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(54) **Title**: USE OF INHIBITORS OF TOLL-LIKE RECEPTORS IN THE PREVENTION AND TREATMENT OF HYPERCHOLESTEROLEMIA AND HYPERLIPIDEMIA AND DISEASES RELATED THERETO

(57) **Abstract**: The invention provides the use of TLR inhibitors or a pharmaceutically acceptable derivative thereof, optionally in combination with one or more lipid lowering composition, cholesterol lowering composition, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof for the prevention or treatment of hypercholesterolemia and/or hyperlipidemia and/or diseases associated therewith.

USE OF INHIBITORS OF TOLL-LIKE RECEPTORS IN THE PREVENTION AND TREATMENT OF HYPERCHOLESTEROLEMIA AND HYPERLIPIDEMIA AND DISEASES RELATED THERETO

This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/102,974, filed on October 6, 2008, the disclosure of which is explicitly incorporated by reference herein.

BACKGROUND OF THE INVENTION

Field of the invention

[0001] The present invention is related to the use of compounds and pharmaceutical compositions that inhibit the Toll-Like Receptor signaling pathway to prevent or treat diseases or disorders. The invention further relates to the treatment of hypercholesterolemia and hyperlipidemia and diseases related thereto.

Summary of the related art

[0002] Due to their insolubility in blood, cholesterol and lipids are transported in the circulatory system in lipoproteins. Cholesterol and lipid in lipoproteins can be deposited in tissues throughout the circulatory system. High concentrations of cholesterol (hypercholesterolemia) and/or lipid (hyperlipidemia) in the circulatory system are conditions known to be associated with many diseases, including, but not limited to, coronary heart disease, arteriosclerosis, atherosclerosis, stroke, peripheral vascular disease, diabetes and high blood pressure. It has been established that lowering low density lipoprotein lipid and/or low density lipoprotein cholesterol concentration in the blood is beneficial for protecting against diseases associated with high blood concentrations of cholesterol and/or lipids. It has been further established that increasing the concentration of high density lipoprotein lipid and/or high density lipoprotein cholesterol concentration in relation to the concentration of low density lipoprotein lipid and/or low density lipoprotein cholesterol concentration in the blood is beneficial for protecting against diseases associated with high blood concentrations of cholesterol and/or lipids.

[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 eleven proteins called TLR1 to TLR11, which are known to recognize pathogen associated molecular patterns (PAMPs) from bacteria, fungi, parasites and viruses and induce an immune response mediated by a number of transcription factors (Poltorak, A. *et al.* (1998) *Science* 282:2085-2088; Underhill, D.M., *et al.* (1999) *Nature* 401:811-815; Hayashi, F. *et al.* (2001) *Nature* 410:1099-1103; Zhang, D. *et al.* (2004) *Science* 303:1522-1526; Meier, A. *et al.* (2003) *Cell. Microbiol.* 5:561-570; Campos, M.A. *et al.* (2001) *J. Immunol.* 167: 416-423; Hoebe, K. *et al.* (2003) *Nature* 424: 743-748; Lund, J. (2003) *J. Exp. Med.* 198:513-520; Heil, F. *et al.* (2004) *Science* 303:1526-1529; Diebold, S.S., *et al.* (2004) *Science* 303:1529-1531; Hornung, V. *et al.* (2004) *J. Immunol.* 173:5935-5943). TLRs are known to be a key means by which mammals recognize and mount an immune response to foreign molecules and are also recognized as providing 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).

[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 "CpG" motifs; DNA-immunoglobulin complexes	Monocytes/macrophages, Plasmacytoid dendritic cells, B lymphocytes

[0005] 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.

[0006] As a result of their involvement in regulating an inflammatory response, activation of TLRs has 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). As a result of their role in inflammation, it is recognized that down-regulating TLR expression and/or activity may provide a useful means for disease intervention.

[0007] To date, investigative strategies aimed at selectively inhibiting TLR activity have involved small molecules (*e.g.*, chloroquine and hydroxychloroquine) (see, for example, WO 2005/007672 and Krieg, A. M. (2002) *Annu. Rev. Immunol.* 20:709), antibodies (see, for example, Duffy, K. *et al.* (2007) *Cell Immunol.* 248:103-114), catalytic RNAi technologies (*e.g.*, small inhibitory RNAs), cyclohexene derivatives (Il *et al.* (2006) *Mol. Pharmcol.* 69:1288-1295), lipid derivatives (Akira *et al.* (2005) *Circulation* 114:270-274, oligonucleotides containing poly-G sequences (Pawar *et al.* (2007) *J. Am. Soc. Nephrol.* 18:1721-1731) and competitive inhibition with methylated or modified oligonucleotides (see, for example, Barrat and Coffman (2008) Immunol. Rev. 223:271-283). Passages of these publications disclosing TLR inhibitors are specifically incorporated by reference.

[0008] As a result of their ability to inhibit a TLR-mediated inflammatory response, TLR antagonists are currently being investigated as possible therapeutics for the treatment and/or prevention of certain diseases. However, the role of TLRs in regulating blood cholesterol concentration and/or blood lipid concentration and/or the diseases associated therewith was heretofore unknown.

BRIEF SUMMARY OF THE INVENTION

[0009] The present inventors have surprisingly discovered that inhibition of TLR pathway signaling can lower blood cholesterol concentration and/or blood lipid concentration and/or increase the ratio of HDL-C to LDL-C in a mammal having elevated blood cholesterol and/or blood lipid.

[0010] Thus, in a first aspect, the invention provides methods for lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid by inhibiting TLR signaling. In certain preferred embodiments, TLR signaling is inhibited by inhibiting the expression or activity of one or more TLR or a downstream protein in the TLR signaling pathway, such as the myeloid differentiation marker 88 (MyD88), IL-1R-associated kinase (IRAK), interferon regulating factor (IRF), TNF-receptor-associated factor (TRAF), transforming growth factor beta (TGFβ)-activated kinase1, IκB kinases, IκB, and NF-κB. Thus, in some

embodiments inhibition of TLR signaling is achieved by administering to a mammal having elevated blood cholesterol and/or blood lipid a TLR antagonist compound. Certain of these TLR antagonist compounds or compositions are small molecules, for example, but not limited to chloroquine or hydroxychloroquine; antibodies; cyclohexene derivatives; lipid derivatives; synthetic oligonucleotides comprising two triplet sequences, a "CCT" triplet and a "GGG" triplet, in which the "CCT" triplet may be considered proximal and the "GGG" triplet may be considered distal; synthetic oligonucleotides comprising regions that contain "GGG" and/or "GGGG" and/or "GC" sequences, in which multiple of such sequences may be present; synthetic oligonucleotides that are methylated; or synthetic, immune inhibitory oligonucleotides, which may have one or more chemical modifications in the sequence flanking an immune stimulatory motif and/or in an oligonucleotide motif that would be immune stimulatory but for the modification. In some embodiments, TLR signaling is inhibited by inhibiting the expression of one or more TLR or a downstream protein in the TLR signaling pathway, such as MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB, and NF-κB using gene expression blocking technologies such as antisense oligonucleotides, decoy RNAs, ribozymes, catalytic RNAi technologies, siRNA or miRNA.

[0011] In some preferred embodiments, the method of lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid comprises administering to the mammal a TLR antagonist composition having the structure 5'-N_m – $N_3N_2N_1CGN^1N^2N^3$ – N^m -3', wherein CG is an oligonucleotide motif and C is cytosine or a pyrimidine nucleotide derivative, and G is guanosine or a purine nucleotide derivative; N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide or nucleotide derivative; N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage; provided that at least one N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative wherein the oligonucleotide motif would be immune stimulatory but for the nucleotide derivative; and wherein m is an integer from 0 to about 30.

[0012] In a further embodiment, the invention provides a method of lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid comprising administering to the mammal a TLR antagonist composition having the structure 5'-N_m – $N_3N_2N_1CGN^1N^2N^3$ – N^m -3', wherein CG is an oligonucleotide motif and C is cytosine or a pyrimidine nucleotide

derivative, and G is guanosine or a purine nucleotide derivative; N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide or a nucleotide derivative; N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage; provided that at least one N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative; and further provided that compound may contain less than 3 consecutive guanosine nucleotides wherein the oligonucleotide motif would be immune stimulatory but for the nucleotide derivative or non-nucleotide linkage; and wherein m is an integer from 0 to about 30.

[0013] In another embodiment, the invention provides for a method of lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid through administration of a pharmaceutical composition comprising one or more TLR antagonist compounds or compositions and a pharmaceutically acceptable carrier.

[0014] In some preferred embodiments, lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid comprises administering one or more TLR antagonists compounds, wherein the TLR is selected from TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.

[0015] In a second aspect, the invention provides a method for therapeutically treating a disease associated with high blood lipid concentration and/or high blood cholesterol concentration in a mammal, such method comprising inhibiting TLR signaling in the mammal. In some embodiments, TLR signaling is inhibited by administering to the mammal one or more TLR antagonist compounds according to the invention in a pharmaceutically effective amount. In some embodiments TLR signaling is inhibited by inhibiting the expression of one or more TLR, or another protein in the TLR signaling pathway in a pharmaceutically effective amount. In certain preferred embodiments of this aspect of the invention, the disease is hypercholesterolemia, hyperlipidemia, coronary heart disease, arteriosclerosis, atherosclerosis, stroke, peripheral vascular disease, diabetes or high blood pressure.

[0016] In a third aspect, the invention provides a method for preventing a disease associated with high blood lipid concentration and/or blood cholesterol concentration in a mammal having elevated blood cholesterol and/or blood lipid, such method comprising inhibiting TLR signaling in the mammal. In some embodiments, TLR signaling is inhibited by administering to the mammal one or more TLR antagonist compounds

according to the invention in a pharmaceutically effective amount. In some embodiments TLR signaling is inhibited by inhibiting the expression of one or more TLR, or another protein in the TLR signaling pathway. In certain preferred embodiments, the disease is hypercholesterolemia, hyperlipidemia, coronary heart disease, arteriosclerosis, atherosclerosis, stroke, peripheral vascular disease, diabetes or high blood pressure.

[0017] In some embodiments, the methods according to the invention further comprise administering to the mammal one or more cholesterol lowering compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1a depicts the total serum cholesterol in animals treated according to Example 2. Briefly, animals were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg), PBS or Lipitor® (20 mg/kg) for 12 weeks. Blood samples were collected over the course of the twelve-week study and used for determining total serum cholesterol. These data demonstrate that administration of a TLR antagonist can inhibit a rise in total serum cholesterol following *in vivo* administration.

[0019] Figure 1b depicts the percent change in total serum cholesterol over time, in animals treated according to Example 2. Briefly, animals were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg), PBS or Lipitor® (20 mg/kg) for 12 weeks. Blood samples were collected over the course of the twelve-week study and used for determining total serum cholesterol. These data demonstrate that a TLR antagonist can inhibit a rise in total serum cholesterol following *in vivo* administration.

[0020] Figure 2a depicts total serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), glucose, and the ratio of HDL-cholesterol to LDL-cholesterol (HDL-C/LDL-C) in animals treated according to Example 3. Briefly, C57/BL/6 mice were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg) or PBS for 12 weeks. Blood samples were collected over the course of the twelve-week study and used for determining total serum cholesterol, HDL, LDL, TG and glucose concentrations. These data demonstrate that a TLR antagonist can inhibit

a rise in total serum cholesterol, LDL and TG and maintain or lower blood glucose concentrations following *in vivo* administration.

[0021] Figure 2b depicts the percent changes in total serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), and glucose in animals treated according to Example 3.

[0022] Figure 2c depicts total serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), glucose, and the ratio of HDL-cholesterol to LDL-cholesterol (HDL-C/LDL-C) in animals treated according to Example 3. Briefly, ApoE-deficient mice were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg) or PBS for 12 weeks. Blood samples were collected over the course of the twelve-week study and used for determining total serum cholesterol, HDL, LDL, TG and glucose concentrations. These data demonstrate that a TLR antagonist can inhibit a rise in total serum cholesterol, LDL, TG and maintain or lower blood glucose concentrations following *in vivo* administration to mammals with a predisposition to developing hypercholesterolemia and/or hyperlipidemia.

[0023] Figure 2d depicts the percent changes in total serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), and glucose in animals treated according to Example 3.

[0024] Figure 3a depicts total body weight in animals treated according to Example 3. Briefly, C57/BL/6 mice were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg) or PBS for 12 weeks. Body weight was measured over the course of the twelve-week study. These data demonstrate that a TLR antagonist can inhibit a rise in total body weight following *in vivo* administration to mammals fed a high fat diet.

[0025] Figure 3b depicts total body weight in animals treated according to Example 3. Briefly, ApoE-deficient mice were fed a Western diet and administered a TLR antagonist (5 mg/kg or 20 mg/kg) or PBS for 12 weeks. Body weight was measured over the course of the twelve-week study. These data demonstrate that a TLR antagonist can inhibit a rise in total body weight following *in vivo* administration to mammals with a predisposition to developing hypercholesterolemia and/or hyperlipoproteinemia that are fed a high fat diet.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0026] The present invention relates to the therapeutic use of TLR antagonists to inhibit and/or suppress hypercholesterolemia and/or hyperlipidemia and/or the diseases associated therewith. The present inventors have surprisingly discovered that inhibition of TLR pathway signaling can lower blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid.

[0027] Thus, in a first aspect, the invention provides methods for lowering blood cholesterol concentration and/or blood lipid concentration in a mammal having elevated blood cholesterol and/or blood lipid by inhibiting TLR signaling. In certain preferred embodiments, TLR signaling is inhibited by inhibiting the expression or activity of one or more TLR or a downstream protein in the TLR signaling pathway, such as MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB, and NF-κB. Thus, in some embodiments inhibition of TLR signaling is achieved by administering to a mammal having elevated blood cholesterol and/or blood lipid a TLR antagonist compound. Certain of these TLR antagonist compounds or compositions are small molecules that down-regulate maturation of endosomes, for example, but not limited to chloroquine or hydroxylchloroquine; antibodies; cyclohexene derivatives; lipid derivatives; synthetic oligonucleotides comprising two triplet sequences, a "CCT" triplet and a "GGG" triplet, in which the "CCT" triplet may be considered proximal and the "GGG" triplet may be considered distal; synthetic oligonucleotides comprising regions that contain "GGG" and/or "GGGG" and/or "GC" sequences, in which multiple of such sequences may be present; synthetic oligonucleotides that are methylated; or synthetic, immune inhibitory oligonucleotides, which may have one or more chemical modifications in the sequence flanking an immune stimulatory motif and/or in an oligonucleotide motif that would be immune stimulatory but for the modification. In some embodiments, TLR signaling is inhibited by inhibiting the expression of one or more TLR or a downstream protein in the TLR signaling pathway, such as MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB, and/or NF-κB using gene expression blocking technologies such as antisense oligonucleotides, decoy RNAs, ribozymes, catalytic RNAi technologies, siRNA or miRNA. In certain embodiments wherein TLR activity or expression is inhibited, the TLR is selected from TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and/or TLR9.

[0028] Furthermore, the present invention provides TLR antagonist compounds having optimal levels of blood cholesterol concentration and/or blood lipid concentration lowering activity and methods for making and using such compounds.

[0029] In addition, inhibition of TLR signaling according to the invention is useful in combination with one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof for preventing and/or treating diseases.

[0030] Subjects in need of preventing an increase of blood cholesterol and/or lipid concentration and/or treatment of a high blood cholesterol concentration and/or high blood lipid concentration would include, for example, those at risk of having a cardiovascular event.

[0031] The term "oligonucleotide" generally refers to a polynucleoside comprising a plurality of linked nucleoside units. Such oligonucleotides can be obtained from existing nucleic acid sources, including genomic or cDNA, but are preferably produced by synthetic methods. In exemplar embodiments each nucleoside unit can encompass various chemical modifications and substitutions as compared to wild-type oligonucleotides, including but not limited to modified nucleoside base and/or modified sugar unit. Examples of chemical modifications are known to the person skilled in the art and are described, for example, in Uhlmann E *et al.* (1990) *Chem. Rev.* 90:543; "Protocols for Oligonucleotides and Analogs" *Synthesis and Properties & Synthesis and Analytical Techniques*, S. Agrawal, ed., Humana Press, Totowa, USA 1993; Hunziker, J. *et al.* (1995) *Mod. Syn. Methods* 7:331-417; and Crooke, S. *et al.* (1996) *Ann. Rev. Pharm. Tox.* 36:107-129. 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, 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" 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, respectively, having any such internucleoside linkage, whether or not the linkage comprises a phosphate group. In certain preferred embodiments, these internucleoside linkages may be phosphodiester, phosphorothioate or phosphorodithioate linkages, or combinations thereof.

[0032] The term "2'-substituted" generally includes nucleosides or in which the hydroxyl group at the 2' position of the pentose moiety is substituted to produce a 2'-substituted or 2'-O-substituted nucleoside. In certain embodiments, such substitution is with a lower hydrocarbyl group containing 1-6 saturated or unsaturated carbon atoms, with a halogen atom, or with an aryl group having 6-10 carbon atoms, wherein such hydrocarbyl, or aryl group may be unsubstituted or may be substituted, for example, but not limited to substitution with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carboalkoxy or amino groups. Examples of 2'-O-substituted nucleosides include, without limitation 2'-amino, 2'-fluoro, 2'-allyl, 2'-O-alkyl and 2'-propargyl ribonucleosides or arabinosides, 2'-O-methylribonucleosides or 2'-O-methylarabinosides and 2'-O-methoxyethoxyribonucleosides or 2'-O-methoxyethoxyarabinosides.

[0033] The term " 3' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 3' (downstream) from another region or position in the same polynucleotide or oligonucleotide.

[0034] The term " 5' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 5' (upstream) from another region or position in the same polynucleotide or oligonucleotide.

[0035] The term "about" generally means that the exact number is not critical. Thus, the number of nucleoside residues in the oligonucleotides according to the invention is not critical, and 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.

[0036] The term "agonist" generally refers to a substance that can bind to a receptor and induce a response. Such response may be an increase in the activity mediated by the receptor. An agonist often mimics the action of a naturally occurring substance such as a ligand.

[0037] The term "antagonist" generally refers to a substance that can bind to a receptor, but does not produce a biological response upon binding. The antagonist can block, inhibit or attenuate the response mediated by an agonist and may compete with agonists for binding to a receptor. Such antagonist activity may be reversible or irreversible.

[0038] The term "antisense oligonucleotide" generally refers to strands of DNA or RNA or combinations thereof that are complementary to a chosen nucleic acid sequence. Such nucleic acid sequence may be in the form of messenger RNA (mRNA). When introduced into an animal or cell, an antisense oligonucleotide can bind to and cause the reduction in the translation of RNA to which it is complementary. If binding takes places, this nucleic acid complex can be degraded by endogenous enzymes. Antisense oligonucleotides include, but are not limited to, traditional antisense oligonucleotides, short interfering RNA (siRNA), micro RNA (miRNA) and ribozymes. Antisense oligonucleotides that would be useful according to the invention include but are not limited to those in Kandimalla *et al.* (U.S. Patent Application Nos. 12/510,469; 12/534,462; 12/534,476; 12/534,911; and 12/537,354; and U.S. Provisional Patent Application Nos. 61/111,143; 61/111,148; and 61/111,160).

[0039] The term "small molecule" generally refers to small organic compounds that are biologically active but are not polymers. Small molecules may exist naturally or may be created synthetically. Generally, small molecules do not include proteins or oligonucleotides. Small molecules may include compounds that down-regulate the maturation of endosomes, for example, but not limited to, chloroquine and hydroxychloroquine.

[0040] The term "physiologically acceptable" generally refers to a material that does not interfere with the effectiveness of the TLR antagonist compound and 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.

[0041] The term "pharmaceutically acceptable" generally refers to compositions that are suitable for use in humans and animals without undue toxicity.

[0042] 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, *e.g.*, *Remington's Pharmaceutical Sciences*, 18th Edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990.

[0043] The term "co-administration" 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.

[0044] The terms an "effective amount," "pharmaceutically effective amount" or "therapeutically effective amount" generally refer to an amount sufficient to affect a desired biological effect, such as beneficial results. Thus, an "effective amount" or "sufficient amount" or "pharmaceutically effective amount" or "therapeutically effective amount" will depend upon the context in which it is being administered. In the context of administering a composition that modulates an immune response to a co-administered antigen, an effective amount of a TLR antagonist compound and antigen is an amount sufficient to achieve the desired modulation as compared to the immune response obtained when the antigen is administered alone. An effective amount may be administered in one or more administrations.

[0045] The term "in combination with" generally means in the course of treating a disease or disorder in a patient, administering a TLR antagonist compound and an agent useful for treating the disease or disorder that does not diminish the immune modulatory effect of the TLR antagonist compound. Such combination treatment may also include more than a single administration of a TLR antagonist compound and/or independently an agent. The administration of the TLR antagonist compound and/or the agent may be by the same or different routes.

[0046] The term "individual" or "patient" or "subject" or "mammal" includes a human. Mammals generally include, but are not limited to, humans, non-human primates, rats, mice, cats, dogs, horses, cattle, cows, pigs, sheep and rabbits.

[0047] The term "nucleoside" generally refers to compounds consisting of a sugar, usually ribose or deoxyribose, and a purine or pyrimidine base.

[0048] The term "nucleotide" generally refers to a nucleoside comprising a phosphate group attached to the sugar.

[0049] As used herein, the term "pyrimidine nucleoside" refers to a nucleoside wherein the base component of the nucleoside is a pyrimidine base (*e.g.*, cytosine (C) or thymine (T) or uracil (U)). Similarly, the term "purine nucleoside" refers to a nucleoside wherein the base component of the nucleoside is a purine base (*e.g.*, adenine (A) or guanine (G)).

[0050] The terms "analog" or "derivative" can be used interchangeable to generally refer to any purine and/or pyrimidine nucleotide or nucleoside that has a modified base and/or sugar. A modified base is a base that is not guanine, cytosine, adenine, thymine or uracil. A modified sugar is any sugar that is not ribose or 2' deoxyribose that can be used in the backbone for an oligonucleotide.

[0051] The term "inhibiting" or "suppressing" generally refers to a decrease in a response or qualitative difference in a response, which could otherwise arise from eliciting and/or stimulation of a response.

[0052] The term "non-nucleotide linker" generally refers to any linkage or moiety that can link or be linked to the oligonucleotides other than through a phosphorous-containing linkage. Preferably such linker is from about 2 angstroms to about 200 angstroms in length.

[0053] The term "nucleotide linkage" generally refers to a direct 3'-5' linkage that directly connects the 3' and 5' hydroxyl groups of two nucleosides through a phosphorous-containing linkage.

[0054] The term "oligonucleotide motif" means an oligonucleotide sequence, including a dinucleotide. An "oligonucleotide motif that would be immune stimulatory, but for one or more modifications [or specifically recited modifications]" means an oligonucleotide motif that is immune stimulatory in a parent oligonucleotide, but not in a derivative oligonucleotide, wherein the derivative oligonucleotide is derived from the parent oligonucleotide by one or more modifications of the parent oligonucleotide.

[0055] The terms CpG, C*pG, C*pG* and CpG* refer to oligonucleotide motifs that are immune stimulatory and comprise cytosine or a cytosine analog and a guanine or a guanine analog.

[0056] The term "hypercholesterolemia" generally refers to the presence of high levels of cholesterol in the blood. Hypercholesterolemia can be secondary to many diseases and can contribute to many other diseases, including but not limited to cardiovascular disease, atherosclerosis and pancreatitis. Elevated blood cholesterol can

be due to elevated levels of lipoproteins, the particles that carry cholesterol and lipids (for example triglycerides) in the bloodstream. Lipoproteins include, for example but not limited to, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotien (VLDL).

[0057] The term "hyperlipidemia" generally refers to the presence of elevated or abnormal levels of triglyceride (TG), lipids or lipoproteins in the blood. Elevated or abnormal levels of triglyceride, lipid and lipoprotein are common in the general population. Substantial effort is exerted to normalize the triglyceride, lipid and lipoprotein levels in patients due to the influence of lipid and cholesterol on cardiovascular and other diseases.

[0058] The term "statin" generally refers to a class of drugs used to lower cholesterol levels in people with or at risk of developing cardiovascular disease. These compounds lower cholesterol by inhibiting the enzyme HMG-CoA reductase, the rate-limiting enzyme in the pathway of cholesterol synthesis. Many statins are well known in the art and include, without limitation atorvastatin, cerivastatin (rivastatin), fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.

[0059] The term "treatment" generally refers to an approach intended to obtain a beneficial or desired results, which may include alleviation of symptoms or delaying or ameliorating a disease progression. As such, and without limitation, "treatment" includes palliative treatment and beneficial results that are temporary (*i.e.*, not permanent). For example, cholesterol levels may decrease during treatment according to the invention but eventually increase once treatment is terminated.

[0060] In some preferred embodiments, the oligonucleotide comprises an oligonucleotide motif and at least one modification, wherein the oligonucleotide motif would be immune stimulatory (*e.g.*, unmethylated CpG), but for the one or more modifications that suppress the activity of the oligonucleotide motif, provided that compound may contain less than 4 consecutive guanosine nucleotides and preferably less than 3 consecutive guanosine nucleotides. Such modifications may be in the oligonucleotide 5' terminus, in a sequence flanking the oligonucleotide motif, and/or within the oligonucleotide motif. These modifications result in a TLR antagonist compound that suppresses TLR-modulated immune stimulation. Such modifications can be to the bases, sugar residues and/or the phosphate backbone of the

nucleotides/nucleosides flanking the oligonucleotide motif or within the oligonucleotide motif. In some embodiments, the modification is a 2'-substitution.

[0061] In some embodiments, when the modification is a 2' alkylation or alkoxylation then the modification is not 5' adjacent to the oligonucleotide motif; when the modification is a non-charged internucleoside linkage then the modification is not 5' adjacent to the oligonucleotide motif; and when the modification is a 3' alkylation or alkoxylation then the modification is not 5' or 3' adjacent to the oligonucleotide motif.

[0062] In other embodiments of the invention, the TLR antagonist compound is an antisense oligonucleotide.

[0063] In some preferred embodiments, the general structure of an oligonucleotide-based TLR antagonist may be represented as, but is not limited to, 5'-N_m – $N_3N_2N_1CGN^1N^2N^3$ – N^m -3' wherein CG is an immune stimulatory motif and C is cytosine or a pyrimidine nucleotide derivative, and G is guanosine or a purine nucleotide derivative; N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide or a nucleotide derivative; N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linker; provided that at least one N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative; and further provided that compound may contain less than 4 consecutive guanosine nucleotides and preferably less than 3 consecutive guanosine nucleotides, wherein the immune stimulatory activity of the CG is suppressed by the nucleotide derivative or non-nucleotide linker; and wherein m is an integer from 0 to about 30. Such oligonucleotide-based TLR antagonists are disclosed in U.S. Application No. 11/549,048 (U.S. Patent Appliction Publication No. 2009/0060898), the disclosure of which is explicitly incorporated by reference herein (to the extent that there are any inconsistencies between the instant application and U.S. Application No. 11/549,048, such inconsistencies shall be resolved in accordance with the instant application).

[0064] In additional preferred embodiments, the oligonucleotide-based TLR antagonist may contain a modified immune stimulatory motif and may be represented as, but is not limited to, the structure 5'-N_m – $N_3N_2N_1CGN^1N^2N^3$ – N^m -3', wherein CG is the modified immune stimulatory motif and C is cytosine, or a pyrimidine nucleotide derivative selected from 5-methyl-dC, 2'-O-substituted-C, 2'-O-methyl-C, 2'-O-methoxyethyl-C, 2'-O-methoxyethyl-5-methyl-C, and 2'-O-methyl-5-methyl-C, and G is guanosine or a purine nucleotide derivative selected from 2'-O-substituted-G, 2'-O-

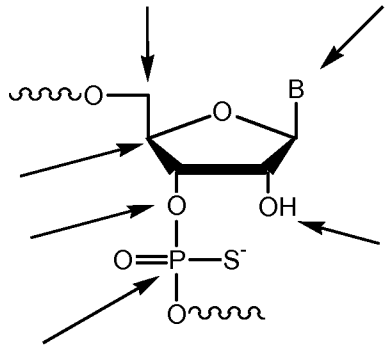
methyl-G, and 2'-O-methoxyethyl-G; N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage; N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage; provided that at least one C and/or G of the modified immune stimulatory motif is a nucleotide derivative specified above; and optionally containing less than 3 consecutive guanosine nucleotides; wherein the modified immune stimulatory motif would be immune stimulatory but for the nucleotide derivative; and wherein m is an integer from 0 to about 30. Such oligonucleotide-based TLR antagonists are disclosed in U.S. Provisional Application No. 61/182,928, the disclosure of which is explicitly incorporated by reference herein (to the extent that there are any inconsistencies between the instant application and U.S. Provisional Application No. 61/182,928, such inconsistencies shall be resolved in accordance with the instant application).

[0065] In further embodiments of this aspect of the invention, the oligonucleotide-based TLR antagonist containing a modified immune stimulatory motif comprises one or more modified immune stimulatory motifs, wherein CG is the modified immune stimulatory motif and C is cytosine, or a pyrimidine nucleotide derivative selected from 5-methyl-dC, 2'-O-substituted-C, 2'-O-methyl-C, 2'-O-methoxyethoxy-C, 2'-O-methoxyethyl-5-methyl-C, 2'-O-methyl-5-methyl-C, and G is guanosine or a purine nucleotide derivative selected from 2'-O-substituted-G, 2'-O-methyl-G, and 2'-O-methoxyethoxy-G; provided that at least one C and/or G of the modified immune stimulatory motif is a nucleotide derivative specified above; and optionally containing less than 3 consecutive guanosine nucleotides; wherein the modified immune stimulatory motif would be immune stimulatory but for the nucleotide derivative.

[0066] In certain embodiments of the invention, the TLR antagonist compounds may comprise at least two oligonucleotides covalently linked by a nucleotide linkage, or a non-nucleotide linker, at their 5'-, 3'- or 2'-ends or by functionalized sugar or by functionalized nucleobase via a non-nucleotide linker or a nucleotide linkage. Such TLR antagonist compounds may be linear or branched. As a non-limiting example, the linker may be attached to the 3'-hydroxyl. In such embodiments, the linker comprises a functional group, which is attached to the 3'-hydroxyl by means of a phosphate-based linkage like, for example, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, or by non-phosphate-based linkages. Possible sites of conjugation

for the nucleotide are indicated in Formula I, below, wherein B represents a heterocyclic base and wherein the arrow pointing to P indicates any attachment to phosphorous.

Formula I



[0067] In some embodiments, the non-nucleotide linker is a small molecule linker, macromolecule or biomolecule, including, without limitation, polypeptides, antibodies, lipids, antigens, allergens, and oligosaccharides. In some other embodiments, the non-nucleotidic linker is a small molecule linker. For purposes of the invention, a small molecule linker is an organic moiety having a molecular weight of less than 1,000 Da. In some embodiments, the small molecule has a molecular weight of less than 750 Da.

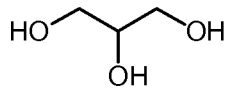
[0068] In some embodiments, the small molecule linker is an aliphatic or aromatic hydrocarbon, either of which optionally can include, either in the linear chain connecting the oligoribonucleotides or appended to it, one or more functional groups including, but not limited to, hydroxy, amino, thiol, thioether, ether, amide, thioamide, ester, urea, or thiourea. The small molecule linker can be cyclic or acyclic. Examples of small molecule linkers include, but are not limited to, amino acids, carbohydrates, cyclodextrins, adamantane, cholesterol, haptens and antibiotics. However, for purposes of describing the non-nucleotidic linker, the term "small molecule linker" is not intended to include a nucleoside.

[0069] In some embodiments, the non-nucleotidic linker is an alkyl linker or amino linker. The alkyl linker may be branched or unbranched, cyclic or acyclic, substituted or unsubstituted, saturated or unsaturated, chiral, achiral or racemic mixture. The alkyl linkers can have from about 2 to about 18 carbon atoms. In some embodiments such alkyl linkers have from about 3 to about 9 carbon atoms. Some alkyl linkers include one or more functional groups including, but not limited to, hydroxy, amino, thiol, thioether, ether, amide, thioamide, ester, urea, and thioether. Such alkyl linkers can

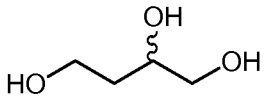
include, but are not limited to, 1,2 propanediol, 1,2,3 propanetriol, 1,3 propanediol, triethylene glycol hexaethylene glycol, polyethylene glycollinkers (*e.g.*, $[-O-CH2-CH2-]_n$ (n= 1-9)), methyl linkers, ethyl linkers, propyl linkers, butyl linkers, or hexyl linkers. In some embodiments, such alkyl linkers may include peptides or amino acids.

[0070] In some embodiments, the non-nucleotide linker may include, but is not limited to, those listed in Table 2, wherein the oligonucleotide-based TLR antagonist is linked through a hydroxyl group present on the non-nucleotidic linker.

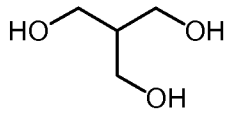
Table 2: Representative Non-Nucleotidic Linkers



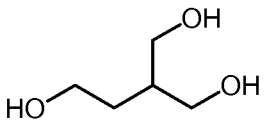
Glycerol (1,2,3-Propanetriol)



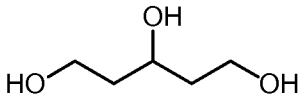
1,2,4-Butanetriol



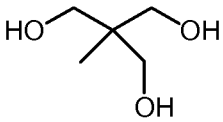
2-(hydroxymethyl)-1,3-propanediol



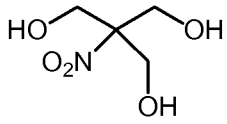
2-(hydroxymethyl)1,4-butanediol



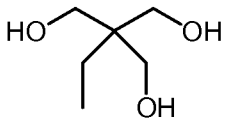
1,3,5-Pentanetriol



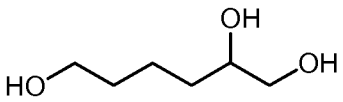
1,1,1-Tris(hydroxymethyl)ethane



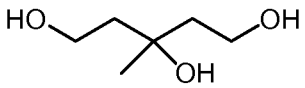
1,1,1-Tris(hydroxymethyl)nitromethane



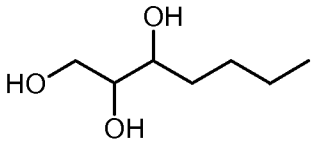
1,1,1-Tris(hydroxymethyl)propane



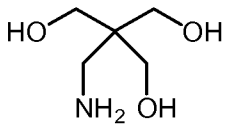
1,2,6-Hexanetriol



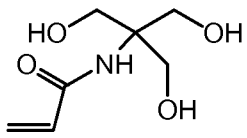
3-Methyl-1,3,5-pentanetriol



1,2,3-Heptanetriol

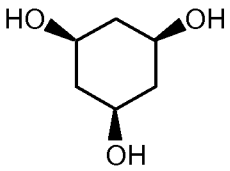


2-Amino-2-(hydroxymethyl)-1,3-propanediol

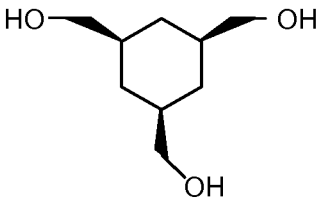


N-[Tris(hydroxymethyl)methyl]acrylamide

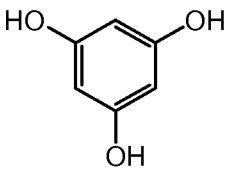
Table 2: Continued



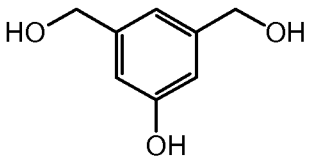
cis-1,3,5-Cyclohexanetriol



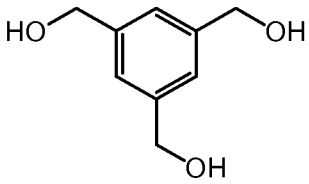
cis-1,3,5-Tri(hydroxymethyl)cyclohexane



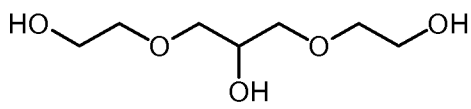
1,3,5,-Trihydroxyl-benzene



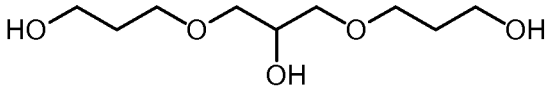
3,5,-Di(hydroxymethyl)phenol



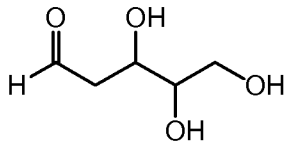
1,3,5,-Tri(hydroxymethyl)benzene



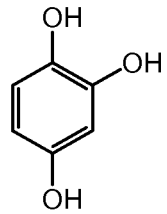
1,3-Di(hydroxyethoxy)-2-hydroxyl-propane



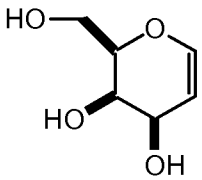
1,3-Di(hydroxypropoxy)-2-hydroxyl-propane



2-Deoxy-D-ribose

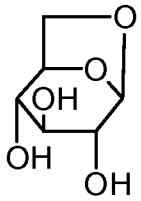


1,2,4,-Trihydroxyl-benzene

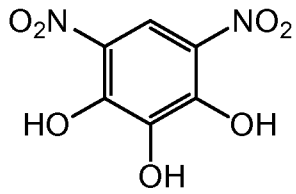


D-Galactoal

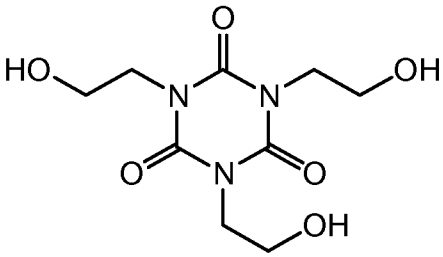
Table 2: Continued



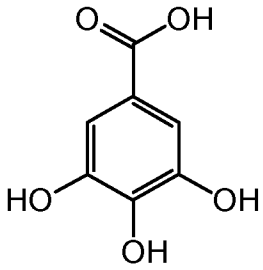
1,6-anhydro-β-D-Glucose



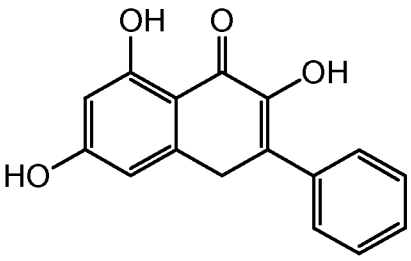
4,6-Nitropyrogallol



1,3,5-Tris(2-hydroxyethyl)-Cyanuric acid

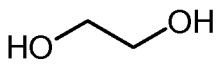


Gallic acid



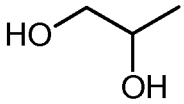
3,5,7-Trihydroxyflavone

Table 2: Continued

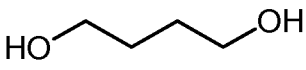


Ethylene glycol

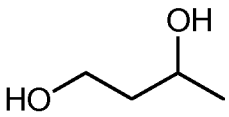
1,3-Propanediol



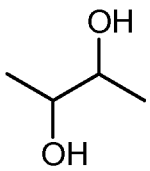
1,2-Propanediol



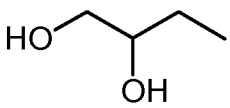
1,4-Butanediol



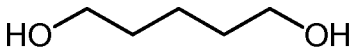
1,3-Butanediol



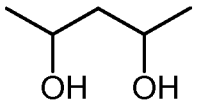
2,3-Butanediol



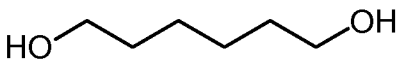
1,4-Butanediol



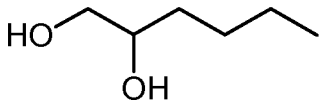
1,5-Pentanediol



2,4-Pentanediol



1,6-Hexanediol



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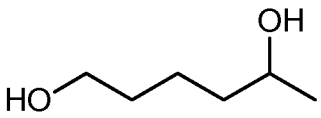

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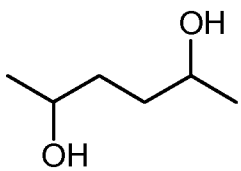
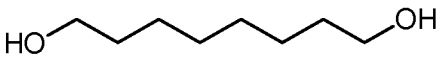


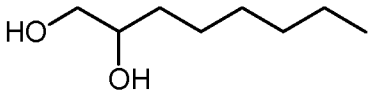
2,5-Hexanediol

Table 2: Continued

1,7-Heptanediol



1,8-Octanediol



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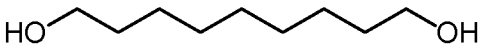
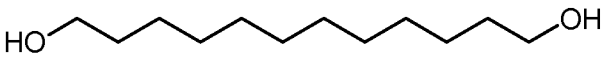
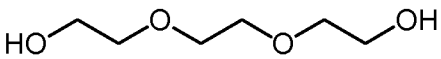


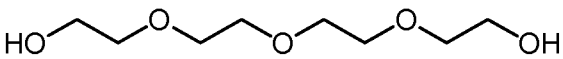
1,9-Nonanediol



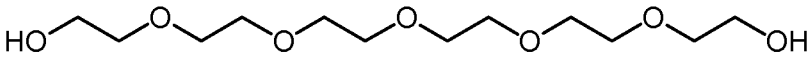
1,12-Dodecanediol



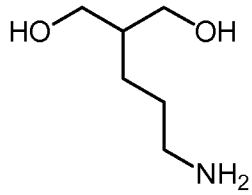
Triethylene glycol



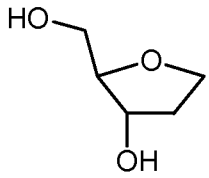
Tetraethylene glycol



Hexaethylene glycol

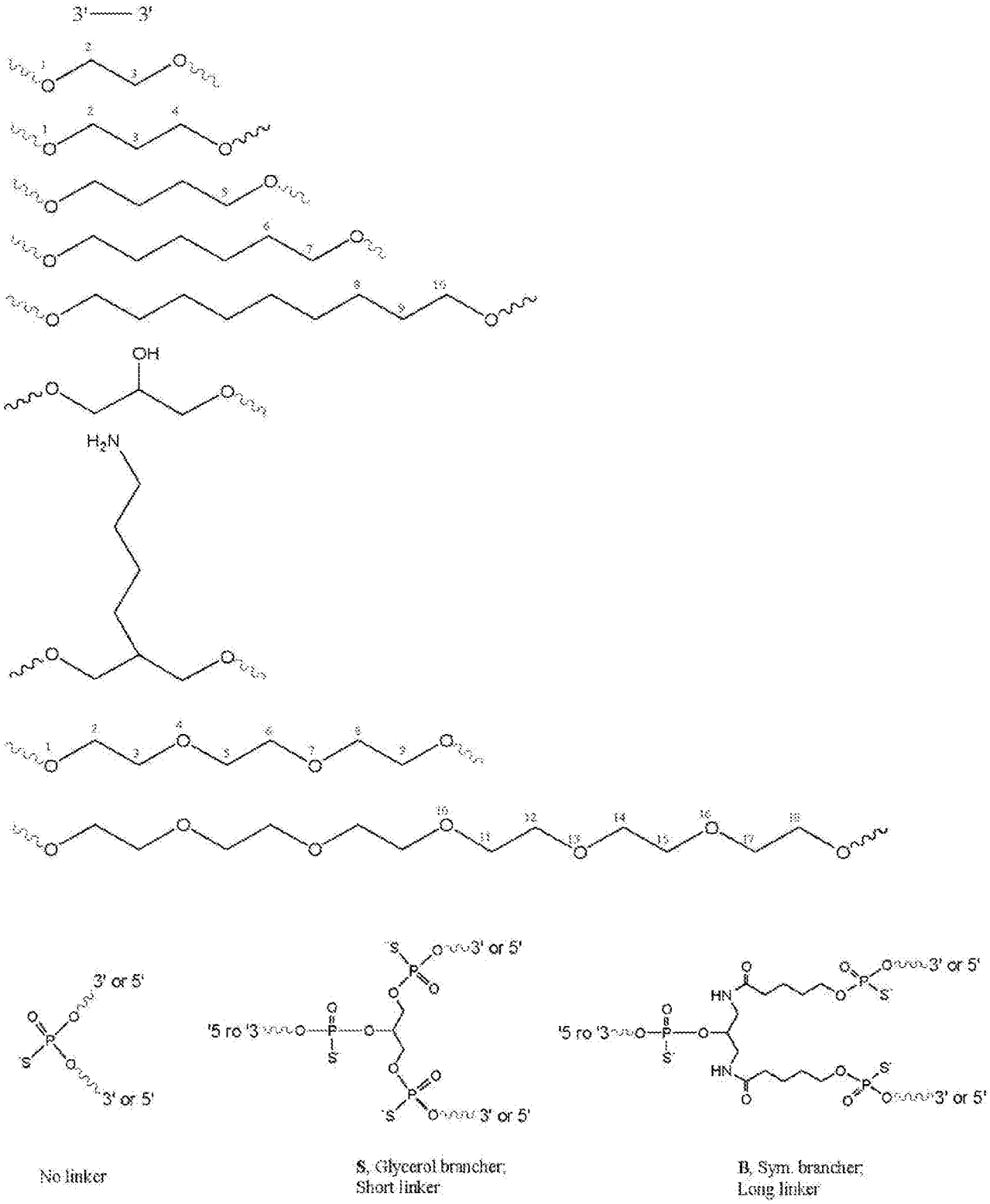


2-(1-Aminopropyl)-1,3-propanediol



1,2-Dideoxyribose

Table 2: Continued



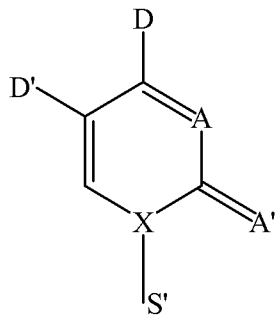
[0071] In some embodiments, the small molecule linker is glycerol or a glycerol homolog of the formula $HO-(CH_2)_o-CH(OH)-(CH_2)_p-OH$, wherein *o* and *p* independently

are integers from 1 to about 6, from 1 to about 4, or from 1 to about 3. In some other embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane. Some such derivatives have the formula $HO\text{-}(CH_2)_m\text{-}C(O)NH\text{-}CH_2\text{-}CH(OH)\text{-}CH_2\text{-}NHC(O)\text{-}(CH_2)_m\text{-}OH$, wherein *m* is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6, or from 2 to about 4.

[0072] Some non-nucleotide linkers according to the invention permit attachment of more than two oligonucleotides. For example, the small molecule linker glycerol has three hydroxyl groups to which oligonucleotides may be covalently attached. Some TLR antagonists according to the invention, therefore, comprise two or more oligonucleotides linked to a nucleotide or a non-nucleotide linker. Such TLR antagonists are referred to as being "branched".

[0073] TLR antagonist compounds may comprise at least two oligonucleotides non-covalently linked, such as by electrostatic interactions, hydrophobic interactions, π-stacking interactions, hydrogen bonding and combinations thereof. Non-limiting examples of such non-covalent linkage includes Watson-Crick base pairing, Hoogsteen base pairing and base stacking.

[0074] In certain embodiments, pyrimidine nucleosides in the immune regulatory oligonucleotides used in the compositions and methods according to the invention have the structure (***II***):



(***II***)

wherein:

D is a hydrogen bond donor;

D' is selected from the group consisting of hydrogen, hydrogen bond donor, hydrogen bond acceptor, hydrophilic group, hydrophobic group, electron withdrawing group and electron donating group;

A is a hydrogen bond acceptor or a hydrophilic group;

A' is selected from the group consisting of hydrogen bond acceptor, hydrophilic group, hydrophobic group, electron withdrawing group and electron donating group;

X is carbon or nitrogen; and

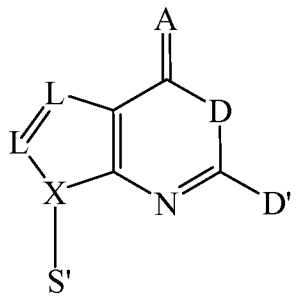
S' is a pentose or hexose sugar ring, or a sugar analog.

[0075] In certain embodiments, the sugar ring is derivatized with a phosphate moiety, modified phosphate moiety, or other linker moiety suitable for linking the pyrimidine nucleoside to another nucleoside or nucleoside analog.

[0076] In some embodiments hydrogen bond donors include, without limitation, -NH-, $-NH_2$, -SH and -OH. Preferred hydrogen bond acceptors include, without limitation, C=O, C=S, and the ring nitrogen atoms of an aromatic heterocycle, *e.g.*, N3 of cytosine.

[0077] In some embodiments, (***II***) is a pyrimidine nucleoside derivative. Examples of pyrimidine nucleoside derivatives include, without limitation, 5-hydroxycytosine, 5-hydroxymethylcytosine, N4-alkylcytosine, or N4-ethylcytosine, araC, 5-OH-dC, N3-Me-dC, and 4-thiouracil. Chemical modified derivatives also include, but are not limited to, thymine or uracil analogues. In some embodiments, the sugar moiety S' in (***II***) is a sugar derivative. Suitable sugar derivatives include, but are not limited to, trehalose or trehalose derivatives, hexose or hexose derivatives, arabinose or arabinose derivatives.

[0078] In some embodiments, the purine nucleosides in the TLR antagonists used in the compositions and methods according to the invention have the structure (***III***):



(***III***)

wherein:

D is a hydrogen bond donor;

D' is selected from the group consisting of hydrogen, hydrogen bond donor, and hydrophilic group;

A is a hydrogen bond acceptor or a hydrophilic group;

X is carbon or nitrogen;

each L is independently selected from the group consisting of C, O, N and S; and

S' is a pentose or hexose sugar ring, or a sugar analog.

[0079] In certain embodiments, the sugar ring is derivatized with a phosphate moiety, modified phosphate moiety, or other linker moiety suitable for linking the pyrimidine nucleoside to another nucleoside or nucleoside analog.

[0080] In certain embodiments hydrogen bond donors include, without limitation, -NH-, $-NH_2$, -SH and -OH. In certain embodiments hydrogen bond acceptors include, without limitation, C=O, C=S, $-NO_2$ and the ring nitrogen atoms of an aromatic heterocycle, *e.g.*, N1 of guanine.

[0081] In some embodiments, (***III***) is a purine nucleoside derivative. Examples of purine nucleoside derivatives include, without limitation, guanine analogues such as 7-deaza-G, 7-deaza-dG, ara-G, 6-thio-G, Inosine, Iso-G, loxoribine, TOG(7-thio-8-oxo)-G, 8-bromo-G, 8-hydroxy-G, 5-aminoformycin B, Oxoformycin, 7-methyl-G, 9-p-chlorophenyl-8-aza-G, 9-phenyl-G, 9-hexyl-guanine, 7-deaza-9-benzyl-G, 6-Chloro-7-deazaguanine, 6-methoxy-7-deazaguanine, 8-Aza-7-deaza-G(PPG), 2-(Dimethylamino)guanosine, 7-Methyl-6-thioguanosine, 8-Benzyloxyguanosine, 9-Deazaguanosine, 1-(B-D-ribofuranosyl)-2-oxo-7-deaza-8-methyl-purine, 1-(2'-deoxy-β-D-ribofuranosyl)-2-oxo-7-deaza-8-methyl-purine. Chemically modified derivatives also include, but are not limited to, adenine analogues such as 9-benzyl-8-hydroxy-2-(2-methoxyethoxy)adenine, 2-Amino-N2-O-, methyladenosine, 8-Aza-7-deaza-A, 7-deaza-A, Vidarabine, 2-Aminoadenosine, N1-Methyladenosine, 8-Azaadenosine, 5-Iodotubercidin, and N1-Me-dG. In some embodiments, the sugar moiety S' in (***III***) is a sugar derivative as defined for Formula II.

[0082] In certain embodiments of the invention, the TLR antagonist comprises a nucleic acid sequence containing at least one B-L-deoxy nucleoside or 3'-deoxy nucleoside.

[0083] In certain embodiments of the invention, the TLR antagonist comprises a nucleic acid sequence containing at least one dinucleotide selected from CpG, C*pG, C*pG* and CpG*, wherein C is cytosine or 2'-deoxycytidine, G is guanosine or 2'-deoxyguanosine, C* is 2'-deoxythymidine, 1-(2'-deoxy-ß-D-ribofuranosyl)-2-oxo-7-deaza-8-methyl-purine, 2'-dideoxy-5-halocytosine, 2'-dideoxy-5-nitrocytosine, arabinocytidine, 2'-deoxy-2'-substituted arabinocytidine, 2'-O-substituted arabinocytidine, 2'-deoxy-5-hydroxycytidine, 2'-deoxy-N4-alkyl-cytidine, 2'-deoxy-4-thiouridine, or other pyrimidine nucleoside analogs, G* is 2'-deoxy-7-deazaguanosine, 2'-deoxy-6-thioguanosine, arabinoguanosine, 2'-deoxy-2'substituted-arabinoguanosine, 2'-O-

substituted-arabinoguanosine, 2'- deoxyinosine, or other purine nucleoside analogs, and p is an internucleoside linkage selected from the group consisting of phosphodiester, phosphorothioate, and phosphorodithioate, and wherein the activity of the at least one dinucleotide is regulated by the flanking sequence.

[0084] The sequences of selected TLR antagonists within these general structures that would be useful for the present invention include, but are not limited to, those shown in Table 3.

Table 3.

SEQ ID NO:	Sequence
5	5'-CTATCTGACGTTCTCTGT-3'
7	5'-CTATCTGACGTTCTCTGT-3'
17	5'-CTATCTGACG$_1$TTCTCTGT-3'
37	5'-CTATCTGACG$_4$TTCTCTGT-3'
39	5'-CTATCTGAC$_4$GTTCTCTGT-3'
41	5'-CTATCTGAC$_5$GTTCTCTGT-3'
43	5'-CTATCTGAC$_6$GTTCTCTGT-3'
45	5'-CTATCTGACG$_5$TTCTCTGT-3'
47	5'-CTATCTGAC$_7$GTTCTCTGT-3'
64	5'-CTATCTAACGTTCTCTGT-3'
67	5'-CTATCTAACG$_1$TTCTCTGT-3'
22	5'-CTATCTGAmCGTTCTCTGT-3'
9	5'-CTATCTGUCGTTCTCTGT-3'
10	5'-CTATCTGUCGTTCTCTGT-3'
19	5'-CTATCTGUCG$_1$TTCTCTGT-3'
38	5'-CTATCTGUCG$_4$TTCTCTGT-3'
40	5'-CTATCTGUC$_4$GTTCTCTGT-3'
42	5'-CTATCTGUC$_5$GTTCTCTGT-3'
44	5'-CTATCTGUC$_6$GTTCTCTGT-3'
46	5'-CTATCTGUCG$_5$TTCTCTGT-3'
48	5'-CTATCTGUC$_7$GTTCTCTGT-3'
66	5'-CTATCTAUCGTTCTCTGT-3'

69	5'-CTATCTAUCG$_1$TTCTCTGT-3'
65	5'-CTATCTAGCGTTCTCTGT-3'
68	5'-CTATCTAGCG$_1$TTCTCTGT-3'
23	5'-CTATCTGmACGTTCTCTGT-3'
24	5'-CTATCTGmAmCGTTCTCTGT-3'
25	5'-CTATCTGAC$_2$GTTCTCTGT-3'
27	5'-CTATCTGTC$_2$GTTCTCTGT-3'
33	5'-CTATCTGAC$_3$GTTCTCTGT-3'
35	5'-CTATCTGTC$_3$GTTCTCTGT-3'
26	5'-CTATCTGACG$_2$TTCTCTGT-3'
28	5'-CTATCTGTCG$_2$TTCTCTGT-3'
34	5'-CTATCTGACG$_3$TTCTCTGT-3'
36	5'-CTATCTGTCG$_3$TTCTCTGT-3'
21	3'-TCTTGCAGTCT-X$_2$-TCTGACGTTCT-3'
52	5'-CCTACTAGCGTX$_1$CTCATC-3'
53	5'-CCTACTAGCGX$_1$TCTCATC-3'
54	5'-CCTACTAG$_3$CGTTCTCATC-3'
55	5'-TCCATGA$_1$CGTTCCTGATGC-3'
56	5'-CTATCTGAC$_2$G$_2$TTCTCTGT-3'
57	5'-C$_2$T$_2$A$_2$T$_2$C$_2$T$_2$G$_2$A$_2$C$_2$G$_2$T$_2$T$_2$C$_2$T$_2$C$_2$T$_2$G$_2$T$_2$-3'
29	5'-CTATCTGAX$_1$GTTCTCTGT-3'
30	5'-CTATCTGACX$_1$TTCTCTGT-3'
31	5'-CTATCTGTX$_1$GTTCTCTGT-3'
32	5'-CTATCTGTCX$_1$TTCTCTGT-3'
61	5'-CTATCTAGCGTX$_1$CTCTGT-3'
62	5'-CTATCTAGCGX$_1$TCTCTGT-3'
63	5'-CTATCTAGCGX$_1$X$_1$CTCTGT-3'
58	5'-CTATCTGACGTX$_3$CTCTGT-3'
59	5'-CTATCTGACGX$_3$TCTCTGT-3'
60	5'-CTATCTGACGX$_3$X$_3$CTCTGT-3'
70	5'-CTATCTAGCGTX$_3$CTCTGT-3'
71	5'-CTATCTAGCGX$_3$TCTCTGT-3'

72	5'-CTATCTAGCGX$_3$X$_3$CTCTGT-3'
74	5'-CTATCTGACGTTCTCTGT-3'
76	5'-CCTACTAG$_6$CGTTCTCATC-3'
77	5'-TCCATGACGU$_1$TCCTGATGC-3'
78	5'-CTATCTGX$_2$CGTTCTCTGT-3'
79	5'-CTATCTX$_2$ACGTTCTCTGT-3'
80	5'-CTATCTU$_2$ACGTTCTCTGT-3'
81	5'-CTATCTGU$_2$CGTTCTCTGT-3'
82	5'-CTATCTGACGX$_2$TCTCTGT-3'
83	5'-CTATCTGACGTX$_2$CTCTGT-3'
84	5'-CTATCTGX$_3$CGTTCTCTGT-3'
85	5'-CTATCTX$_3$ACGTTCTCTGT-3'
86	(5'-TCTGACGTTCT)$_2$X$_2$
87	(5'-TCTGACG$_1$TTCT)$_2$X$_2$
88	(5'-TCTGACG$_4$TTCT)$_2$X$_2$
89	(5'-TCTCTGACGTT)$_2$X$_2$
90	5'-TCTGACG$_1$TTCT-X$_3$-TGACCGGTCA-3'
93	(5'-TCTGUCGTTCT)$_2$X$_2$
94	(5'-TCTGUCG$_1$TTCT)$_2$X$_2$
93	(5'-TCTGACG$_4$TTCT)X$_2$
96	(5'-TCTGACG$_1$TT)$_2$X$_2$
95	5'-TCTGACG$_1$TTCT-X$_3$-TCAACCACACA-3'
98	5'-CTATCTGACG$_1$TTCTCUGU-3'
99	5'-CTATCTGUCG$_1$TTCTCUGU-3'
100	(5'-UGUCG$_1$TTCT)X$_2$
101	(5'-UGACG$_1$TTCT)$_2$X$_2$
102	5'-CTATCTGAC^{1}GTTCTCTGT-3'
103	5'-CTATCTGAC^{2}GTTCTCTGT-3'
104	5'-CTATCTGAC^{3}GTTCTCTGT-3'
105	5'-CTATCTGAC^{2}G^{1}TTCTCTGT-3'

Underlined G, A or U = 2'-OMe; Underlined T = 3'-OMe; A_1 = 3'-OMe; G_1=7-deaza-dG; m= P-Me; A_2, T_2, C_2, and G_2 = B-L-deoxy nucleoside; X_1 = abasic; X_2 = glycerol linker, X_3 = C3-linker; C_3 and G_3 = 3'-deoxy-nucleoside; G_4 = araG; C_4 = araC; C_5 = 5-OH-dC; C_6 = 1-(2'-deoxy-β-D-ribofuranosyl)-2-oxo-7-deaza-8-methyl-purine; G_5 = N1-Me-dG; C_7 = N3-Me-dC; U_1=3'-OMe; U_2=dU; C^1 = 2'-O-methyl-C; C^2 = 5-methyl-dC; C^3 = 2'-O-methyl-5-methyl-C; G^1 = 2'-O-methyl-G

[0085] In some embodiments, the TLR antagonists each have from about 6 to about 35 nucleoside residues, preferably from about 9 to about 30 nucleoside residues, more preferably from about 11 to about 23 nucleoside residues. In some embodiments, the TLR antagonists have from about 6 to about 18 nucleoside residues.

[0086] In a second aspect, the invention provides a method for therapeutically treating a disease associated with high blood lipid concentration and/or high blood cholesterol concentration in a mammal, such method comprising inhibiting TLR signaling in the mammal, as described in the first aspect of the invention. In some embodiments, TLR signaling is inhibited by administering to the mammal one or more TLR antagonist compounds according to the invention in a pharmaceutically effective amount. In some embodiments TLR signaling is inhibited by inhibiting the expression of one or more TLR, or another protein in the TLR signaling pathway. In certain preferred embodiments of this aspect of the invention, the disease is hypercholesterolemia, hyperlipidemia, coronary heart disease, arteriosclerosis, atherosclerosis, stroke, peripheral vascular disease, diabetes or high blood pressure.

[0087] In a third aspect, the invention provides a method for preventing a disease associated with high blood lipid concentration and/or blood cholesterol concentration in a mammal having elevated blood cholesterol and/or blood lipid, such method comprising inhibiting TLR signaling in the mammal as described in the first aspect of the invention. In some embodiments, TLR signaling is inhibited by administering to the mammal one or more TLR antagonist compounds according to the invention in a pharmaceutically effective amount. In some embodiments TLR signaling is inhibited by inhibiting the expression of one or more TLR, or another protein in the TLR signaling pathway. In certain preferred embodiments, the disease is hypercholesterolemia, hyperlipidemia, coronary heart disease, arteriosclerosis, atherosclerosis, stroke, peripheral vascular disease, diabetes or high blood pressure.

[0088] In some embodiments, the methods according to the invention further comprise administering to the mammal one or more cholesterol lowering compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

[0089] In the methods according to the invention, administration of the TLR antagonist compound can 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 the TLR antagonist compound can be carried out using known procedures at dosages and for periods of time effective to reduce symptoms or surrogate markers of the disease. When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of the TLR antagonist compound from about 0.0001 micromolar to about 10 micromolar. For localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. In some preferred embodiments, a total dosage of the TLR antagonist compound ranges from about 0.001 mg per patient per day to about 200 mg per kg body weight per day. It may be desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the TLR antagonists to an individual as a single treatment episode.

[0090] The TLR antagonist compound may be administered or used in combination with other compounds including, without limitation, one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

[0091] The methods according to the invention are useful for the prophylactic or therapeutic treatment of human and/or animal disease. For example, the methods may be useful for adult, pediatric and veterinary applications.

[0092] In any of the methods according to the invention, the TLR antagonist compound can be administered in combination with any other agent useful for treating the disease or condition that does not diminish the antagonist activity of the TLR antagonist

compound. 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 lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof, which can enhance the specificity or magnitude of the response to the TLR antagonist. For example, in the treatment of hyperlipidemia and/or hypercholesterolemia, it is contemplated that the TLR antagonist compound may be administered in combination with one or more lipid and/or cholesterol lowering compounds, including, without limitation, statins, targeted therapeutic agents and/or monoclonal antibodies.

[0093] The following examples are intended to further illustrate certain exemplary embodiments of the invention and are not intended to limit the scope of the invention. For example, representative TLR antagonists are shown in the following examples, but do not limit the scope of TLRs to which the antagonist of the invention are applicable.

[0094] The patents and publications cited herein represent common knowledge in the field and are incorporated by reference in their entirety. Any conflict between the teachings of the cited references and the instant specification shall be resolved in favor of the latter.

Example 1

Synthesis of Oligonucleotides Containing Immune regulatory Moieties.

[0095] All synthetic oligonucleotides were synthesized according to standard procedures (*see*, *e.g.*, U.S. Patent Publication No. 2004/0097719).

[0096] Oligonucleotides were synthesized on a 1 μM scale using an automated DNA synthesizer (Expedite 8909; PerSeptive Biosystems, Framingham, Mass.), following standard linear synthesis or parallel synthesis procedures (*see*, *e.g.*, Figures 5 and 6 of U.S. Patent Publication No. 2004/0097719).

[0097] Deoxyribonucleoside phosphoramidites were obtained from (Aldrich-Sigma, St Louis, Mo). 1',2'-dideoxyribose phosphoramidite, propyl-1-phosphoramidite, 2-deoxyuridine phosphoramidite, 1,3-bis-[5-(4,4'-dimethoxytrityl)pentylamidyl]-2-propanol phosphoramidite and methyl phosponamidite were obtained from Glen Research (Sterling, Va.). ß-L-2'-deoxyribonucleoside phosphoramidite, alpha-2'-

deoxyribonucleoside phosphoramidite, mono-DMT-glycerol phosphoramidite and di-DMT-glycerol phosphoramidite were obtained from ChemGenes (Willmington, Mass.). (4-Aminobutyl)-1,3-propanediol phosphoramidite was obtained from Clontech (Palo Alto, Calif.). Arabinocytidine phosphoramidite, arabinoguanosine, arabinothymidine and arabinouridine were obtained from Reliable Pharmaceutical (St. Louis, Mo.). Arabinoguanosine phosphoramidite, arabinothymidine phosphoramidite and arabinouridine phosphoramidite were synthesized at Idera Pharmaceuticals, Inc. (Cambridge, Mass.) (Noronha *et al.* (2000) *Biochem.* 39:7050-7062).

[0098] All nucleoside phosphoramidites were characterized by ^{31}P and ^{1}H NMR spectra. Modified nucleosides were incorporated at specific sites using normal coupling cycles. After synthesis, oligonucleotides were deprotected using concentrated ammonium hydroxide and purified by reverse phase HPLC, followed by dialysis. Purified oligonucleotides as sodium salt form were lyophilized prior to use. Purity was tested by CGE and MALDI-TOF MS.

Example 2

Cholesterol and lipid lowering activities of TLR antagonists *in vivo*

[0099] Female C57BL/6 mice of 5 weeks age were obtained from The Jackson Laboratory. All animal experiments were performed as per guidelines of Idera Pharmaceuticals ICAUC approved protocols. Mice were fed a Western diet consisting of 60% lard (Research Diets, New Brunswick, NJ). TLR antagonist was administered to mice at doses of 5 and 20 mg/kg, s.c two times a week for five weeks starting at week 6 to week 10. Lipitor® was administered to mice at a dose of 20 mg/kg i.g five times a week for five weeks. Control group of mice were injected with PBS. Each group had five mice. Blood was collected during the course of the study and at week 12 when the experiment was terminated. The level of serum cholesterol was determined in each sample by "EnzyChrom™ Cholesterol Assay Kit" from BioAssay System.

Example 3

Cholesterol, lipoprotein, lipid and glucose lowering activities of TLR antagonists *in vivo*

[00100] Female C57BL/6 or ApoE-deficient mice of 5 weeks old were obtained from The Jackson Laboratory. All animal experiments were performed as per guidelines of Idera Pharmaceuticals ICAUC approved protocols. Mice were fed a Western diet

consisting of 60% lard (Research Diets, New Brunswick, NJ). Antagonist was administered to mice at doses of 15 mg/kg, s.c two times a week for five weeks starting at week 6 to week 10. Control group of mice were injected with PBS. Each group had 15 mice. Blood was collected every week after overnight fasting of mice until at week 12 when the experiment was terminated and serum levels of cholesterol, HDL, LDL, ALT, AST, triglyceride and glucose were determined at Quest Laboratories.

Example 4

Body weight lowering activities of TLR antagonists *in vivo*

[00101] Female C57BL/6 or ApoE-deficient mice of 5 weeks old were obtained from The Jackson Laboratory. All animal experiments were performed as per guidelines of Idera Pharmaceuticals ICAUC approved protocols. Mice were fed a Western diet consisting of 60% lard (Research Diets, New Brunswick, NJ). Antagonist was administered to mice at doses of 15 mg/kg, s.c two times a week for five weeks starting at week 6 to week 10. Control group of mice were injected with PBS. Each group had 15 mice. Body weight was measured.

What is claimed is

1. A method for lowering blood concentrations of cholesterol, lipids and/or low density lipoprotein in a mammal having elevated concentrations thereof, the method comprising inhibiting TLR signaling in the mammal.
2. The method according to claim 1, comprising administering to the mammal an inhibitor of the activity of one or more TLR or a protein involved in TLR signaling.
3. The method according to claim 2, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.
4 The method according to claim 2, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.
5. The method according to claim 2, wherein the inhibitor is a compound selected from the group consisting of a small molecule; an antibody; a cyclohexene derivative; a lipid derivative; a synthetic oligonucleotide comprising a "CCT" triplet and a "GGG" triplet; a synthetic oligonucleotide comprising regions containing "GGG" and/or "GGGG" and/or "GC" sequences; a synthetic oligonucleotide that is methylated; a synthetic, immune inhibitory oligonucleotide, having one or more chemical modifications in the sequence flanking an immune stimulatory motif that would be immune stimulatory but for the modification; and a synthetic immune inhibitory oligonucleotide, containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein the modified immune stimulatory motif would be immune stimulatory but for the modification.
6. The method according to claim 5, wherein the small molecule is chloroquine or hydroxychloroquine.
7. The method according to claim 2, wherein the inhibitor is a TLR antagonist compound having the structure

$$5'\text{-}N_m - N_3N_2N_1CGN^1N^2N^3 - N^m\text{-}3':$$

wherein:

CG is an oligonucleotide motif, C is cytosine or a pyrimidine nucleotide derivative or non-nucleotide linkage, and G is guanosine a purine nucleotide derivative or non-nucleotide linkage;

N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

provided that at least one of N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative or non-nucleotide linkage;

wherein the oligonucleotide motif would be immune stimulatory but for the nucleotide derivative or non-nucleotide linkage;

and wherein m is an integer from 0 to about 30.

8. The method according to claim 2, wherein the inhibitor is a TLR antagonist compound containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein CG is the modified immune stimulatory motif, wherein C is cytosine, or a pyrimidine nucleotide derivative selected from 5-methyl-dC, 2'-O-substituted-C, 2'-O-methyl-C, 2'-O-methoxyethoxy-C, 2'-O-methoxyethyl-5-methyl-C, and 2'-O-methyl-5-methyl-C, and G is guanosine or a purine nucleotide derivative selected from 2'-O-substituted-G, 2'-O-methyl-G, and 2'-O-methoxyethoxy-G; provided that at least one C and/or G of the modified immune stimulatory motif is a specified nucleotide derivative; and optionally containing less than 3 consecutive guanosine nucleotides; wherein the modified immune stimulatory motif would be immune stimulatory but for the nucleotide derivative.

9. The method according to claim 2, wherein the route of administration is 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.

10. The method according to claim 2, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

11. The method according to claim 10, wherein the cholesterol lowering composition is a statin.

12. The method according to claim 1, comprising administering to the mammal an inhibitor of the expression of one or more TLR or a protein involved in TLR signaling.
13. The method according to claim 12, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.
14. The method according to claim 12, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.
15. The method according to claim 12, wherein the route of administration is 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.
16. The method according to claim 12, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.
17. The method according to claim 16, wherein the cholesterol lowering composition is a statin.
18. A method for therapeutically treating a mammal having a disease selected from coronary heart disease, arteriosclerosis, atherosclerosis stroke, peripheral vascular disease, diabetes and high blood pressure, the method comprising inhibiting TLR signaling in the mammal.
19. The method according to claim 18, comprising administering to the mammal an inhibitor of the activity of one or more TLR or a protein involved in TLR signaling.
20. The method according to claim 19, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.
21. The method according to claim 19, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.

22. The method according to claim 19, wherein the inhibitor is a compound selected from the group consisting of a small molecule; an antibody; a cyclohexene derivative; a lipid derivative; a synthetic oligonucleotide comprising a "CCT" triplet and a "GGG" triplet; a synthetic oligonucleotide comprising regions containing "GGG" and/or "GGGG" and/or "GC" sequences; a synthetic oligonucleotide that is methylated or a synthetic; immune inhibitory oligonucleotides, having one or more chemical modifications in the sequence flanking an immune stimulatory motif that would be immune stimulatory but for the modification; and a synthetic immune inhibitory oligonucleotide, containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein the modified immune stimulatory motif would be immune stimulatory but for the modification.

23. The method according to claim 22, wherein the small molecule is chloroquine or hydroxychloroquine.

24. The method according to claim 19, wherein the inhibitor is a TLR antagonist compound having the structure

$$5'\text{-}N_m - N_3N_2N_1CGN^1N^2N^3 - N^m\text{-}3':$$

wherein:

CG is an oligonucleotide motif and C is cytosine or a pyrimidine nucleotide derivative or non-nucleotide linkage, and G is guanosine a purine nucleotide derivative or non-nucleotide linkage;

N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

provided that at least one N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative or non-nucleotide linkage;

wherein the oligonucleotide motif would be immune stimulatory but for the nucleotide derivative or non-nucleotide linkage;

and wherein m is an integer from 0 to about 30.

25. The method according to claim 19, wherein the inhibitor is a TLR antagonist compound containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein CG is the modified immune

stimulatory motif, wherein C is cytosine, or a pyrimidine nucleotide derivative selected from 5-methyl-dC, 2'-O-substituted-C, 2'-O-methyl-C, 2'-O-methoxyethoxy-C, 2'-O-methoxyethyl-5-methyl-C, and 2'-O-methyl-5-methyl-C, and G is guanosine or a purine nucleotide derivative selected from 2'-O-substituted-G, 2'-O-methyl-G, and 2'-O-methoxyethoxy-G; provided that at least one C and/or G of the modified immune stimulatory motif is a specified nucleotide derivative; and optionally containing less than 3 consecutive guanosine nucleotides; wherein the modified immune stimulatory motif would be immune stimulatory but for the nucleotide derivative.

26. The method according to claim 19, wherein the route of administration is 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.

27. The method according to claim 19, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

28. The method according to claim 27, wherein the cholesterol lowering composition is a statin.

29. The method according to claim 18, comprising administering to the mammal an inhibitor of the expression of one or more TLR or a protein involved in TLR signaling.

30. The method according to claim 29, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.

31. The method according to claim 29, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.

32. The method according to claim 29, wherein the route of administration is 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.

33. The method according to claim 29, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.
34. The method according to claim 33, wherein the cholesterol lowering composition is a statin.
35. A method for preventing disease in a mammal, wherein the disease is selected from coronary heart disease, arteriosclerosis, atherosclerosis stroke, peripheral vascular disease, diabetes and high blood pressure, the method comprising inhibiting TLR signaling in the mammal.
36. The method according to claim 35, comprising administering to the mammal an inhibitor of the activity of one or more TLR or a protein involved in TLR signaling.
37. The method according to claim 35, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.
38. The method according to claim 36, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.
39. The method according to claim 36, wherein the inhibitor is a compound selected from the group consisting of a small molecule; an antibody; a cyclohexene derivative; a lipid derivative; a synthetic oligonucleotide comprising a "CCT" triplet and a "GGG" triplet; a synthetic oligonucleotide comprising regions containing "GGG" and/or "GGGG" and/or "GC" sequences; a synthetic oligonucleotide that is methylated or a synthetic, immune inhibitory oligonucleotides, having one or more chemical modifications in the sequence flanking an immune stimulatory motif that would be immune stimulatory but for the modification; and a synthetic immune inhibitory oligonucleotide, containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein the modified immune stimulatory motif would be immune stimulatory but for the modification.

40. The method according to claim 39, wherein the small molecule is chloroquine or hydroxychloroquine.

41. The method according to claim 36, wherein the inhibitor is a TLR antagonist compound having the structure

$$5'\text{-}N_m - N_3N_2N_1CGN^1N^2N^3 - N^m\text{-}3':$$

wherein:

CG is an oligonucleotide motif and C is cytosine or a pyrimidine nucleotide derivative or non-nucleotide linkage, and G is guanosine a purine nucleotide derivative or non-nucleotide linkage;

N_1-N_3 and N^1-N^3, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

N_m and N^m, at each occurrence, is independently a nucleotide, nucleotide derivative or non-nucleotide linkage;

provided that at least one N_1-N_3 and/or N^1-N^3 and/or C and/or G is a nucleotide derivative or non-nucleotide linkage;

wherein the oligonucleotide motif would be immune stimulatory but for the nucleotide derivative or non-nucleotide linkage;

and wherein m is an integer from 0 to about 30.

42. The method according to claim 36, wherein the inhibitor is a TLR antagonist compound containing a modified immune stimulatory motif comprising one or more modified immune stimulatory motifs, wherein CG is the modified immune stimulatory motif, wherein C is cytosine, or a pyrimidine nucleotide derivative selected from 5-methyl-dC, 2'-O-substituted-C, 2'-O-methyl-C, 2'-O-methoxyethoxy-C, 2'-O-methoxyethyl-5-methyl-C, and 2'-O-methyl-5-methyl-C, and G is guanosine or a purine nucleotide derivative selected from 2'-O-substituted-G, 2'-O-methyl-G, and 2'-O-methoxyethoxy-G; provided that at least one C and/or G of the modified immune stimulatory motif is a specified nucleotide derivative; and optionally containing less than 3 consecutive guanosine nucleotides; wherein the modified immune stimulatory motif would be immune stimulatory but for the nucleotide derivative.

43. The method according to claim 36, wherein the route of administration is 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.

44. The method according to claim 36, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

45. The method according to claim 44, wherein the cholesterol lowering composition is a statin.

46. The method according to claim 35, comprising administering to the mammal an inhibitor of the expression of one or more TLR or a protein involved in TLR signaling.

47. The method according to claim 46, wherein the TLR is selected from the group consisting of TLR2, TLR3, TLR4, TLR5, TLR7, TLR8 and TLR9.

48. The method according to claim 46, wherein the protein involved in TLR signaling is selected from the group consisting of MyD88, IRAK, IRF, TRAF, TGFβ, IκB kinase, IκB and NF-κB.

49. The method according to claim 46, wherein the route of administration is 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.

50. The method according to claim 46, further comprising administering to the mammal one or more lipid lowering compounds or compositions, cholesterol lowering compounds or compositions, diuretics, non-steroidal anti-inflammatory compounds (NSAIDs), statins, antibodies, antisense oligonucleotides, TLR agonists, TLR antagonists, peptides, proteins or gene therapy vectors or combinations thereof.

51. The method according to claim 50, wherein the cholesterol lowering composition is a statin.

FIG. 1a

Serum total cholesterol levels (mg/dl ± SD)

Week	PBS	TLR Antagonist		Lipitor®
		5 mg/kg	20 mg/kg	
6	145 ± 6	135 ±16	155 ± 1	140 ± 6
8	185 ± 2	167 ± 1	132 ± 7	164 ± 1
10	197 ± 8	177 ± 5	145 ± 3	182 ± 5
12	234 ± 11	179 ± 7	162 ± 8	189 ± 23

FIG. 1b

% Change over week 6

Week	PBS	TLR Antagonist		Lipitor®
		5 mg/kg	20 mg/kg	
8	28	24	-15	17
10	36	31	-7	30
12	61	32	5	35

FIG. 2a

C57BL/6 mice serum analysis

	Chol, mg/dl		HDL, mg/dl		LDL mg/dl		TG, mg/dl		Glucose, mg/dl		HDL-C/LDL-C	
Week	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta
6	81	82	59	57	5	3	86	108	149	159	11.8	19
7	114	104	86	77	7	13	105	72	184	163	12.3	5.9
8	91	75	77	55	5	6	42	72	128	62	15.4	9.2
9	84	84	70	72	6	-	40	49	70	47	11.7	--
10	122	62	96	41	15	14	55	37	119	117	6.4	2.9
11	95	62	85	47	-	7	55	41	53	85	--	6.7
12	101	60	91	46	-	6	64	40	48	86	--	7.7

FIG. 2b

57BL/6 mice serum: % Change

	Chol, mg/dl		HDL, mg/dl		LDL, mg/dl		TG, mg/dl		Glucose, mg/dl	
Week	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta
7	40.7	26.8	45.7	35.1	40	333.3	22.1	-33.3	23.5	2.5
8	12.3	-8.5	30.5	-3.5	0	100	-45.3	-33.3	14.1	-61
9	3.7	2.4	18.6	26.3	20	-100	-53.5	-54.6	-53	-70.4
10	50.6	-24.4	62.7	-28.1	200	366.7	-36.1	-65.7	-20.1	-26.4
11	17.3	-24.4	44.1	-17.5	-100	133.3	-36.1	-62	-64.4	-46.5
12	24.7	-26.8	54.2	-19.3	-100	100	-25.6	-63	-67.8	-45.9

FIG. 2c

ApoE-deficient mice serum analysis_

	Chol, mg/dl		HDL, mg/dl		LDL, mg/dl		TG, mg/dl		Glucose, mg/dl		HDL-C/LDL-C	
Week	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta
6	458	461	102	105	335	335	103	103	178	174	0.3	0.3
7	1161	951	357	318	782	609	110	119	179	153	0.45	0.52
8	1068	963	360	321	694	630	68	62	49	59	0.52	0.51
9	1008	948	315	312	683	626	50	50	54	86	0.46	0.5
10	1026	590	369	207	646	380	54	48	134	88	0.57	0.54
11	990	585	318	225	650	352	108	41	159	89	0.49	0.64
12	1596	624	396	225	1170	391	151	41	160	71	0.34	0.56

FIG. 2d

ApoE-deficient mice: % Change

	Chol, mg/dl		HDL, mg/dl		LDL, mg/dl		TG, mg/dl		Glucose, mg/dl	
Week	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta	PBS	Anta
7	153.5	106.3	250	202.9	133.4	81.8	6.8	15.5	0.6	-12.1
8	133.2	108.9	252.9	205.7	107.1	88.1	-34	-39.8	-72.5	-66.1
9	120.1	105.6	208.8	197.4	103.9	86.9	-51.5	-51.5	-69.7	-50.6
10	124	28	261.8	97.1	92.8	13.4	-47.6	-53.4	-24.7	-49.4
11	116.2	26.9	211.8	114.3	94	5.1	4.9	-60.2	-10.7	-48.9
12	248.5	35.4	288.2	114.3	249.3	16.7	46.6	-60.2	-10.1	-59.2

FIG. 3a

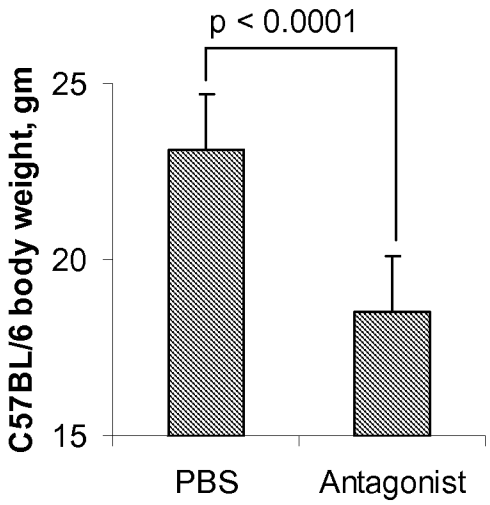
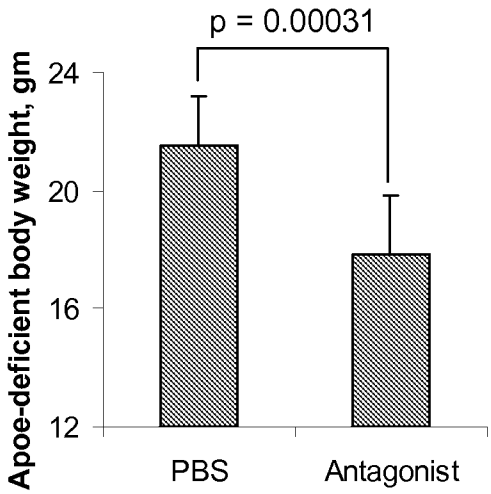


FIG. 3b


p = 0.00031
Apoe-deficient body weight, gm
24
20
16
12
PBS
Antagonist