive, Shrewsbury, MA 01545 (US).

(74) Agent: **ZUCCHERO, Joseph, C.**; Keown & Zucchero, LLP, 500 West Cummings Park, Suite 1200, Woburn, MA 01801 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2010/017152 A2

(54) **Title**: MODULATION OF TOLL-LIKE RECEPTOR 8 EXPRESSION BY ANTISENSE OLIGONUCLEOTIDES

(57) **Abstract**: Antisense oligonucleotide compounds, compositions and methods are provided for down regulating the expression of TLR8. The compositions comprise antisense oligonucleotides targeted to nucleic acids encoding TLR8. The compositions may also comprise antisense oligonucleotides targeted to nucleic acids encoding TLR8 in combination with other therapeutic and/or prophylactic compounds and/or compositions. Methods of using these compounds and compositions for down-regulating TLR8 expression and for prevention or treatment of diseases wherein modulation of TLR8 expression would be beneficial are provided.

MODULATION OF TOLL-LIKE RECEPTOR 8 EXPRESSION BY ANTISENSE OLIGONUCLEOTIDES

(Atty. Docket No. IDR-050PC)

BACKGROUND OF THE INVENTION

Related Applications

[0001] This application claims the benefit of prior U.S. Provisional Patent Application Serial No. 61/086,017, filed on August 4, 2008, the contents of which are incorporated by reference in its entirety.

Field of the invention

[0002] The present invention relates to Toll-Like Receptor 8 (TLR8). In particular, the invention relates to antisense oligonucleotides that specifically hybridize with nucleic acids encoding TLR8, thus modulating TLR8 expression and activity, and their use in treating or preventing diseases associated with TLR8 or wherein modulation of TLR8 expression would be beneficial.

Summary of the related art

[0003] Toll-like receptors (TLRs) are present on many cells of the immune system and have been shown to be involved in the innate immune response (Hornung, V. et al., (2002) J. Immunol. 168:4531-4537). TLRs are a key means by which mammals recognize and mount an immune response to foreign molecules and also provide a means by which the innate and adaptive immune responses are linked (Akira, S. et al. (2001) Nature Immunol. 2:675-680; Medzhitov, R. (2001) Nature Rev. Immunol. 1:135-145). In mammals, this family consists of at least 11 proteins called TLR1 to TLR11, which are known to recognize pathogen associated molecular patterns (PAMP) from bacteria, fungi, parasites and viruses and induce an immune response mediated by a number of transcription factors.

[0004] Some TLRs are located on the cell surface to detect and initiate a response to extracellular pathogens and other TLRs are located inside the cell to detect and initiate a response to intracellular pathogens. Table 1 provides a representation of TLRs, the known agonists therefore and the cell types known to contain the TLR (Diebold, S.S. et al. (2004)

Science 303:1529-1531; Liew, F. et al. (2005) Nature 5:446-458; Hemmi H et al. (2002) Nat Immunol 3:196-200; Jurk M et al., (2002) Nat Immunol 3:499; Lee J et al. (2003) Proc. Natl. Acad. Sci. USA 100:6646-6651); (Alexopoulou, L. (2001) Nature 413:732-738).

Table 1:

TLR Molecule	**Agonist**	**Cell Types Containing Receptor**
Cell Surface TLRs:		
TLR2	bacterial lipopeptides	• Monocytes/macrophages • Myeloid dendritic cells • Mast cells
TLR4	gram negative bacteria	• Monocytes/macrophages • Myeloid dendritic cells • Mast cells • Intestinal epithelium
TLR5	motile bacteria	• Monocyte/macrophages • Dendritic cells • Intestinal epithelium
TLR6	gram positive bacteria	• Monocytes/macrophages • Mast cells • B lymphocytes
Endosomal TLRs:		
TLR3	double stranded RNA viruses	• Dendritic cells • B lymphocytes
TLR7	single stranded RNA viruses; RNA-immunoglobulin complexes	• Monocytes/macrophages • Plasmacytoid dendritic cells • B lymphocytes
TLR8	single stranded RNA viruses; RNA-immunoglobulin complexes	• Monocytes/macrophages • Dendritic cells • Mast cells
TLR9	DNA containing unmethylated	• Monocytes/macrophages

	"CpG" motifs; DNA-immunoglobulin complexes	• Plasmacytoid dendritic cells • B lymphocytes

[0005] The signal transduction pathway mediated by the interaction between a ligand and a TLR is shared among most members of the TLR family and involves a toll/IL-1 receptor (TIR domain), the myeloid differentiation marker 88 (MyD88), IL-1R-associated kinase (IRAK), interferon regulating factor (IRF), TNF-receptor-associated factor (TRAF), TGFβ-activated kinase1, IκB kinases, IκB, and NF-κB (see for example: Akira, S. (2003) J. Biol. Chem. 278:38105 and Geller at al. (2008) Curr. Drug Dev. Tech. 5:29-38). More specifically, for TLRs 1, 2, 4, 5, 6, 7, 8, 9 and 11, this signaling cascade begins with a PAMP ligand interacting with and activating the membrane-bound TLR, which exists as a homo-dimer in the endosomal membrane or the cell surface. Following activation, the receptor undergoes a conformational change to allow recruitment of the TIR domain containing protein MyD88, which is an adapter protein that is common to all TLR signaling pathways except TLR3. MyD88 recruits IRAK4, which phosphorylates and activates IRAK1. The activated IRAK1 binds with TRAF6, which catalyzes the addition of polyubiquitin onto TRAF6. The addition of ubiquitin activates the TAK/TAB complex, which in turn phosphorylates IRFs, resulting in NF-kB release and transport to the nucleus. NF-kB in the nucleus induces the expression of proinflammatory genes (see for example, Trinchieri and Sher (2007) Nat. Rev. Immunol. 7:179-190).

[0006] The selective localization of TLRs and the signaling generated therefrom, provides some insight into their role in the immune response. The immune response involves both an innate and an adaptive response based upon the subset of cells involved in the response. For example, the T helper (Th) cells involved in classical cell-mediated functions such as delayed-type hypersensitivity and activation of cytotoxic T lymphocytes (CTLs) are Th1 cells. This response is the body's innate response to antigen (e.g. viral infections, intracellular pathogens, and tumor cells), and results in a secretion of IFN-gamma and a concomitant activation of CTLs.

[0007] As a result of their involvement in regulating an inflammatory response, TLRs have been shown to play a role in the pathogenesis of many diseases, including autoimmunity, infectious disease and inflammation (Papadimitraki et al. (2007) J. Autoimmun. 29: 310-318; Sun et al. (2007) Inflam. Allergy Drug Targets 6:223-235; Diebold (2008) Adv. Drug Deliv. Rev. 60:813-823; Cook, D.N. et al. (2004) Nature Immunol. 5:975-979; Tse and Horner (2008)

Semin. Immunopathol. 30:53-62; Tobias & Curtiss (2008) Semin. Immunopathol. 30:23-27; Ropert et al. (2008) Semin. Immunopathol. 30:41-51; Lee et al. (2008) Semin. Immunopathol. 30:3-9; Gao et al. (2008) Semin. Immunopathol. 30:29-40; Vijay-Kumar et al. (2008) Semin. Immunopathol. 30:11-21). While activation of TLRs is involved in mounting an immune response, an uncontrolled or undesired stimulation of the immune system through TLRs may exacerbate certain diseases in immune compromised subjects or may cause unwanted immune stimulation. Thus, down-regulating TLR expression and/or activity may provide a useful means for disease intervention.

[0008] To date, investigative strategies aimed selectively at inhibiting TLR activity have involved small molecules (WO/2005/007672), antibodies (see for example: Duffy, K. et al. (2007) Cell Immunol. 248:103-114), catalytic RNAi technologies (e.g. small inhibitory RNAs), certain antisense molecules (Caricilli et al. (2008) J. Endocrinology 199:399), and competitive inhibition with modified or methylated oligonucleotides (see for example: Kandimalla et al. US2008/0089883; Barrat and Coffman (2008) Immunol. Rev. 223:271-283). For example, chloroquine and hydroxylchloroquine have been shown to block endosomal-TLR signaling by down-regulating the maturation of endosomes (Krieg, A. M. (2002) Annu. Rev. Immunol. 20:709). Also, Huang et al. have shown the use of TLR4 siRNA to reverse the tumor-mediated suppression of T cell proliferation and natural killer cell activity (Huang et al. (2005) Cancer Res. 65:5009-5014), and the use of TLR9 siRNA to prevent bacterial-induced inflammation of the eye (Huang et al. (2005) Invest. Opthal. Vis. Sci. 46:4209-4216).

[0009] Additionally, several groups have used synthetic oligodeoxynucleotides having two triplet sequences, a proximal "CCT" triplet and a distal "GGG" triplet, a poly "G" (e.g. "GGGG" or "GGG") or "GC" sequences that interact with certain intracellular proteins, resulting in the inhibition of TLR signaling and the concomitant production and release of pro-inflammatory cytokines (see for example: Lenert, P. et al. (2003) DNA Cell Biol. 22(10):621-631; Patole, P. et al. (2005) J. Am. Soc. Nephrol. 16:3273-3280; Gursel, I., et al. (2003) J. Immunol., 171: 1393-1400 ; Shirota, H., et al. (2004) J. Immunol., 173: 5002-5007 ; Chen, Y., et al. (2001) Gene Ther. 8: 1024-1032; Stunz, L.L. (2000) Eur. J. Immunol. 32: 1212-1222 ; Kandimalla et al. WO2007/7047396). However, oligonucleotides containing guanosine strings have been shown to form tetraplex structures, act as aptamers and inhibit thrombin activity (Bock LC et al.,

Nature, 355:564-6, 1992; Padmanabhan, K et al., J Biol Chem., 268(24):17651-4, 1993). Thus, the utility of these inhibitory oligodeoxynucleotide molecules may not be achievable in patients.

[0010] As an alternative to interacting with the receptor protein and directly inhibiting receptor activation, some studies have suggested the utility of "knock down" or silencing technologies, for example siRNA, miRNA, ddRNA and eiRNA technologies, for inhibiting the activity of a receptor. These technologies rely upon administration or expression of double stranded RNA (dsRNA). However, RNAi molecules act through a catalytic process, these molecules are recognized as being distinct from other technologies that target RNA molecules and inhibit their translation (see for example: Opalinska and Gewirtz (2002) Nature Reviews 1:503-514). Moreover, siRNA molecules have been recognized to induce non-specific immune stimulation through interaction with TLRs (Kleinman et al., (2008) Nature 452:591-597; De Veer et. al. (2005) Immun. Cell Bio. 83:224-228; Kariko et al. (2004) J. Immunol. 172:6545-6549).

[0011] A promising approach to suppressing the activity of TLR8 is the use of oligonucleotide-based antagonists (see Kandimalla et al., WO2007/7047396).

[0012] Yet another potential approach to "knock down" expression of TLRs is antisense technology. The history of antisense technology has revealed that while discovery of antisense oligonucleotides that inhibit gene expression is relatively straight forward, the optimization of antisense oligonucleotides that have true potential as clinical candidates is not. Accordingly, if an antisense approach to down-regulating TLR8 is to be successful, there is a need for optimized antisense oligonucleotides that most efficiently achieve this result. Such optimized antisense oligonucleotides could be used alone, or in conjunction with the antagonists of Kandimalla et al., or other therapeutic approaches.

BRIEF SUMMARY OF THE INVENTION

[0013] The present invention is directed to optimized synthetic antisense oligonucleotides that are targeted to a nucleic acid encoding TLR8 and that efficiently inhibit the expression of TLR8 through inhibition of mRNA translation and/or through an RNase H mediated mechanism.

[0014] In a first aspect, the invention provides for optimized antisense oligonucleotides including those having SEQ ID NOs: 26, 46, 53, 84, 85, 91, 102, 116, 131, 143, 146, 152, 157, 180, 182, 189 or 197.

[0015] In a second aspect, the invention provides a composition comprising at least one optimized antisense oligonucleotide according to the invention and a physiologically acceptable carrier, diluent or excipient.

[0016] In a third aspect, the invention provides a method of inhibiting TLR8 expression. In this method, an oligonucleotide or multiple oligonucleotides of the invention are specifically contacted or hybridized with TLR8 mRNA either *in vitro* or in a cell.

[0017] In a fourth aspect, the invention provides methods for inhibiting the expression of TLR8 in a mammal, particularly a human, such methods comprising administering to the mammal a compound or composition according to the invention.

[0018] In a fifth aspect, the invention provides a method for inhibiting a TLR8-mediated immune response in a mammal, the method comprising administering to the mammal a TLR8 antisense oligonucleotide according to the invention in a pharmaceutically effective amount.

[0019] In a sixth aspect, the invention provides a method for therapeutically treating a mammal having a disease mediated by TLR8, such method comprising administering to the mammal, particularly a human, a TLR8 antisense oligonucleotide of the invention, or a composition thereof, in a pharmaceutically effective amount.

[0020] In a seventh aspect, the invention provides methods for preventing a disease or disorder in a mammal, particularly a human, at risk of contracting or developing a disease or disorder mediated by TLR8. The method according to this aspect of the invention comprises administering to the mammal an antisense oligonucleotide according to the invention, or a composition thereof, in a prophylactically effective amount.

[0021] In an eighth aspect, the invention provides methods for down-regulating TLR8 expression and thus preventing the "off-target" activity of certain other RNA-based molecules, or other compounds or drugs that have a side effect of activating TLR8. For example, the TLR8 antisense oligonucleotide according to the invention can be administered in combination with one or more RNA-based oligonucleotides or other nucleic acid containing compounds, which are not targeted to the same target as the antisense molecule of the invention, and which comprise an immunostimulatory motif that would activate a TLR8-mediated immune response but for the presence of the TLR8 antisense oligonucleotide according to the invention.

[0022] In a ninth aspect, the invention provides a method for inhibiting TLR8 expression and activity in a mammal, comprising administering to the mammal an antisense oligonucleotide complementary to TLR8 mRNA and an antagonist of TLR8 protein, a kinase inhibitor or an inhibitor of STAT (signal transduction and transcription) protein.

[0023] The subject oligonucleotides and methods of the invention are also useful for examining the function of the TLR8 gene in a cell or in a control mammal or in a mammal afflicted with a disease associated with TLR8 or immune stimulation through TLR8. The cell or mammal is administered the oligonucleotide, and the expression of TLR8 mRNA or protein is examined.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figure 1 is a synthetic scheme for the linear synthesis of antisense oligonucleotides of the invention. DMTr = 4,4'-dimethoxytrityl; CE = cyanoethyl.

[0025] Figure 2 is a graphical representation of the activity of exemplar human TLR8 antisense oligonucleotides according to the invention in HEK293XL cells expressing human TLR8. The data demonstrate the ability of exemplar oligonucleotides according to the invention to inhibit TLR8 expression and activation in HEK293 cells that were cultured and treated according to Example 2.

[0026] Figure 3 shows the nucleotide sequence for TLR8 mRNA [SEQ. ID. NO.:223] (Genbank Accession No. AF246971; NM 138636).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0027] The invention relates to optimized TLR8 antisense oligonucleotides, compositions comprising such oligonucleotides and methods of their use for inhibiting or suppressing a TLR8-mediated immune response. The antisense oligonucleotides according to the invention are stable, specific and do not activate an innate immune response, thereby overcoming the problems of certain previously attempted approaches. Pharmaceutical and other compositions comprising the compounds according to the invention are also provided. Further provided are methods of down-regulating the expression of TLR8 in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention alone or in combination with other prophylactic or therapeutic compositions.

[0028] Specifically, the invention provides antisense oligonucleotides designed to be complementary to a genomic region or an RNA molecule transcribed therefrom. These TLR8 antisense oligonucleotides have unique sequences that target specific, particularly available mRNA sequences, resulting in maximally effective inhibition or suppression of TLR8-mediated signaling in response to endogenous and/or exogenous TLR8 ligands or TLR8 agonists.

[0029] The TLR8 antisense oligonucleotides according to the invention inhibit immune responses induced by natural or artificial TLR8 agonists in various cell types and in various in vitro and in vivo experimental models. As such, the antisense compositions according to the invention are useful as tools to study the immune system, as well as to compare the immune systems of various animal species, such as humans and mice.

[0030] Further provided are methods of treating an animal, particularly a human, having, suspected of having, or being prone to develop a disease or condition associated with TLR8 activation by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention. These can be used for immunotherapy applications such as, but not limited to, treatment of cancer, autoimmune disorders, asthma, respiratory allergies, food allergies, skin allergies, systemic lupus erythematosus (SLE), arthritis, pleurisy, chronic infections, inflammatory diseases, inflammatory bowel syndrome, sepsis, malaria, and bacteria, parasitic, and viral infections in adult and pediatric human and veterinary applications. In addition, the TLR8 antisense oligonucleotides according to the invention are also useful in the prevention and/or treatment of various diseases,

either alone, in combination with or co-administered with other drugs or prophylactic or therapeutic compositions, for example, DNA vaccines, antigens, antibodies, and allergens; and in combination with chemotherapeutic agents (both traditional chemotherapy and modern targeted therapies) and/or TLR8 antagonists for prevention and treatment of diseases. TLR8 antisense oligonucleotides of the invention are useful in combination with compounds or drugs that have unwanted TLR8-mediated immune stimulatory properties.

[0031] The patents and publications cited herein reflect the level of knowledge in the art and are hereby incorporated by reference in their entirety. Any conflict between the teachings of these patents and publications and this specification shall be resolved in favor of the latter.

[0032] The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

[0033] The term "2'-O-substituted" means substitution of the 2' position of the pentose moiety with an -O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms (for example, but not limited to, 2'-O-methyl), or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, (for example, with 2'-O-ethoxy-methyl, halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups); or with a hydroxy, an amino or a halo group, but not with a 2'-H group. In some embodiments the oligonucleotides of the invention include four or five ribonucleotides 2'-O-alkylated at their 5' terminus (i.e., 5' 2-O-alkylated ribonucleotides), and/or four or five ribonucleotides 2'-O-alkylated at their 3' terminus (i.e., 3' 2-O-alkylated ribonucleotides). In exemplar embodiments, the nucleotides of the synthetic oligonucleotides are linked by at least one phosphorothioate internucleotide linkage. The phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be stereoregular or substantially stereoregular in either Rp or Sp form (see Iyer et al. (1995) Tetrahedron Asymmetry 6:1051-1054).

[0034] The term " 3' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 3' (toward the 3'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0035] The term " 5' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 5' (toward the 5'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0036] The term "about" generally means that the exact number is not critical. Thus, oligonucleotides having one or two fewer nucleoside residues, or from one to several additional nucleoside residues are contemplated as equivalents of each of the embodiments described above.

[0037] The term "agonist" generally refers to a substance that binds to a receptor of a cell and induces a response. An agonist often mimics the action of a naturally occurring substance such as a ligand.

[0038] The term "antagonist" generally refers to a substance that attenuates the effects of an agonist.

[0039] The term "kinase inhibitor" generally refers to molecules that antagonize or inhibit phosphorylation-dependent cell signaling and/or growth pathways in a cell. Kinase inhibitors may be naturally occurring or synthetic and include small molecules that have the potential to be administered as oral therapeutics. Kinase inhibitors have the ability to rapidly and specifically inhibit the activation of the target kinase molecules. Protein kinases are attractive drug targets, in part because they regulate a wide variety of signaling and growth pathways and include many different proteins. As such, they have great potential in the treatment of diseases involving kinase signaling, including cancer, cardiovascular disease, inflammatory disorders, diabetes, macular degeneration and neurological disorders. Examples of kinase inhibitors include sorafenib (Nexavar®), Sutent®, dasatinib, Dasatinib™, Zactima™, Tykerb™ and STI571.

[0040] The term "airway inflammation" generally includes, without limitation, inflammation in the respiratory tract caused by allergens, including asthma.

[0041] The term "allergen" generally refers to an antigen or antigenic portion of a molecule, usually a protein, which elicits an allergic response upon exposure to a subject. Typically the subject is allergic to the allergen as indicated, for instance, by the wheal and flare test or any method known in the art. A molecule is said to be an allergen even if only a small subset of subjects exhibit an allergic (e.g., IgE) immune response upon exposure to the molecule.

[0042] The term "allergy" generally includes, without limitation, food allergies, respiratory allergies and skin allergies.

[0043] The term "antigen" generally refers to a substance that is recognized and selectively bound by an antibody or by a T cell antigen receptor. Antigens may include but are not limited to peptides, proteins, nucleosides, nucleotides and combinations thereof. Antigens may be natural or synthetic and generally induce an immune response that is specific for that antigen.

[0044] The term "autoimmune disorder" generally refers to disorders in which "self" antigen undergo attack by the immune system. Such term includes, without limitation, lupus erythematosus, multiple sclerosis, type I diabetes mellitus, irritable bowel syndrome, Chron's disease, rheumatoid arthritis, septic shock, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, chagas disease, chronic obstructive pulmonary disease, coeliac disease, dermatomyositis, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, interstitial cystitis, morphea, myasthenia gravis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, schizophrenia, Sjögren's syndrome, temporal arteritis ("giant cell arteritis"), vasculitis, vitiligo, vulvodynia and Wegener's granulomatosis autoimmune asthma, septic shock and psoriasis.

[0045] The term "cancer" generally refers to, without limitation, any malignant growth or tumor caused by abnormal or uncontrolled cell proliferation and/or division. Cancers may occur in humans and/or mammals and may arise in any and all tissues. Treating a patient having cancer may include administration of a compound, pharmaceutical formulation or vaccine according to the invention such that the abnormal or uncontrolled cell proliferation and/or division, or metastasis is affected.

[0046] The term "carrier" generally encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microspheres, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient, or diluent will depend on the route of administration for a particular application. The preparation of pharmaceutically acceptable

formulations containing these materials is described in, for example, *Remington's Pharmaceutical Sciences*, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA, 1990.

[0047] The term "co-administration" or "co-administered" generally refers to the administration of at least two different substances sufficiently close in time to modulate an immune response. Co-administration refers to simultaneous administration, as well as temporally spaced order of up to several days apart, of at least two different substances in any order, either in a single dose or separate doses.

[0048] The term "in combination with" generally means administering a compound according to the invention and another agent useful for treating the disease or condition that does not abolish TLR8 antisense activity of the compound in the course of treating a patient. Such administration may be done in any order, including simultaneous administration, as well as temporally spaced order from a few seconds up to several days apart. Such combination treatment may also include more than a single administration of the compound according to the invention and/or independently the other agent. The administration of the compound according to the invention and the other agent may be by the same or different routes.

[0049] The term "individual" or "subject" or "vertebrate" generally refers to a mammal, such as a human.

[0050] The term "linear synthesis" generally refers to a synthesis that starts at one end of an oligonucleotide and progresses linearly to the other end. Linear synthesis permits incorporation of either identical or non-identical (in terms of length, base composition and/or chemical modifications incorporated) monomeric units into an oligonucleotide.

[0051] The term "mammal" is expressly intended to include warm blooded, vertebrate animals, including, without limitation, humans, non-human primates, rats, mice, cats, dogs, horses, cattle, cows, pigs, sheep and rabbits.

[0052] The term "nucleoside" generally refers to compounds consisting of a sugar, usually ribose or deoxyribose, and a purine or pyrimidine base.

[0053] The term "nucleotide" generally refers to a nucleoside comprising a phosphorous-containing group attached to the sugar.

[0054] The term "modified nucleoside" generally is a nucleoside that includes a modified heterocyclic base, a modified sugar moiety, or any combination thereof. In some embodiments, the modified nucleoside is a non-natural pyrimidine or purine nucleoside, as herein described. For purposes of the invention, a modified nucleoside, a pyrimidine or purine analog or non-naturally occurring pyrimidine or purine can be used interchangeably and refers to a nucleoside that includes a non-naturally occurring base and/or non-naturally occurring sugar moiety. For purposes of the invention, a base is considered to be non-natural if it is not guanine, cytosine, adenine, thymine or uracil and a sugar is considered to be non-natural if it is not β-ribo-furanoside or 2'-deoxyribo-furanoside.

[0055] The term "modified oligonucleotide" as used herein describes an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any number of chemical groups. The term "modified oligonucleotide" also encompasses oligonucleotides having at least one nucleotide with a modified base and/or sugar, such as a 2'-O-substituted, a 5'-O-substituted and/or a 3'-O-substituted ribonucleotide.

[0056] The term "nucleic acid" encompasses a genomic region or an RNA molecule transcribed therefrom. In some embodiments, the nucleic acid is mRNA.

[0057] The term "nucleotidic linkage" generally refers to a chemical linkage to join two nucleosides through their sugars (e.g. 3'-3', 2'-3', 2'-5', 3'-5') consisting of a phosphorous atom and a charged, or neutral group (e.g., phosphodiester, phosphorothioate, phosphorodithioate or methylphosphonate) between adjacent nucleosides.

[0058] The term "oligonucleotide" refers to a polynucleoside formed from a plurality of linked nucleoside units. The nucleoside units may be part of viruses, bacteria, cell debris or oligonucleotide-based compositions (for example, siRNA and microRNA). Such oligonucleotides can also be obtained from existing nucleic acid sources, including genomic or cDNA, but are preferably produced by synthetic methods. In certain embodiments each nucleoside unit includes a heterocyclic base and a pentofuranosyl, trehalose, arabinose, 2'-deoxy-2'-substituted nucleoside, 2'-deoxy-2'-substituted arabinose, 2'-O-substitutedarabinose or hexose sugar group. The nucleoside residues can be coupled to each other by any of the

numerous known internucleoside linkages. Such internucleoside linkages include, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboalkoxy, acetamidate, carbamate, morpholino, borano, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, and sulfone internucleoside linkages. The term "oligonucleotide-based compound" also encompasses polynucleosides having one or more stereospecific internucleoside linkage (e.g., (R_P)- or (S_P)-phosphorothioate, alkylphosphonate, or phosphotriester linkages). As used herein, the terms "oligonucleotide" and "dinucleotide" are expressly intended to include polynucleosides and dinucleosides having any such internucleoside linkage, whether or not the linkage comprises a phosphate group. In certain exemplar embodiments, these internucleoside linkages may be phosphodiester, phosphorothioate or phosphorodithioate linkages, or combinations thereof.

[0059] The term "complementary to a genomic region or an RNA molecule transcribed therefrom" is intended to mean an oligonucleotide that binds to the nucleic acid sequence under physiological conditions, for example, by Watson-Crick base pairing (interaction between oligonucleotide and single-stranded nucleic acid) or by Hoogsteen base pairing (interaction between oligonucleotide and double-stranded nucleic acid) or by any other means, including in the case of an oligonucleotide, binding to RNA and causing pseudoknot formation. Binding by Watson-Crick or Hoogsteen base pairing under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence.

[0060] The term "peptide" generally refers to polypeptides that are of sufficient length and composition to affect a biological response, for example, antibody production or cytokine activity whether or not the peptide is a hapten. The term "peptide" may include modified amino acids (whether or not naturally or non-naturally occurring), where such modifications include, but are not limited to, phosphorylation, glycosylation, pegylation, lipidization and methylation.

[0061] The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of a compound according to the invention or the biological activity of a compound according to the invention.

[0062] The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. Preferably, the biological system is a living organism, such as a mammal, particularly a human.

[0063] The term "prophylactically effective amount" generally refers to an amount sufficient to prevent or reduce the development of an undesired biological effect.

[0064] The term "therapeutically effective amount" or "pharmaceutically effective amount" generally refers to an amount sufficient to affect a desired biological effect, such as a beneficial result, including, without limitation, prevention, diminution, amelioration or elimination of signs or symptoms of a disease or disorder. Thus, the total amount of each active component of the pharmaceutical composition or method is sufficient to show a meaningful patient benefit, for example, but not limited to, healing of chronic conditions characterized by immune stimulation. Thus, a "pharmaceutically effective amount" will depend upon the context in which it is being administered. A pharmaceutically effective amount may be administered in one or more prophylactic or therapeutic administrations. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0065] The term "treatment" generally refers to an approach intended to obtain a beneficial or desired result, which may include alleviation of symptoms, or delaying or ameliorating a disease progression.

[0066] In a first aspect, the invention provides antisense oligonucleotides that are complementary to a nucleic acid that is specific for human TLR8 (SEQ ID NO: 223). The antisense oligonucleotides according to the invention are optimized with respect to the targeted region of the TLR8 mRNA coding sequence or 5' untranslated region or the 3' untranslated region, in their chemical modification and/or both. In some embodiments of this aspect, the compounds are complementary to a region within nucleobases 69 through 3149 of the coding region, or 1-68 of the 5' untranslated region, or 3150-4197 of the 3' untranslated region of TLR8 mRNA. (SEQ ID NO: 223).

[0067] Antisense oligonucleotides according to the invention are useful in treating and/or preventing diseases wherein inhibiting a TLR8-mediated immune response would be beneficial.

TLR8-targeted antisense oligonucleotides according to the invention that are useful include, but are not limited to, antisense oligonucleotides comprising naturally occurring nucleotides, modified nucleotides, modified oligonucleotides and/or backbone modified oligonucleotides. However, antisense oligonucleotides that inhibit the translation of mRNA encoded proteins may produce undesired biological effects, including but not limited to insufficiently active antisense oligonucleotides, inadequate bioavailability, suboptimal pharmacokinetics or pharmacodynamics, and immune stimulation. Thus, the optimal design of an antisense oligonucleotide according to the invention requires many considerations beyond simple design of a complementary sequence. Thus, preparation of TLR8-targeted antisense oligonucleotides according to the invention is intended to incorporate changes necessary to limit secondary structure interference with antisense activity, enhance the oligonucleotide's target specificity, minimize interaction with binding or competing factors (for example, proteins), optimize cellular uptake, stability, bioavailability, pharmacokinetics and pharmacodynamics, and/or inhibit, prevent or suppress immune cell activation. Such inhibition, prevention or suppression of immune cell activation may be accomplished in a number of ways without compromising the antisense oligonucleotide's ability to hybridize to nucleotide sequences contained within the mRNA for TLR8, including, without limitation, incorporation of one or more modified nucleotides or nucleotide linkages, wherein such modified nucleotides are a 2'-O-methyl, a 3'-O-methyl, a 5—methyl, a 2'-O-methoxyethyl-C, a 2'-O-methoxyethyl-5-methyl-C and/or a 2'-O-methyl-5-methyl-C on the "C" of a "CpG" dinucleotide, a 2'-O-substituted-G, a 2'-O-methyl-G and/or a 2'-O-methoxyethoxy-G on the "G" of the CpG, and such modified nucleotide linkages are a non-phosphate or non-phosphorothioate internucleoside linkage between the C and G of a "CpG" dinucleotide, a methylphosphonate linkage and/or a 2'-5' internucleotide linkage between the C and G of a "CpG" dinucleotide.

[0068] It has been determined that the human TLR8 mRNA coding region is comprised of approximately 3.1kB, and the transcript corresponding to the 1041 amino acid protein have also been identified in humans (Chuang and Ulevitch, Eur. Cytokine Network (2000) 3:372-378). The sequence of the gene encoding TLR8 has been reported in mice (Hemmi et al., Nature (2000) 408:740-745) and for humans (Chuang and Ulevitch, Eur. Cytokine Network (2000) 3:372-378). The oligonucleotides of the invention are directed to optimally available portions of the TLR8 nucleic acid sequence that most effectively act as a target for inhibiting TLR8

expression. These targeted regions of the TLR8 gene include portions of the known exons or 5' untranslated region. In addition, intron-exon boundaries, 3' untranslated regions and introns are potentially useful targets for antisense inhibition of TLR8 expression. The nucleotide sequences of some representative, non-limiting oligonucleotides specific for human TLR8 have SEQ ID NOS: 1 - 222. The nucleotide sequences of optimized oligonucleotides according to the invention include those having SEQ ID NOS: 26, 46, 53, 84, 85, 91, 102, 116, 131, 143, 146, 152, 157, 180, 182, 189 or 197.

[0069] The oligonucleotides of the invention are composed of ribonucleotides, deoxyribonucleotides or a combination of both, with the 5' end of one nucleotide and the 3' (or in limited cases 2') end of another nucleotide being covalently linked. These oligonucleotides are at least 14 nucleotides in length, but are preferably 15 to 60 nucleotides long, preferably 20 to 50 nucleotides in length. In some embodiments, these oligonucleotides contain from about 14 to 28 nucleotides or from about 16 to 25 nucleotides or from about 18 to 22 nucleotides or 20 nucleotides. These oligonucleotides can be prepared by the art recognized methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer. The synthetic TLR8 antisense oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to TLR8 mRNA. Such modifications may include at least one internucleotide linkage of the oligonucleotide being an alkylphosphonate, phosphorothioate, phosphorodithioate, methyl phosphonate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate or carboxymethyl ester or a combination of these and other internucleotide linkages between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphodiester linkage has been replaced with any number of chemical groups.

[0070] For example, U.S. Pat. No. 5,149,797 describes traditional chimeric oligonucleotides having a phosphorothioate core region interposed between methylphosphonate or phosphoramidate flanking regions. U.S. Pat. No. 5,652,356 discloses "inverted" chimeric oligonucleotides comprising one or more nonionic oligonucleotide region (e.g. alkylphosphonate and/or phosphoramidate and/or phosphotriester internucleoside linkage) flanked by one or more region of oligonucleotide phosphorothioate. Various oligonucleotides with modified internucleotide linkages can be prepared according to standard methods. Phosphorothioate

linkages may be mixed Rp and Sp enantiomers, or they may be made stereoregular or substantially stereoregular in either Rp or Sp form according to standard procedures.

[0071] Oligonucleotides which are self-stabilized are also considered to be modified oligonucleotides useful in the methods of the invention (Tang et al. (1993) Nucleic Acids Res. 20:2729-2735). These oligonucleotides comprise two regions: a target hybridizing region; and a self-complementary region having an oligonucleotide sequence complementary to a nucleic acid sequence that is within the self-stabilized oligonucleotide.

[0072] Other modifications include those which are internal or at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesterol, cholesteryl, or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions, is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position).

[0073] Other examples of modifications to sugars include modifications to the 2' position of the ribose moiety which include but are not limited to 2'-O-substituted with an -O-alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl, or –O-allyl group having 2-6 carbon atoms wherein such -O-alkyl, -O-aryl or –O-allyl group may be unsubstituted or may be substituted, for example with halo, hydroxy, trifluoromethyl cyano, nitro acyl acyloxy, alkoxy, carboxy, carbalkoxyl or amino groups. None of these substitutions are intended to exclude the native 2'-hydroxyl group in the case of ribose or 2'1-H- in the case of deoxyribose.

[0074] US Pat No. 5,652,355 discloses traditional hybrid oligonucleotides having regions of 2'-O-substituted ribonucleotides flanking a DNA core region. U.S. Pat. No. 5,652,356 discloses an "inverted" hybrid oligonucleotide which includes an oligonucleotide comprising a 2'-O-substituted (or 2' OH, unsubstituted) RNA region which is in between two oligodeoxyribonucleotide regions, a structure that "inverted relative to the "traditional" hybrid oligonucleotides. Non-limiting examples of particularly useful oligonucleotides of the invention

have 2'-O-alkylated ribonucleotides at their 3', 5', or 3' and 5' termini, with at least four or five contiguous nucleotides being so modified. Non-limiting examples of 2'-O-alkylated groups include 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-butyls and 2'-O-ethoxy-methyl.

[0075] Other modified oligonucleotides are capped with a nuclease resistance-conferring bulky substituent at their 3' and/or 5' end(s), or have a substitution in one non-bridging oxygen per nucleotide. Such modifications can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule.

[0076] The oligonucleotides of the invention can be administered in combination with one or more antisense oligonucleotides or other nucleic acid containing compounds, which are not the same target as the antisense molecule of the invention, and which comprise an immunostimulatory motif that would activate a TLR8-mediated immune response but for the presence of the TLR8 antisense oligonucleotide according to the invention. In addition, the oligonucleotides of the invention can be administered in combination with one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, TLR antagonists, siRNA, miRNA, antisense oligonucleotides, aptamers, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants, kinase inhibitors or co-stimulatory molecules or combinations thereof.

[0077] A non-limiting list of TLR8 antisense oligonucleotides are shown in SEQ ID NO. 1 through SEQ ID NO 222 and Table 2 below. Optimized antisense oligonucleotides according to the invention include those having SEQ ID NOS: 26, 46, 53, 84, 85, 91, 102, 116, 131, 143, 146, 152, 157, 180, 182, 189 or 197. In Table 2, the oligonucleotide-based TLR8 antisense compounds have all phosphorothioate (PS) linkages. Those skilled in the art will recognize, however, that phosphodiester (PO) linkages, or a mixture of PS and PO linkages can be used.

Table 2

SEQ ID NO.	Position of Binding	Antisense Sequence Orientation is 5'-3'
1	1	TGGTACCCTC TATGCAGGAG
2	21	TAACTTGCAG CAGCGCAGAA
3	41	TGTTCTAATT TTTCATTCCG
4	61	CATGTTTTCC ATGTTTCTGT
5	81	AGCATTGACG ACTGAAGGAA
6	101	TTAGCAGGAA AATGCAGGTC
7	121	TAACTCACAG GAACCAGATA
8	141	GAAAAATTTT CTTCGGCGCA
9	161	CATCACAAGG ATAGCTTCTA

10	181	TGAGTCATTT TGCTTTTTCT
11	201	TTGCTGCACT CTGCAATAAC
12	221	GAACTTCCTG TAGTCGACGA
13	241	ATATTTGCCC ACCGTTTGGG
14	261	GACAGGTCTA GTTCTGTCAC
15	281	TGTGTGTGAT GAAATTATCA
16	301	TTGAAATGAT TCATTCGTTA
17	321	TTAGTGAGAT TTTGCAGCCC
18	341	GGTTGTGGTT TAGATTTATT
19	361	GTTCTGGTGC TGTACATTGG
20	381	GATTGTATAC CGGGATTTCC
21	401	CTGTGATATT CAAGCCATTT
22	421	TAGGTTGAGG AATGCCCCGT
23	441	AGTAACTCCC TTAGGTTTTT
24	461	GTAACTGGTT GTCTTCAAGC
25	481	CAAACCAGAG GGTATTTGGG
26	500	GTTCTGTCAA AGACTCTGGC
27	521	TATTGTTTTG AATTAGACTA
28	541	CTCTTTAGTT ATGTTGTATA
29	561	TTTATAAGTC TTGAAATGCC
30	581	CCAAATAGAG ATTTTTCAAG
31	601	GTTAAAATAG CAGTTCCAGG
32	621	TTAGTTTTCT CGCAAACTTT
33	641	CAAATACTCC ATCTTCTATG
34	661	CTCCAAATTT GTCAGCGTTT
35	681	TTGAAAGATA GTGATAGCAA
36	701	GTGGCACGTG TGAAAGAGAA
37	714	CTTGGCAGTT TGGGTGGCAG
38	721	TAGGGAGCTT GGCAGTTTGG
39	741	TTGCTCAGAA AAAGTTTGCG
40	761	TAATGTATTT GATCTGGGTG
41	781	TCCCTTGAAA TCTTCTTCAC
42	801	AGTAATGTTA AATTTATCAA
43	821	GACAGTTCCC GCTTAAATCT
44	841	TGGGGCATTG AAGCACCTCG
45	861	TCACAAGGCA CGCATGGAAA
46	870	GCACCACCAT CACAAGGCAC
47	881	TATTAATTGA AGCACCACCA
48	901	TTGAAAAGCA AAACGATCTA
49	921	TATCGAAGTT GGGTCAAGTT
50	941	AAGTGCTAGA GAGGTTTAGG
51	961	AGCATTAATC TTCCTGAGGG
52	981	GGCATATTTT TAAACCAGGC
53	998	CCAGCACCTT CAGATGAGGC
54	1001	GATCCAGCAC CTTCAGATGA
55	1021	CACTAAATAG TTGAATTCAA
56	1041	GCCCCAGAGG CTATTTCTCC
57	1061	GGGGCAGCAT CGTTAAAAAT
58	1081	CAAGTCAAGT ATTTCTAAGC
59	1101	CCCTTTATAT AGTTAAAAGA
60	1121	TAATATGCTG TGGATAACTC
61	1141	AGAGAAGTTT CTGGAAATAT
62	1161	GCCCGTAGAG ACAAAAGTTT

63	1181	CATAACCTCT TAAATGCAAT
64	1201	TTCTCTGAGT TCCTGGAACA
65	1221	ATCAGGGGCT GGAAATCATC
66	1241	TCGATAAGTT TGGAAGCTGC
67	1261	ATTAATACCC AAGTTGATAG
68	1281	AAATCGATTT GCTTAATAAA
69	1301	AGAAATTTTG GAAAAGTTTG
70	1321	GTAAATAATT TCCAGATTGG
71	1341	GATATTCTGT TTTCTGACAA
72	1361	GGGTATCTTT TACCAACGGT
73	1381	ACTATTTGCA TAACTCTGCC
74	1401	ATATGACGTT GAAAAGAGGA
75	1421	CTGTTGAGCG TCGTTTCCGG
76	1441	ATGTGGGTCA AACTCAAAAT
77	1461	GTGAAATGAT AAAAGTTCGA
78	1481	GTGGCTTTAT TAAAGGACGG
79	1501	TTTTCCATAA GCAGCACATT
80	1521	TTGAGGCTTA AATCTAAGGC
81	1541	GCCCAATGAA GAAAATACTG
82	1561	AAGATTTTCA AATTGGTTTG
83	1581	TTTAAACAGG CAATGTCAGG
84	1604	GAGCATTGCT ATTTGCAGAC
85	1620	AGTTCCACTT AACACTTGAG
86	1641	TGAGGAATGG CTGAAAATTC
87	1661	TCAAATCCAA ATATTTGACA
88	1681	AAAGTCTAGT CTATTGTTTG
89	1701	GTAAGAGCAC TAGCATTATC
90	1721	CTTCCAAGTC GGACAATTCA
91	1727	CTAGAACTTC CAAGTCGGAC
92	1741	ATTATAGCTG AGATCTAGAA
93	1761	GCTATTCTGA AATAGTGTGA
94	1781	CTAGATGATG TGTTACGCCT
95	1801	TGTGAAATTT TGAATAAATT
96	1821	AAGTTTAAAA CTTTTAGATT
97	1841	TATAAATGTT GTTGTGGCTC
98	1861	GTTATACTTA TCTGTTAAAG
99	1881	ACCAGGGACT TGCTTTCCAG
100	1901	TGCCACTGAA AACTAATTCT
101	1921	CCACAAAATG TCAAGGCGAT
102	1939	CCTGTTGTCA TCATCATTCC
103	1961	GACCTTTGAA AATGGAGATA
104	1981	CAGACGTGTC AGATTCTTGA
105	2001	AGCCTATTAA GGGATAAATC
106	2021	CTTCATTTGG GATGTGCTTC
107	2041	CGCTGGCAAA TTAAGGAATG
108	2061	ATATGTAGTT CAGTGAGACT
109	2081	ACTTTAACAT ATTATCATTT
110	2101	GAGTAATGTC CAGTTAAAAA
111	2121	TCGAGACGAG GAAACTGCTG
112	2141	TTCCACGTAA GTCAAGCAAC
113	2161	AGTTAAAAAG AGTAGTTTGT
114	2181	GTAAAGTCAG ATAGGCTATC
115	2201	GCAGTGTCCG AAGGGAAGAT

116	2212	ATGACTCAGC AGCAGTGTCC
117	2221	AATCCTGTTA TGACTCAGCA
118	2241	AAGCCAGAGG GTAGGTGGGA
119	2261	GACTACTGAC TTCAGAAAGA
120	2281	ACTTAAATCG AGGTGCTTCA
121	2301	ATTGTTTTTA GCAGATTGGA
122	2321	TTTCAAGTGC GGATTTGTTG
123	2341	TAATTTGGTG GTGGTCTTAG
124	2361	CCGTGTAGTT CCAACATAGA
125	2381	AGGTGCATTC AAAGGGGTTT
126	2401	TCGGAAATCT CCAATGTCAC
127	2421	AGATGTTCAT CCATCCATCT
128	2441	GTCTGGGAAT TTTGACATTC
129	2461	GGCACAAATG ACATCTACCA
130	2481	CCTCTTTGAT CCCCAGGACT
131	2504	GCTCCAGACT CACAATACTC
132	2521	TGAAACACAA GTTGTTAGCT
133	2541	AATATCACTG CAGTGACATC
134	2561	TAAAGAACGT GAAGAAAAAT
135	2581	CAACATAACC ATGGTGGTGA
136	2601	AAATGGTGAG CCAGGGCAGC
137	2621	ACCAAACATC CCAGTAAAAC
138	2641	TAAACACACA TTATATATAA
139	2661	CTGTAGCCTT TTACCTTAGC
140	2681	TTTGGGATGT GGAAAGAGAC
141	2701	AATGTAAGCA TCATAGAAAG
142	2721	GCATCTTTGG TGTCATAAGA
143	2727	ACAGAGGCAT CTTTGGTGTC
144	2741	TCACCCAGTC AGTAACAGAG
145	2761	GTGGTAGCGC AGCTCATTTA
146	2773	GCTCTCTTCA AGGTGGTAGC
147	2781	TTGTCTCGGC TCTCTTCAAG
148	2801	CTAGACAAAG GAGAACGTTT
149	2821	CGGATCCCAA TCCCTCTCCT
150	2841	TTGTCGATGA TGGCCAATCC
151	2861	GGTTGATGCT CTGCATGAGG
152	2867	TGCTTTGGTT GATGCTCTGC
153	2881	AAATACTGTT TTCTTGCTTT
154	2901	GCATATTTTT TGGTTAAAAC
155	2921	TTTTAAAGTT CCAGCTTTTT
156	2941	CAAAGCCAAG TAAAAAGCTG
157	2954	CCATTAGCCT CTGCAAAGCC
158	2961	TTCTCATCCA TTAGCCTCTG
159	2981	TAAATATAAT CACATCCATG
160	3001	TAACACTGGC TCCAGCAGGA
161	3005	GCTGTAACAC TGGCTCCAGC
162	3021	CTCAAATACT GAGAATGCTG
163	3041	TACAGATCCG CTGCCGTAGC
164	3061	CCACTGGAGG ATGGAGCTCT
165	3081	TCTGCCTTCG GGTTGTCAGG
166	3101	GAGTTTGCCA AAACAAGCCT
167	3121	AGTCAAGACC ACATTTCTCA
168	3141	TTATACCGTG AATCATTTTC

169	3161	TGGAATCGAC ATACATATTG
170	3181	CGTCAGTTAG TATTGCTTAA
171	3201	GGCGCGAAAT CATGACTTAA
172	3221	TTCCTTTGCA TCTTTATTAT
173	3241	TAACTAATAC AGAAATGTCA
174	3261	ATTTGTTACA TAGCAATAGA
175	3281	AACCACTAAG TTTTGGGATA
176	3301	CCAGCAAATG TGTTGTTTTA
177	3321	TGACCCTCAA AACTGTGGG
178	3341	TTATGCTGGG CTGGACTCC
179	3361	ACCCTGAGCA AGGACCCAG
180	3379	CATTGCAGCC TCTGCGACAC
181	3381	TACATTGCAG CCTCTGAGAC
182	3402	CCTATGTCTC TGGTGAACAC
183	3421	GAGTGTGACC CCAGTGATGC
184	3441	AATCCAGAAA ACAACCACAT
185	3461	CAATAGCCCA GGAGGAATTG
186	3481	ACATGAGTAT AGCCTTTGGC
187	3501	GGGAGAGGCT CGCATGGCTT
188	3521	GATGAAGCAA GCTGCCTTGT
189	3525	CTCTGATGAA GCAAGCTGCC
190	3541	CTCTCTTTTT TGCTAGCTCT
191	3561	GACTTCATCT TGCTAGCAAC
192	3581	ATTCGATTAC AAAAGATTGT
193	3601	GATGAGATAT CACTTTTTTG
194	3621	AAATAGAATA TGGCCAAAGT
195	3641	ACCTGTGGTT TACTTCTAAC
196	3661	ACTCCCATGG AGCTGGTGGG
197	3677	CTGGACTGAG GTGGTCACTC
198	3681	TTCCCTGGAC TGAGGTGGTC
199	3701	CATCTTGGTC TTCAGCTGTT
200	3721	CTGAAGCAAT CAGAGCTCAC
201	3741	GGAAAATAGT TGATGACCAA
202	3761	ATCCCAGGAC AGCAGTCAAG
203	3781	TATCATCAAG ATAGCAGGCC
204	3801	GCCTCCTGAT ATTCACAATC
205	3821	GATGGTCCAC AGTGATCCCT
206	3841	TGTGTTAGGT CAACTGCTAA
207	3861	CTTAGATATT GAAAAGAAGA
208	3881	TAGTCACAGT GGCAAAAGTT
209	3901	CAGCTTAATA TTAGGACCAT
210	3921	TATATGATAA ATATAAACAA
211	3941	TATAACCATG TAGCCATAGA
212	3961	CGAACGCAAC CACAGCATAA
213	3981	AAAGCAACTG TAAATAAAAC
214	4001	TGTTACAGCA AATATTTGTA
215	4021	TCTAAACCTT AGAAGTCAAA
216	4041	ATCTCAGTTC TTAAATGGCA
217	4061	AGATGCTTTA AAAGCTATCC
218	4081	AAAAAATGGT AAGAAGTAAA
219	4101	GAATTTAGCT GCATACTTTT
220	4121	CAATATAGAC CAAAAGCTTC
221	4141	TTTACAGCAA TGGCAATTAA

222	4161	TTTATTCATT CATTTTAAGA
224	952 (mouse)	GGAGTTCCTTCAGATTTGAC (MOUSE)
225	1562 (mouse)	GTGCCATTAAACACTTGAGT (MOUSE)
226	2153 (mouse)	TGGCTCAGTAGCAGTGTCTC (MOUSE)
227	2715 (mouse)	ACTCTCTTCAAGGTGGTAGC (MOUSE)

[0078] Underlined nucleotides are 2'-O-methylribonucleotides; all others are 2'-deoxyribonucleotides. All sequences are phosphorothioate backbone modified. In the exemplar antisense oligonucleotides according to the invention, when a "CG" dinucleotide is contained in the sequence, such oligonucleotide is modified to remove or prevent the immune stimulatory properties of the oligonucleotide.

[0079] In a second aspect, the invention provides a composition comprising at least one optimized antisense oligonucleotide according to the invention and a physiologically acceptable carrier, diluent or excipient. The characteristics of the carrier will depend on the route of administration. Such a composition may contain, in addition to the synthetic oligonucleotide and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance inhibition of TLR8 expression. For example, combinations of synthetic oligonucleotides, each of which is directed to different regions of the TLR8 mRNA, may be used in the pharmaceutical compositions of the invention. The pharmaceutical composition of the-invention may further contain nucleotide analogs such as azidothymidine, dideoxycytidine, dideoxyinosine, and the like. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic, additive or enhanced effect with the synthetic oligonucleotide of the invention, or to minimize side-effects caused by the synthetic oligonucleotide of the invention. The pharmaceutical composition of the invention may be in the form of a liposome in which the synthetic oligonucleotides of the invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. One particularly useful lipid carrier is lipofectin. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323. The pharmaceutical

composition of the invention may further include compounds such as cyclodextrins and the like that enhance delivery of oligonucleotides into cells or slow release polymers.

[0080] In a third aspect, the invention provides a method of inhibiting TLR8 expression. In this method, an oligonucleotide or multiple oligonucleotides of the invention are specifically contacted or hybridized with TLR8 mRNA either *in vitro* or in a cell.

[0081] In a fourth aspect, the invention provides methods for inhibiting the expression of TLR8 in an mammal, particularly a human, such methods comprising administering to the mammal a compound or composition according to the invention.

[0082] In a fifth aspect, the invention provides a method for inhibiting a TLR-mediated immune response in a mammal, the method comprising administering to the mammal a TLR8 antisense oligonucleotide according to the invention in a pharmaceutically effective amount, wherein routes of administration include, but are not limited to, parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form.

[0083] In a sixth aspect, the invention provides a method for therapeutically treating a mammal having a disease mediated by TLR8, such method comprising administering to the mammal, particularly a human, a TLR8 antisense oligonucleotide of the invention in a pharmaceutically effective amount.

[0084] In certain embodiments, the disease is cancer, an autoimmune disorder, airway inflammation, inflammatory disorders, infectious disease, malaria, Lyme disease, ocular infections, conjunctivitis, skin disorders, psoriasis, scleroderma, cardiovascular disease, atherosclerosis, chronic fatigue syndrome, sarcoidosis, transplant rejection, allergy, asthma or a disease caused by a pathogen. Preferred autoimmune disorders include without limitation lupus erythematosus, multiple sclerosis, type I diabetes mellitus, irritable bowel syndrome, Chron's disease, rheumatoid arthritis, septic shock, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, chagas disease, chronic obstructive pulmonary disease, coeliac disease, dermatomyositis, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, interstitial cystitis, morphea,

myasthenia gravis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, schizophrenia, Sjögren's syndrome, temporal arteritis ("giant cell arteritis"), vasculitis, vitiligo, vulvodynia and Wegener's granulomatosis. In certain embodiments, inflammatory disorders include without limitation airway inflammation, asthma, autoimmune diseases, chronic inflammation, chronic prostatitis, glomerulonephritis, Behçet's disease, hypersensitivities, inflammatory bowel disease, reperfusion injury, rheumatoid arthritis, transplant rejection, ulcerative colitis, uveitis, conjunctivitis and vasculitis.

[0085] In a seventh aspect, the invention provides methods for preventing a disease or disorder in a mammal, particularly a human, at risk of contracting or developing a disease or disorder mediated by TLR8. The method according to this aspect comprises administering to the mammal a prophylactically effective amount of an antisense oligonucleotide or composition according to the invention. Such diseases and disorders include, without limitation, cancer, an autoimmune disorder, airway inflammation, inflammatory disorders, infectious disease, malaria, Lyme disease, ocular infections, conjunctivitis, skin disorders, psoriasis, scleroderma, cardiovascular disease, atherosclerosis, chronic fatigue syndrome, sarcoidosis, transplant rejection, allergy, asthma or a disease caused by a pathogen in a mammal. Autoimmune disorders include, without limitation, lupus erythematosus, multiple sclerosis, type I diabetes mellitus, irritable bowel syndrome, Chron's disease, rheumatoid arthritis, septic shock, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, chagas disease, chronic obstructive pulmonary disease, coeliac disease, dermatomyositis, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, interstitial cystitis, morphea, myasthenia gravis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, schizophrenia, Sjögren's syndrome, temporal arteritis ("giant cell arteritis"), vasculitis, vitiligo, vulvodynia and Wegener's granulomatosis. Inflammatory disorders include, without limitation, airway inflammation, asthma, autoimmune diseases, chronic inflammation, chronic prostatitis, glomerulonephritis, Behçet's disease, hypersensitivities, inflammatory bowel disease, reperfusion injury, rheumatoid arthritis, transplant rejection, ulcerative colitis, uveitis, conjunctivitis and vasculitis.

[0086] In an eighth aspect of the invention, the invention provides methods for down-regulating TLR8 expression and thus preventing the "off-target" activity of certain other antisense molecules, or other compounds or drugs that have a side effect of activating TLR8. Certain antisense and other DNA and/or RNA-based compounds that are designed to down-regulate expression of targets other than TLR8 also are recognized by TLR8 proteins and induce an immune response. This activity can be referred to as "off-target" effects. The TLR8 antisense oligonucleotides according to the invention have the ability to down-regulate TLR8 expression and thus prevent the TLR8-mediated off-target activity of the non-TLR8 targeted antisense molecules. For example, the TLR8 antisense oligonucleotide according to the invention can be administered in combination with one or more antisense oligonucleotides, which are not the same target as the antisense molecule of the invention, and which comprise an immunostimulatory motif that would activate a TLR8-mediated immune response but for the presence the TLR8 antisense oligonucleotide according to the invention. Thus, for example, the TLR8 antisense oligonucleotide according to the invention may be administered in combination with one or more antisense oligonucleotides or RNAi molecules (for example: siRNA, miRNA, ddRNA and eiRNA), which are not targeted to the same molecule as the antisense oligonucleotides of the invention.

[0087] In a ninth aspect, the invention provides a method for inhibiting TLR8 expression and activity in a mammal, comprising administering to the mammal an antisense oligonucleotide complementary to TLR8 mRNA and an antagonist of TLR8 protein, a kinase inhibitor or an inhibitor of STAT (signal transduction and transcription) protein. According to this aspect, TLR8 expression is inhibited by the antisense oligonucleotide, while any TLR8 protein residually expressed is inhibited by the antagonist. Preferred antagonists include anti-TLR8 antibodies or binding fragments or peptidomimetics thereof, RNA-based compounds, oligonucleotide-based compounds, and/or small molecule inhibitors of TLR8 activity or of a signaling protein's activity.

[0088] In the various methods according to the invention, a therapeutically or prophylactically effective amount of a synthetic oligonucleotide of the invention and effective in inhibiting the expression of TLR8 is administered to a cell. This cell may be part of a cell culture, a neovascularized tissue culture, or may be part or the whole body of a mammal such as a human or other mammal. Administration may be by any suitable route, including, without

limitation, parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form. Administration of the therapeutic compositions of TLR8 antisense oligonucleotide can be carried out using known procedures at dosages and for periods of time effective to reduce symptoms or surrogate markers of the disease, depending on the condition and response, as determined by those with skill in the art. It may be desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the therapeutic TLR8 antisense oligonucleotides of the invention to an individual as a single treatment episode. In some exemplar embodiments of the methods of the invention described above, the oligonucleotide is administered locally and/or systemically. The term "administered locally" refers to delivery to a defined area or region of the body, while the term "systemic administration" is meant to encompass delivery to the whole organism.

[0089] In any of the methods according to the invention, one or more of the TLR8 antisense oligonucleotide can be administered either alone or in combination with any other agent useful for treating the disease or condition that does not diminish the immune modulatory effect of the TLR8 antisense oligonucleotide. In any of the methods according to the invention, the agent useful for treating the disease or condition includes, but is not limited to, one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, siRNA, miRNA, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants or kinase inhibitors to enhance the specificity or magnitude of the immune response, or co-stimulatory molecules such as cytokines, chemokines, protein ligands, trans-activating factors, peptides and peptides comprising modified amino acids. For example, in the treatment of autoimmune disease, it is contemplated that the TLR8 antisense oligonucleotide may be administered in combination with one or more targeted therapeutic agents and/or monoclonal antibodies. Alternatively, the agent can include DNA vectors encoding for antigen or allergen. In these embodiments, the TLR8 antisense oligonucleotide of the invention can produce direct immune modulatory or suppressive effects. When co-administered with one or more other therapies, the synthetic oligonucleotide of the invention may be administered either simultaneously with the other treatment(s), or sequentially.

[0090] In the various methods according to the invention the route of administration may be, without limitation, parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation,

intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form.

[0091] When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered orally, the synthetic oligonucleotide will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide or from about 1 to 50% synthetic oligonucleotide.

[0092] When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered by parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form, the synthetic antisense oligonucleotide will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. An pharmaceutical composition for parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form should contain, in addition to the synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants or other additives known to those of skill in the art.

[0093] When administered parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form, doses ranging from 0.01% to 10% (weight/volume) may be used. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil or synthetic oils may be added. Topical administration may be by liposome or transdermal time-release patch.

[0094] The amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patent has undergone. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 10 micrograms to about 20 mg of synthetic oligonucleotide per kg body or organ weight.

[0095] The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient.

[0096] Some diseases lend themselves to acute treatment while others require longer term therapy. Both acute and long term intervention in diseases are worthy goals. Injections of antisense oligonucleotides against TLR8 can be an effective means of inhibiting certain diseases in an acute situation. However for long term therapy over a period of weeks, months or years, systemic delivery (intraperitoneal, intramuscular, subcutaneous, intravenous) either with carriers such as saline, slow release polymers or liposomes are likely to be considered.

[0097] In some chronic diseases, systemic administration of oligonucleotides may be preferable. The frequency of injections is from continuous infusion to once a month, several times per month or less frequently will be determined based on the disease process and the biological half life of the oligonucleotides.

[0098] The oligonucleotides and methods of the invention are also useful for examining the function of the TLR8 gene in a cell or in a control mammal or in a mammal afflicted with a disease associated with TLR8 or immune stimulation through TLR8. In such use, the cell or mammal is administered the oligonucleotide, and the expression of TLR8 mRNA or protein is examined.

[0099] Without being limited to any theory or mechanism, it is generally believed that the activity of oligonucleotides according to the invention depends on the hybridization of the oligonucleotide to the target nucleic acid (e.g. to at least a portion of a genomic region, gene or mRNA transcript thereof), thus disrupting the function of the target. Such hybridization under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence. Thus, an exemplar oligonucleotide used in accordance with the invention is capable of forming a stable duplex (or triplex in the Hoogsteen or other hydrogen bond pairing mechanism) with the target nucleic acid; activating RNase H or other *in vivo* enzymes thereby causing effective destruction of the target RNA molecule; and is capable of resisting nucleolytic degradation (e.g. endonuclease and exonuclease activity) *in vivo*. A number of the modifications to oligonucleotides described above and others which are known in the art specifically and successfully address each of these exemplar characteristics.

[00100] In the various methods of treatment or use of the present invention, a therapeutically or prophylactically effective amount of one, two or more of the synthetic oligonucleotides of the invention is administered to a subject afflicted with or at risk of developing a disease or disorder. The antisense oligonucleotide(s) of the invention may be administered in accordance with the method of the invention either alone or in combination with other known therapies, including but not limited to, one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, siRNA, miRNA, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants or kinase inhibitors to enhance the specificity or magnitude of the immune response, or co-stimulatory molecules such as cytokines, chemokines, protein ligands, trans-activating factors, peptides and peptides comprising modified amino acids. When co-administered with one or more other therapies, the synthetic oligonucleotide of the invention may be administered either simultaneously with the other treatment(s), or sequentially.

[00101] The following examples illustrate the exemplar modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

Example 1:

Preparation of TLR8-Specific Antisense Oligonucleotides

[00102] Chemical entities according to the invention were synthesized on a 1 μmol to 0.1 mM scale using an automated DNA synthesizer (OligoPilot II, AKTA, (Amersham) and/or Expedite 8909 (Applied Biosystem)), following the linear synthesis procedure outlined in Figure 1.

[00103] 5'-DMT dA, dG, dC and T phosphoramidites were purchased from Proligo (Boulder, CO). 5'-DMT 7-deaza-dG and araG phosphoramidites were obtained from Chemgenes (Wilmington, MA). DiDMT-glycerol linker solid support was obtained from Chemgenes. 1-(2'-deoxy-β-D-ribofuranosyl)-2-oxo-7-deaza-8-methyl-purine amidite was obtained from Glen Research (Sterling, VA), 2'-O-methylribonuncleoside amidites were obtained from Promega (Obispo, CA). All compounds according to the invention were phosphorothioate backbone modified.

[00104] All nucleoside phosphoramidites were characterized by ^{31}P and ^{1}H NMR spectra. Modified nucleosides were incorporated at specific sites using normal coupling cycles recommended by the supplier. After synthesis, compounds were deprotected using concentrated ammonium hydroxide and purified by reverse phase HPLC, detritylation, followed by dialysis. Purified compounds as sodium salt form were lyophilized prior to use. Purity was tested by CGE and MALDI-TOF MS. Endotoxin levels were determined by LAL test and were below 1.0 EU/mg.

Example 2:

Cell Culture Conditions and Reagents

HEK293 Cell Culture assays for TLR8 antisense activity

[00105] HEK293 XL cells stably expressing human TLR8 (Invivogen, San Diego, CA) were plated in 48-well plates in 250 μL/well DMEM supplemented with 10% heat-inactivated FBS in a 5% CO2 incubator. At 80% confluence, cultures were transiently transfected with 400 ng/mL of the secreted form of human embryonic alkaline phosphatase (SEAP) reporter plasmid (pNifty2-Seap) (Invivogen) in the presence of 4 μL/mL of lipofectamine (Invitrogen, Carlsbad, CA) in culture medium. Plasmid DNA and lipofectamine were diluted separately in serum-free

medium and incubated at room temperature for 5 min. After incubation, the diluted DNA and lipofectamine were mixed and the mixtures were incubated further at room temperature for 20 min. Aliquots of 25 μL of the DNA/lipofectamine mixture containing 100 ng of plasmid DNA and 1 μL of lipofectamine were added to each well of the cell culture plate, and the cells were transfected for 6 h. After transfection, medium was replaced with fresh culture medium (no antibiotics), antisense compounds were added to the wells, and incubation continued for 18-20 h. Cells were then stimulated with the TLR8 agonist for 24 h.

[00106] At the end of the treatment, 20 μL of culture supernatant was taken from each well and assayed for SEAP assay by the Quanti Blue method according to the manufacturer's protocol (Invivogen). The data are shown in Figure 2 as fold increase in NF-κB activity over PBS control.

Example 3:

In vivo activity of TLR8 antisense oligonucleotide

[00107] Female C57BL/6 mice of 5-6 weeks age (N = 3/group) were injected with exemplar murine TLR8 antisense oligonucleotides according to the invention at 5 mg/kg, or PBS, subcutaneously once a day for three days. Subsequent to administration of the TLR8 antisense oligonucleotide, mice were injected with 0.25mg/kg of a TLR8 agonist subcutaneously. Two hours after administration of the TLR8 agonist, blood was collected and IL-12 concentration was determined by ELISA.

EQUIVALENTS

[00108] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. For example, antisense oligonucleotides that overlap with the oligonucleotides may be used. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

What is claimed is:

1. A synthetic antisense oligonucleotide 20 to 50 nucleotides in length targeted to TLR8 mRNA (SEQ ID NO: 223), wherein the antisense oligonucleotide has a sequence comprising SEQ ID NOs: 26, 46, 53, 84, 85, 91, 102, 116, 131, 143, 146, 152, 157, 180, 182, 189 or 197, and wherein the oligonucleotide specifically hybridizes to and inhibits the expression of human TLR8.

2. The antisense oligonucleotide of claim 1, wherein the oligonucleotide has at least one internucleotide linkage selected from the group consisting of alkylphosphonates, phosphorothioates, phosphorodithioates and methylphosphonates.

3. The antisense oligonucleotide of claim 2, wherein the oligonucleotide has at least one phosphorothioate internucleotide linkage.

4. The antisense oligonucleotide of claim 1, wherein the oligonucleotide comprises a ribonucleotide, a deoxyribonucleotide or a combination thereof.

5. The antisense oligonucleotide of claim 4, wherein the oligonucleotide comprises at least one 2'-O-substituted ribonucleotide.

6. A composition comprising a synthetic antisense oligonucleotide according to any one of claims 1-5 and a physiologically acceptable carrier.

7. A method for inhibiting the expression of TLR8, the method comprising administering a synthetic antisense oligonucleotide according to any one of claims 1-5.

8. A method for inhibiting the expression of TLR8, the method comprising administering a composition according to claim 6.

9. A method for inhibiting the expression of TLR8 in a mammal, the method comprising administering to the mammal a synthetic antisense oligonucleotide according to any one of claims 1-5.

10. A method for inhibiting the expression of TLR8 in a mammal, the method comprising administering to the mammal a composition according to claim 6.

11. A method for inhibiting a TLR8-mediated immune response in a mammal, the method comprising administering to the mammal a synthetic antisense oligonucleotide according to any one of claims 1-5 in a pharmaceutically effective amount.

12. A method for inhibiting a TLR8-mediated immune response in a mammal, the method comprising administering to the mammal a composition according to claim 6 in a pharmaceutically effective amount.

13. A method for therapeutically treating a mammal having a disease mediated by TLR8, the method comprising administering to the mammal a synthetic antisense oligonucleotide according to any one of claims 1-5 in a pharmaceutically effective amount.

14. A method for therapeutically treating a mammal having a disease mediated by TLR8, the method comprising administering to the mammal a composition according to claim 6 in a pharmaceutically effective amount.

15. A method for preventing a disease or disorder in a mammal having a disease or disorder mediated by TLR8, the method comprising administering to the mammal a synthetic antisense oligonucleotide according to any one of claims 1-5 in a prophylactically effective amount.

16. A method for preventing a disease or disorder in a mammal having a disease or disorder mediated by TLR8, the method comprising administering to the mammal a composition according to claim 6 in a prophylactically effective amount.

17. A method for down-regulating TLR8 expression and thus prevent undesired TLR8-mediated immune stimulation by a compound that activates TLR8, the method comprising administering a synthetic antisense oligonucleotide according to any one of claims 1-5 in combination with one or more compounds which comprise an immunostimulatory motif that would activate a TLR8-mediated immune response but for the presence the antisense oligonucleotide.

18. A method for down-regulating TLR8 expression and thus prevent undesired TLR8-mediated immune stimulation by a compound that activates TLR8, the method comprising administering a composition according to claim 6 in combination with one or more compounds which comprise an immunostimulatory motif that would activate a TLR8-mediated immune response but for the presence the composition.

19. The method according to any one of claims 9-16, wherein the mammal is a human.

20. The method according to any one of claims 13-16, wherein the disease is selected from cancer, an autoimmune disorder, airway inflammation, inflammatory disorders, infectious disease, malaria, Lyme disease, ocular infections, conjunctivitis, skin disorders, psoriasis, scleroderma, cardiovascular disease, atherosclerosis, chronic fatigue syndrome, sarcoidosis, transplant rejection, allergy, asthma or a disease caused by a pathogen.

21. The method according to claim 20, wherein the autoimmune disorder is selected from lupus erythematosus, multiple sclerosis, type I diabetes mellitus, irritable bowel syndrome, Chron's disease, rheumatoid arthritis, septic shock, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, chagas disease, chronic obstructive pulmonary disease, coeliac disease, dermatomyositis, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, interstitial cystitis, morphea, myasthenia gravis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis,

primary biliary cirrhosis, schizophrenia, Sjögren's syndrome, temporal arteritis ("giant cell arteritis"), vasculitis, vitiligo, vulvodynia and Wegener's granulomatosis.

22. The method according to claim 20, wherein the inflammatory disorder is selected from airway inflammation, asthma, autoimmune diseases, chronic inflammation, chronic prostatitis, glomerulonephritis, Behçet's disease, hypersensitivities, inflammatory bowel disease, reperfusion injury, rheumatoid arthritis, transplant rejection, ulcerative colitis, uveitis, conjunctivitis and vasculitis.

23. The method according to claims 17 or 18, wherein the compound is one or more non-TLR8 antisense oligonucleotides comprising an immunostimulatory motif that would otherwise activate a TLR8-mediated immune response.

24. The method according to any one of claims 7-18, wherein the route of administration is selected from parenteral, intramuscular, subcutaneous, intraperitoneal, intravenous, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, gene gun, dermal patch, eye drop or mouthwash.

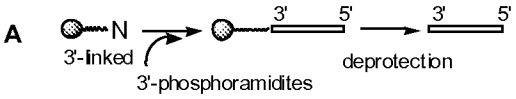
25. The method according to any one of claims 7-18, comprising further administering one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, siRNA, miRNA, antisense oligonucleotides, aptamers, proteins, gene therapy vectors, DNA vaccines, adjuvants, co-stimulatory molecules or combinations thereof.

26. A method for inhibiting TLR8 expression and activity in a mammal, comprising administering to the mammal an antisense oligonucleotide complementary to TLR8 mRNA and an antagonist of TLR8 protein.

27. The method according to claim 26, wherein the TLR 8 antagonist is selected from the group consisting of anti-TLR8 antibodies or binding fragments or peptidomimetics thereof,

RNA-based compounds, oligonucleotide-based compounds, and or small molecule inhibitors of TLR8 activity.

Linear Synthesis



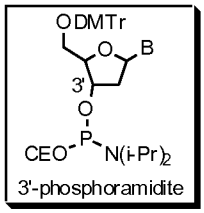


Figure 1

NF-κB activation expressed as fold control (Mean+/-SD) in human TLR8-HEK293XL cells

Treatment / SEQ ID NO.		Antisense alone	Antisense + human TLR8 agonist (50 μg/ml)	Antisense + human TLR8 agonist (100 μg/ml)
PBS		1.00+/- 0.75	15.67+/-0.39	30.08+/-1.91
53	1 μg/ml	0.86+/- 0.15	11.02+/-0.02	23.57+/-0.14
53	10 μg/ml	0.58+/- 0.00	4.55+/-0.07	10.95+/-0.07
116	1 μg/ml	3.55+/- 0.00	2.60+/-0.28	6.05+/-0.26
116	10 μg/ml	0.33+/- 0.00	1.15+/-0.31	1.87+/-0.19
146	1 μg/ml	0.30+/- 0.00	5.47+/-0.57	14.15+/-0.07
146	10 μg/ml	3.27+/- 0.00	4.23+/-0.05	8.42+/-0.02
189	1 μg/ml	1.52+/- 0.56	14.78+/-0.87	36.58+/-0.64
189	10 μg/ml	2.94+/- 0.00	8.78+/-0.54	16.63+/-0.42
197	1 μg/ml	0.74+/-0.24	6.60+/-0.28	17.48+/-0.26
197	10 μg/ml	0.39+/-0.00	4.72+/-0.21	9.55+/-0.07

Figure 2

Figure 3
SEQ ID NO 223: 4197 bp

1	CTCCTGCATA	GAGGGTACCA	TTCTGCGCTG	CTGCAAGTTA	CGGAATGAAA	AATTAGAACA
61	ACAGAAACAT	GGAAAACATG	TTCCTTCAGT	CGTCAATGCT	GACCTGCATT	TTCCTGCTAA
121	TATCTGGTTC	CTGTGAGTTA	TGCGCCGAAG	AAAATTTTTC	TAGAAGCTAT	CCTTGTGATG
181	AGAAAAAGCA	AAATGACTCA	GTTATTGCAG	AGTGCAGCAA	TCGTCGACTA	CAGGAAGTTC
241	CCCAAACGGT	GGGCAAATAT	GTGACAGAAC	TAGACCTGTC	TGATAATTTC	ATCACACACA
301	TAACGAATGA	ATCATTTCAA	GGGCTGCAAA	ATCTCACTAA	AATAAATCTA	AACCACAACC
361	CCAATGTACA	GCACCAGAAC	GGAAATCCCG	GTATACAATC	AAATGGCTTG	AATATCACAG
421	ACGGGGCATT	CCTCAACCTA	AAAAACCTAA	GGGAGTTACT	GCTTGAAGAC	AACCAGTTAC
481	CCCAAATACC	CTCTGGTTTG	CCAGAGTCTT	TGACAGAACT	TAGTCTAATT	CAAAACAATA
541	TATACAACAT	AACTAAAGAG	GGCATTTCAA	GACTTATAAA	CTTGAAAAAT	CTCTATTTGG
601	CCTGGAACTG	CTATTTTAAC	AAAGTTTGCG	AGAAAACTAA	CATAGAAGAT	GGAGTATTTG
661	AAACGCTGAC	AAATTTGGAG	TTGCTATCAC	TATCTTTCAA	TTCTCTTTCA	CACGTGCCAC
721	CCAAACTGCC	AAGCTCCCTA	CGCAAACTTT	TTCTGAGCAA	CACCCAGATC	AAATACATTA
781	GTGAAGAAGA	TTTCAAGGGA	TTGATAAATT	TAACATTACT	AGATTTAAGC	GGGAACTGTC
841	CGAGGTGCTT	CAATGCCCCA	TTTCCATGCG	TGCCTTGTGA	TGGTGGTGCT	TCAATTAATA
901	TAGATCGTTT	TGCTTTTCAA	AACTTGACCC	AACTTCGATA	CCTAAACCTC	TCTAGCACTT
961	CCCTCAGGAA	GATTAATGCT	GCCTGGTTTA	AAAATATGCC	TCATCTGAAG	GTGCTGGATC
1021	TTGAATTCAA	CTATTTAGTG	GGAGAAATAG	CCTCTGGGGC	ATTTTTAACG	ATGCTGCCCC
1081	GCTTAGAAAT	ACTTGACTTG	TCTTTTAACT	ATATAAAGGG	GAGTTATCCA	CAGCATATTA
1141	ATATTTCCAG	AAACTTCTCT	AAACTTTTGT	CTCTACGGGC	ATTGCATTTA	AGAGGTTATG
1201	TGTTCCAGGA	ACTCAGAGAA	GATGATTTCC	AGCCCCTGAT	GCAGCTTCCA	AACTTATCGA
1261	CTATCAACTT	GGGTATTAAT	TTTATTAAGC	AAATCGATTT	CAAACTTTTC	CAAAATTTCT
1321	CCAATCTGGA	AATTATTTAC	TTGTCAGAAA	ACAGAATATC	ACCGTTGGTA	AAAGATACCC
1381	GGCAGAGTTA	TGCAAATAGT	TCCTCTTTTC	AACGTCATAT	CCGGAAACGA	GCTCAACAG
1441	ATTTTGAGTT	TGACCCACAT	TCGAACTTTT	ATCATTTCAC	CCGTCCTTTA	ATAAAGCCAC
1501	AATGTGCTGC	TTATGGAAAA	GCCTTAGATT	TAAGCCTCAA	CAGTATTTTC	TTCATTGGGC
1561	CAAACCAATT	TGAAAATCTT	CCTGACATTG	CCTGTTTAAA	TCTGTCTGCA	AATAGCAATG
1621	CTCAAGTGTT	AAGTGGAACT	GAATTTTCAG	CCATTCCTCA	TGTCAAATAT	TTGGATTTGA
1681	CAAACAATAG	ACTAGACTTT	GATAATGCTA	GTGCTCTTAC	TGAATTGTCC	GACTTGGAAG
1741	TTCTAGATCT	CAGCTATAAT	TCACACTATT	TCAGAATAGC	AGGCGTAACA	CATCATCTAG
1801	AATTTATTCA	AAATTTCACA	AATCTAAAAG	TTTTAAACTT	GAGCCACAAC	AACATTTATA
1861	CTTTAACAGA	TAAGTATAAC	CTGGAAAGCA	AGTCCCTGGT	AGAATTAGTT	TTCAGTGGCA
1921	ATCGCCTTGA	CATTTTGTGG	AATGATGATG	ACAACAGGTA	TATCTCCATT	TTCAAAGGTC
1981	TCAAGAATCT	GACACGTCTG	GATTTATCCC	TTAATAGGCT	GAAGCACATC	CCAAATGAAG
2041	CATTCCTTAA	TTTGCCAGCG	AGTCTCACTG	AACTACATAT	AAATGATAAT	ATGTTAAAGT
2101	TTTTTAACTG	GACATTACTC	CAGCAGTTTC	CTCGTCTCGA	GTTGCTTGAC	TTACGTGGAA
2161	ACAAACTACT	CTTTTTAACT	GATAGCCTAT	CTGACTTTAC	ATCTTCCCTT	CGGACACTGC
2221	TGCTGAGTCA	TAACAGGATT	TCCCACCTAC	CCTCTGGCTT	TCTTTCTGAA	GTCAGTAGTC
2281	TGAAGCACCT	CGATTTAAGT	TCCAATCTGC	TAAAAACAAT	CAACAAATCC	GCACTTGAAA
2341	CTAAGACCAC	CACCAAATTA	TCTATGTTGG	AACTACACGG	AAACCCCTTT	GAATGCACCT
2401	GTGACATTGG	AGATTTCCGA	AGATGGATGG	ATGAACATCT	GAATGTCAAA	ATTCCCAGAC
2461	TGGTAGATGT	CATTTGTGCC	AGTCCTGGGG	ATCAAAGAGG	GAAGAGTATT	GTGAGTCTGG
2521	AGCTAACAAC	TTGTGTTTCA	GATGTCACTG	CAGTGATATT	ATTTTTCTTC	ACGTTCTTTA
2581	TCACCACCAT	GGTTATGTTG	GCTGCCCTGG	CTCACCATTT	GTTTTACTGG	GATGTTTGGT
2641	TTATATATAA	TGTGTGTTTA	GCTAAGGTAA	AAGGCTACAG	GTCTCTTTCC	ACATCCCAAA
2701	CTTTCTATGA	TGCTTACATT	TCTTATGACA	CCAAAGATGC	CTCTGTTACT	GACTGGGTGA
2761	TAAATGAGCT	GCGCTACCAC	CTTGAAGAGA	GCCGAGACAA	AAACGTTCTC	CTTTGTCTAG
2821	AGGAGAGGGA	TTGGGATCCG	GGATTGGCCA	TCATCGACAA	CCTCATGCAG	AGCATCAACC
2881	AAAGCAAGAA	AACAGTATTT	GTTTTAACCA	AAAAATATGC	AAAAAGCTGG	AACTTTAAAA
2941	CAGCTTTTTA	CTTGGCTTTG	CAGAGGCTAA	TGGATGAGAA	CATGGATGTG	ATTATATTTA
3001	TCCTGCTGGA	GCCAGTGTTA	CAGCATTCTC	AGTATTTGAG	GCTACGGCAG	CGGATCTGTA
3061	AGAGCTCCAT	CCTCCAGTGG	CCTGACAACC	CGAAGGCAGA	AGGCTTGTTT	TGGCAAACTC
3121	TGAGAAATGT	GGTCTTGACT	GAAAATGATT	CACGGTATAA	CAATATGTAT	GTCGATTCCA
3181	TTAAGCAATA	CTAACTGACG	TTAAGTCATG	ATTTCGCGCC	ATAATAAAGA	TGCAAAGGAA
3241	TGACATTTCT	GTATTAGTTA	TCTATTGCTA	TGTAACAAAT	TATCCCAAAA	CTTAGTGGTT
3301	TAAAACAACA	CATTTGCTGG	CCCACAGTTT	TTGAGGGTCA	GGAGTCCAGG	CCCAGCATAA
3361	CTGGGTCCTC	TGCTCAGGGT	GTCTCAGAGG	CTGCAATGTA	GGTGTTCACC	AGAGACATAG
3421	GCATCACTGG	GGTCACACTC	ATGTGGTTGT	TTTCTGGATT	CAATTCCTCC	TGGGCTATTG
3481	GCCAAAGGCT	ATACTCATGT	AAGCCATGCG	AGCCTCTCCC	ACAAGGCAGC	TTGCTTCATC
3541	AGAGCTAGCA	AAAAAGAGAG	GTTGCTAGCA	AGATGAAGTC	ACAATCTTTT	GTAATCGAAT

3601	CAAAAAAGTG	ATATCTCATC	ACTTTGGCCA	TATTCTATTT	GTTAGAAGTA	AACCACAGGT
3661	CCCACCAGCT	CCATGGGAGT	GACCACCTCA	GTCCAGGGAA	AACAGCTGAA	GACCAAGATG
3721	GTGAGCTCTG	ATTGCTTCAG	TTGGTCATCA	ACTATTTTCC	CTTGACTGCT	GTCCTGGGAT
3781	GGCCTGCTAT	CTTGATGATA	GATTGTGAAT	ATCAGGAGGC	AGGGATCACT	GTGGACCATC
3841	TTAGCAGTTG	ACCTAACACA	TCTTCTTTTC	AATATCTAAG	AACTTTTGCC	ACTGTGACTA
3901	ATGGTCCTAA	TATTAAGCTG	TTGTTTATAT	TTATCATATA	TCTATGGCTA	CATGGTTATA
3961	TTATGCTGTG	GTTGCGTTCG	GTTTTATTTA	CAGTTGCTTT	TACAAATATT	TGCTGTAACA
4021	TTTGACTTCT	AAGGTTTAGA	TGCCATTTAA	GAACTGAGAT	GGATAGCTTT	TAAAGCATCT
4081	TTTACTTCTT	ACCATTTTTT	AAAAGTATGC	AGCTAAATTC	GAAGCTTTTG	GTCTATATTG
4141	TTAATTGCCA	TTGCTGTAAA	TCTTAAAATG	AATGAATAAA	AATGTTTCAT	TTTACAA

Figure 3 cont.