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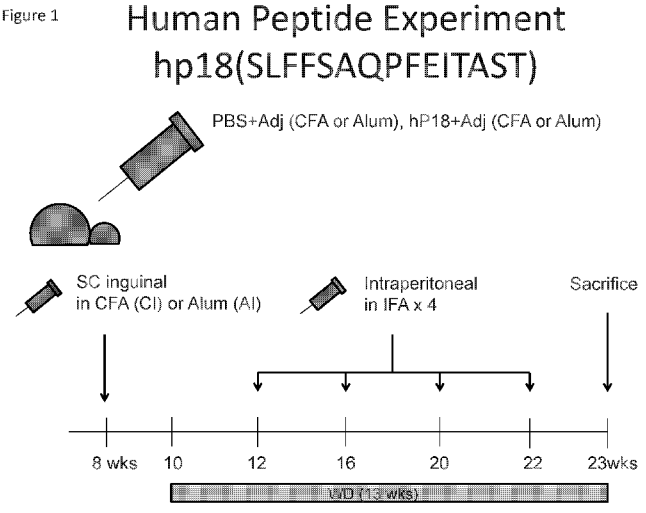
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(54) Title: HUMAN APOB100 EPITOPES, METHODS AND USES FOR MODULATING INFLAMMATORY RESPONSES, AND TREATING ADVERSE CARDIOVASCULAR EVENTS, DISEASE AND ATHEROSCLEROSIS

Figure 1



(57) Abstract: Provided herein are composition comprising novel epitopes of ApoB100, as well as sub-sequences, portions and modifications thereof, and uses thereof for treating adverse cardiovascular events, cardiovascular disease, atherosclerosis and certain liver disorders.

HUMAN APOB100 EPITOPES, METHODS AND USES FOR MODULATING INFLAMMATORY RESPONSES, AND TREATING ADVERSE CARDIOVASCULAR EVENTS, DISEASE AND ATHEROSCLEROSIS

RELATED PATENT APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/163,841 filed on May 19, 2015, entitled *Human Apob100 Epitopes, Methods And Uses For Modulating Inflammatory Responses, And Treating Adverse Cardiovascular Events, Disease And Atherosclerosis*, naming Klaus Ley as inventor, and designated by Attorney Docket No. 051501-0436352. The entire content of the foregoing application is incorporated herein by reference, including all text, tables and drawings.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under contract/grant numbers R01 HL126543 and NIH R01 HL121697 awarded by NIH. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention relates to novel epitopes of ApoB100, or sub-sequences, portions or modifications thereof and methods and compounds thereof for treatment an adverse cardiovascular event or cardiovascular disease, including atherosclerosis.

INTRODUCTION

[0004] Atherosclerosis is an inflammatory disease of the arterial wall characterized by monocytes entering the subendothelial space where they differentiate into macrophages and foam cells (Lusis, Nature 407: 233 (2000); Glass & Witztum, Cell 104: 503 (2001); and Galkina & Ley, Annu. Rev. Immunol. 27: 165 (2009); Li & Glass, Nat. Med. 8: 1235 (2002)). Foam cell formation induced by oxidized low density lipoprotein (oxLDL) leads to induction of pro-inflammatory factors that initiate plaque formation and finally plaque rupture with deleterious clinical consequences like myocardial infarction or stroke. oxLDL-induced foam cell formation is promoted by scavenger receptors like CD36 and SR-A, which allow uncontrolled accumulation of modified LDL cholesterol in foam cells (Libby et al., Am. J. Med. 104: 14S (1998); and Kunjathoor et al., J. Biol. Chem. 277: 49982 (2002)).

[0005] ApoB-100 is an apolipoprotein of about 4500 amino acids long and has been shown to be a component involved in the development of atherosclerosis. ApoB100 has previously been suggested as being an autoantigen relevant to atherosclerosis (1). However the regions of this apolipoprotein that activate T cells through T cell receptors to induce inflammatory responses in atherosclerosis are hitherto not known.

SUMMARY

[0006] The invention is based, in part, on the discovery of novel ApoB100 T cell epitopes and use of such epitopes in atherogenic and vaccination methods. In particular embodiments, the invention provides proteins and peptides comprising an amino acid sequence of ApoB100, and sub-sequences, portions or modifications, and methods and compounds comprising such protein and peptides for the treatment of an adverse cardiovascular event or cardiovascular disease, including in certain embodiments atherosclerosis.

[0007] Thus, in one aspect there is provided a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1 or a sub-sequence, portion, homologue, variant or derivative thereof. In some embodiments, a peptide comprises the amino acid sequence SLFFSAQPFEITAST (p18) (SEQ ID NO: 9), the amino acid sequence IKHIYAISSAALSAS (p9) (SEQ ID NO: 13) or the amino acid sequence of P101, P102 or P103.

[0008] In different particular embodiments, a protein or peptide of the present invention modifies an immune or inflammatory response; modifies a T cell response; induces, promotes, increases or enhances an immune response; elicits, stimulates, induces, promotes, increases or enhances a T cell response; decreases, reduces, inhibits, suppresses or disrupts an immune or inflammatory response; or elicits, stimulates, induces, promotes, increases or enhances an anti- immune or anti- inflammatory response.

[0009] In other embodiments, a protein or peptide of the present invention has prophylactic properties against an adverse cardiovascular event or cardiovascular disease, including atherosclerosis, or against liver disease, disorder or damage.

[0010] In another aspect, the protein or peptide has a length from about 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, 50-75 or 75-100 amino acids. In another aspect, the protein or peptide further comprises a heterologous domain.

[0011] In another aspect, the protein or peptide has a function or activity distinct from wild-type full length ApoB100. In an additional embodiment, the function or activity comprises protecting against or reducing the risk of atherosclerosis or protecting against liver disease, disorder or damage. In a further embodiment, the function or activity comprises protecting against an adverse cardiovascular event or cardiovascular disease or reducing the risk of a subject to an adverse cardiovascular event or cardiovascular disease. In an additional aspect, the protein or peptide is isolated or purified.

[0012] In another aspect, there is provided a nucleic acid encoding a protein or peptide of the present invention. In yet another aspect, there is provided a cell expressing a protein or peptide of the present invention. In different embodiments, the cell may be a eukaryotic or prokaryotic cell including for example a mammalian, insect, fungal or bacterium cell. In another embodiment, the cell is a dendritic cell.

[0013] In another aspect there is provided, a composition comprising a protein or peptide of the present invention. In particular embodiments, a composition of the present invention may comprise an adjuvant. In additional embodiments, the composition may further comprise a statin. In different embodiments, the composition may be a solid or a liquid. In further embodiments, the composition may be a microparticle or a nanoparticle. In particular embodiments, the composition may be a vaccine. In further embodiments, the composition is sterile. In another embodiment, the composition further comprises a salt, a chelating agent, a buffering agent or an anti-microbial agent.

[0014] In yet another aspect, there is provided, a method of vaccinating a subject against an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to vaccinate the subject against the adverse cardiovascular event or cardiovascular disease.

[0015] In another embodiment, there is provided a method of providing a subject with protection against an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any

one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to provide the subject with protection against the adverse cardiovascular event or cardiovascular disease.

[0016] In another embodiment, there is provided a method of reducing the risk of an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage in a subject, comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to reduce the risk of the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage in the subject.

[0017] In certain embodiments, the methods of vaccinating a subject against an adverse cardiovascular event or cardiovascular disease described herein comprise prophylactic vaccination against the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage. In particular embodiments, the methods of the present invention comprise vaccinating the subject against an adverse symptom of the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, including for example atherosclerosis.

[0018] In another aspect, there is provided, a method of treating a subject for an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of: the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to treat the subject for the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage.

[0019] In certain embodiments, the method of treating a subject for an adverse cardiovascular event or cardiovascular disease described herein comprises prophylactic treatment against the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage. In certain embodiments, the method of treating a subject for an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage described herein comprises treating the subject for an adverse symptom of the adverse cardiovascular

event, cardiovascular disease or liver disease, disorder or damage, including for example atherosclerosis.

[0020] In another embodiment, there is provided, a method of treating a subject for an adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to treat the subject for the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage. In further aspects, the adverse cardiovascular event or cardiovascular comprises coronary artery disease, peripheral artery disease, cerebrovascular disease, renal artery disease, stroke, myocardial infarction (heart attack), ischemic heart failure, transient ischemic attack or brain trauma. In additional aspects, the method reduces an adverse symptom of the adverse cardiovascular event, cardiovascular disease or liver disease, disorder or damage, including atherosclerosis. In further aspects, the method comprises plaque regression.

[0021] In some aspects, presented herein is a method of determining whether a subject has, has had, is at risk of having or is need of treatment for an adverse cardiovascular event or cardiovascular disease, the method comprising contacting a biological sample of the subject with an agent that comprises a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof and detecting binding of immune cells in the biological sample to the agent, wherein detection of binding of immune cells in the biological sample to the agent indicates that the subject has, has had, is at risk of having or is need of treatment for an adverse cardiovascular event or cardiovascular disease.

[0022] In certain aspects, presented herein is a method of determining the efficacy of the method of any one of claims 33 to 47, the method comprising: a) contacting a biological sample of the subject prior to performance of the method of any one of claims 33 to 47 with an agent that comprises a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof; b) detecting binding

of immune cells in the biological sample to the agent; c) performing the method of any one of claims 33 to 47; d) contacting a biological sample of the subject after performance of the method of any one of claims 33 to 47 with the agent; e) detecting binding of immune cells in the biological sample of the subject to the agent after performance of the method of any one of claims 33 to 47; and f) comparing the amount of binding of immune cells to the agent after performance of the method of any one of claims 33 to 47 to the amount of binding of immune cells in the biological sample to the agent prior to performance of the method of any one of claims 33 to 47; wherein an increase in the binding of immune cells to the agent after performance of the method of any one of claims 33 to 47 when compared to the biological sample of the subject prior to performance of the method of any one of claims 33 to 47 indicates that there is efficacy in the method of any one of claims 33 to 47.

[0023] In some embodiments, the agent is a multimer comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof and appropriate MHC-II molecule.

[0024] The some embodiments, a multimer comprises a MHC-II molecule that is DRB1*01:01, DRB1*01:02, DRB1*03:01, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05, DRB1*07:01, DRB1*09:01, DRB1*10:01, DRB1*11:01, DRB1*11:04, DRB1*12:01, DRB1*13:01, DRB1*13:02, DRB1*14:01, DRB1*15:01, DRB3*01:01, DRB3*02:02, DRB4*01:01, DRB5*01:01, DPB1*04:01, DPB1*05:01, or DQB1*06:02. In some embodiments, a multimer is a tetramer. In certain embodiments, an agent comprises a protein or peptide comprising, consisting of, or consisting essentially of IKHIYAISSAALSAS (SEQ ID NO: 13). In certain embodiments, an agent comprises a protein or peptide comprising, consisting of, or consisting essentially of an MHC-II molecule that is DPB1*02:01, DPB1*03:01, DPB1*04:02, DPB1*14:01, DQB1*02:01, DQB1*03:01, DQB1*06:02, DRB1*01:01, DRB1*04:01, DRB1*04:05, DRB1*07:01, DRB1*08:02, DRB1*09:01, DRB1*1101, DRB1*12:01, DRB1*13:02, DRB1*15:01, DRB3*01:01, DRB3*02:02, DRB4*01:01 or DRB5*01:01.

[0025] In certain embodiments, the method comprises detecting immune cell activity in the biological sample. In certain embodiments, the method comprises detecting the amount of immune cell activation, immune cell exhaustion, immune cell polarization or apoptosis of

immune cells in the biological sample. In certain embodiments, the method comprises detecting the amount of T cell activation, T cell exhaustion, T cell polarization or apoptosis of T cells in the biological sample.

[0026] In certain embodiments, a method described herein comprises administering a polypeptide to a subject, the polypeptide comprising or consisting of the amino acid sequence of SLFFSAQPFEITAST (p18) (SEQ ID NO: 9), and/or IKHIYAISSAALSAS (SEQ ID NO: 13).

[0027] In different embodiments of the presently described methods, the treatment may be administered with a statin. In further embodiments of the presently described methods, the subject may be for example a mouse or a human.

DRAWINGS

[0028] **Figure 1** shows a study diagram of human peptide 18 (SLFFSAQPFEITAST, hP18) (SEQ ID NO: 9) administration.

[0029] **Figure 2** shows reduction of aortic plaque lesion by human p18 (SLFFSAQPFEITAST, hP18) (SEQ ID NO: 9).

[0030] **Figure 3** shows a gating strategy for CD4 T cells from frozen human PBMCs. Gating for lymphocyte-sized cells (top left), singlets (top middle), live CD3+ cells (top right), CD4 and CD8 (bottom left) and P9 tetramer (bottom right).

[0031] **Figure 4** shows CD3+CD4+ human T cells from frozen PBMCs from a DRB1*0101+ (right) and a mismatched (DRB1*0101-) CVD patient were stained for CD4 (x-axis) and P9 tetramer-PE (y-axis).

[0032] **Figure 5** shows CD3+CD4+ human T cells from frozen PBMCs from DRB1*0101+ CVD patients and healthy controls (right) and mismatched (DRB1*0101-) CVD patients and healthy controls were stained for P9 tetramer-PE, MFI ± SD.

[0033] **Figure 6** shows CD3+CD4+ human T cells from frozen PBMCs were stained with P9 tetramer-PE. P9 tetramer-PE+ (red histogram) cells were back-gated (red dots) into the CD4-dump plot (left).

[0034] **Figure 7** shows expression of activation and exhaustion markers CD69, Ox40 and PD-1 on P9 tetramer+ (tet+, squares) and – (tet-, circles) CD4 T cells from two DRB1*0101 donors with preclinical cardiovascular disease. Gated on CD3+CD4+live cells. All data are percent of tet+ and tet- CD4 T cells.

[0035] **Figure 8A-B** shows plasma ALT levels determined by chemistry analyser RefloVet Plus (Roche, **Fig. 8A**) and Reflotron GPT (Roche, **Fig. 8B**) for Western diet-fed ApoE-/- mice immunized with the peptides indicated (y-axis, P101, P102 and P103) at 50 μg per injection, or were untreated (Untreated) or immunized with adjuvant alone (Adjuvant) according to the schematic shown in **Figure 8C**. Plasma was collected and stored at -80C. 8 untreated mice, 5 adjuvant mice, 5 P101 mice, 5 P102 mice, and 3 P103 mice.

DETAILED DESCRIPTION

[0036] The present inventors have discovered novel epitopes of ApoB100. In different embodiments, the proteins and peptides of the present invention comprise novel ApoB100 epitopes which may have atherosclerosis promoting (atherogenic) properties or therapeutic (protective) properties.

[0037] T cells are involved in the development of atherosclerosis and all the subsets of T cells are found in the arterial plaques. In different embodiments, the proteins and peptides of the present invention comprise T cell epitopes and may potentiate or suppress plaque formation. Thus, T cell populations reactive to the T cell epitopes of the present invention may be targets for immunotherapy. In certain embodiments of the present invention, using specific peptides comprising T cell epitopes of the present invention instead of whole ApoB-100 or LDL (low-density lipoprotein) enables modulation of the specifically activated T cells that participate in the disease processes. Previous studies using whole ApoB-100 or LDL for immunization or vaccination involve a collection of various ApoB-100 peptides that might have opposite effects.

[0038] In another aspect, the present inventors have discovered that vaccination with the proteins or peptides of the present invention may provide protection against adverse cardiovascular event or cardiovascular disease, including in certain embodiments atherosclerosis.

[0039] Thus there are presently provided proteins and peptides comprising an ApoB100 amino acid sequence, and sub-sequences, portions or modifications thereof, and methods and compounds for treating an adverse cardiovascular event or cardiovascular disease. In certain embodiments the methods of the present invention comprise protecting or vaccinating against

an adverse cardiovascular event or cardiovascular disease, including but not limited to atherosclerosis.

[0040] The present invention is based at least in part on ApoB100 peptides, sub-sequences and portions, and amino acid modifications of ApoB100 peptides, sub-sequences and portions. ApoB100 peptides, sub-sequences, portions, homologues, variants or derivatives thereof, including T cell epitopes that elicit, stimulate, induce, promote, increase or enhance an immune or inflammatory response *in vitro* or *in vivo*, are useful in vaccination and immunization (e.g., prophylactic), as well as treatment uses and methods. For example, an invention ApoB100 peptide, sub-sequence, portion or modification thereof, can be used to immunize or vaccinate a subject, or to treat a subject having or at risk of having an adverse cardiovascular event or cardiovascular disease.

[0041] The proteins and peptides of the present invention, and sub-sequences, portions, homologues, variants or derivatives thereof, include T cell epitopes. As used herein an "epitope" refers to a peptide or part thereof that elicits an immune response when administered to a subject. In certain embodiments of the present invention, the epitope is a region of an ApoB100 protein that elicits an immune response when administered to a subject. As will be understood by a person skilled in the art, an immune response may be a cellular or humoral immune response and may comprise an antibody response, a T cell response or both an antibody and T cell response. In particular embodiments of the present invention, a protein or peptide of the present invention comprises an amino acid sequence of ApoB100 that is a T cell epitope.

[0042] A non-limiting example of an ApoB100 polypeptide sequence from which peptides, sub-sequences, portions, homologues, variants, derivatives and T cell epitopes may be derived is as follows (SEQ ID NO.: 1):

MDPPRPALLALLALPALLLLLLAGARAEEEMLENVSLVCPKDATRFKHLRKYTYNY
EAESSSGVPGTADSRSATRINCKVELEVPQLCSFILKTSQCTLKEVYGFNPEGKALLK
KTKNSEEFAAAMSRYELKLAIPEGKQVFLYPEKDEPTYILNIKRGIISALLVPPETEEA
KQVLFLDTVYGNCSTHFTVKTRKGNVATEISTERDLGQCDRFKPIRTGISPLALIKGM
TRPLSTLISSSQSCQYTLDAKRKHVAEAICKEQHLFLPFSYKNKYGMVAQVTQTLKL
EDTPKINSRFFGEGTKKMGLAFESTKSTSPPKQAEAVLKTLQELKKLTISEQNIQRAN
LFNKLVTELRGLSDEAVTSLLPQLIEVSSPITLQALVQCGQPQCSTHILQWLKRVHAN

PLLIDVVTYLVALIPEPSAQQLREIFNMARDQRSRATLYALSHAVNNYHKTNPTGTQ
ELLDIANYLMEQIQDDCTGDEDYTYLILRVIGNMGQTMEQLTPELKSSILKCVQSTKP
SLMIQKAAIQALRKMEPKDKDQEVLLQTFLDDASPGDKRLAAYLMLMRSPSQADIN
KIVQILPWEQNEQVKNFVASHIANILNSEELDIQDLKKLVKEALKESQLPTVMDFRKF
SRNYQLYKSVSLPSLDPASAKIEGNLIFDPNNYLPKESMLKTTLTAFGFASADLIEIGL
EGKGFEPTLEALFGKQGFFPDSVNKALYWVNGQVPDGVSKVLVDHFGYTKDDKHE
QDMVNGIMLSVEKLIKDLKSKEVPEARAYLRILGEELGFASLHDLQLLGKLLLMGAR
TLQGIPQMIGEVIRKGSKNDFFLHYIFMENAFELPTGAGLQLQISSSGVIAPGAKAGV
KLEVANMQAELVAKPSVSVEFVTNMGIIIPDFARSGVQMNTNFFHESGLEAHVALK
AGKLKFIIPSPKRPVKLLSGGNTLHLVSTTKTEVIPPLIENRQSWSVCKQVFPGLNYCT
SGAYSNASSTDSASYYPLTGDTRLELELRPTGEIEQYSVSATYELQREDRALVDTLKF
VTQAEGAKQTEATMTFKYNRQSMTLSSEVQIPDFDVDLGTILRVNDESTEGKTSYRL
TLDIQNKKITEVALMGHLSCDTKEERKIKGVISIPRLQAEARSEILAHWSPAKLLLQM
DSSATAYGSTVSKRVAWHYDEEKIEFEWNTGTNVDTKKMTSNFPVDLSDYPKSLH
MYANRLLDHRVPQTDMTFRHVGSKLIVAMSSWLQKASGSLPYTQTLQDHLNSLKE
FNLQNMGLPDFHIPENLFLKSDGRVKYTLNKNSLKIEIPLPFGGKSSRDLKMLETVRT
PALHFKSVGFHLPSREFQVPTFTIPKLYQLQVPLLGVLDLSTNVYSNLYNWSASYSG
GNTSTDHFSLRARYHMKADSVVDLLSYNVQGSGETTYDHKNTFTLSCDGSLRHKFL
DSNIKFSHVEKLGNNPVSKGLLIFDASSSWGPQMSASVHLDSKKKQHLFVKEVKIDG
QFRVSSFYAKGTYGLSCQRDPNTGRLNGESNLRFNSSYLQGTNQITGRYEDGTLSLT
STSDLQSGIIKNTASLKYENYELTLKSDTNGKYKNFATSNKMDMTFSKQNALLRSEY
QADYESLRFFSLLSGSLNSHGLELNADILGTDKINSGAHKATLRIGQDGISTSATTNLK
CSLLVLENELNAELGLSGASMKLTTNGRFREHNAKFSLDGKAALTELSLGSAYQAMI
LGVDSKNIFNFKVSQEGLKLSNDMMGSYAEMKFDHTNSLNIAGLSLDFSSKLDNIYS
SDKFYKQTVNLQLQPYSLVTTLNSDLKYNALDLTNNGKLRLEPLKLHVAGNLKGAY
QNNEIKHIYAISSAALSASYKADTVAKVQGVEFSHRLNTDIAGLASAIDMSTNYNSD
SLHFSNVFRSVMAPFTMTIDAHTNGNGKLALWGEHTGQLYSKFLLKAEPLAFTFSH
DYKGSTSHHLVSRKSISAALEHKVSALLTPAEQTGTWKLKTQFNNNEYSQDLDAYN
TKDKIGVELTGRTLADLTLLDSPIKVPLLLSEPINIIDALEMRDAVEKPQEFTIVAFVK
YDKNQDVHSINLPFFETLQEYFERNRQTIIVVLENVQRNLKHINIDQFVRKYRAALGK
LPQQANDYLNSFNWERQVSHAKEKLTALTKKYRITENDIQIALDDAKINFNEKLSQL

QTYMIQFDQYIKDSYDLHDLKIAIANIIDEIIEKLKSLDEHYHIRVNLVKTIHDLHLFIE
NIDFNKSGSSTASWIQNVDTKYQIRIQIQEKLQQLKRHIQNIDIQHLAGKLKQHIEAID
VRVLLDQLGTTISFERINDVLEHVKHFVINLIGDFEVAEKINAFRAKVHELIERYEVD
QQIQVLMDKLVELAHQYKLKETIQKLSNVLQQVKIKDYFEKLVGFIDDAVKKLNEL
SFKTFIEDVNKFLDMLIKKLKSFDYHQFVDETNDKIREVTQRLNGEIQALELPQKAEA
LKLFLEETKATVAVYLESLQDTKITLIINWLQEALSSASLAHMKAKFRETLEDTRDR
MYQMDIQQELQRYLSLVGQVYSTLVTYISDWWTLAAKNLTDFAEQYSIQDWAKRM
KALVEQGFTVPEIKTILGTMPAFEVSLQALQKATFQTPDFIVPLTDLRIPSVQINFKDL
KNIKIPSRFSTPEFTILNTFHIPSFTIDFVEMKVKIIRTIDQMLNSELQWPVPDIYLRDLK
VEDIPLARITLPDFRLPEIAIPEFIIPTLNLNDFQVPDLHIPEFQLPHISHTIEVPTFGKLYS
ILKIQSPLFTLDANADIGNGTTSANEAGIAASITAKGESKLEVLNFDFQANAQLSNPKI
NPLALKESVKFSSKYLRTEHGSEMLFFGNAIEGKSNTVASLHTEKNTLELSNGVIVKI
NNQLTLDSNTKYFHKLNIPKLDFSSQADLRNEIKTLLKAGHIAWTSSGKGSWKWAC
PRFSDEGTHESQISFTIEGPLTSFGLSNKINSKHLRVNQNLVYESGSLNFSKLEIQSQVD
SQHVGHSVLTAKGMALFGEGKAEFTGRHDAHLNGKVIGTLKNSLFFSAQPFEITAST
NNEGNLKVRFPLRLTGKIDFLNNYALFLSPSAQQASWQVSARFNQYKYNQNFSAGN
NENIMEAHVGINGEANLDFLNIPLTIPEMRLPYTIITTPPLKDFSLWEKTGLKEFLKTT
KQSFDLSVKAQYKKNKHRHSITNPLAVLCEFISQSIKSFDRHFEKNRNNALDFVTKSY
NETKIKFDKYKAEKSHDELPRTFQIPGYTVPVVNVEVSPFTIEMSAFGYVFPKAVSMP
SFSILGSDVRVPSYTLILPSLELPVLHVPRNLKLSLPDFKELCTISHIFIPAMGNITYDFS
FKSSVITLNTNAELFNQSDIVAHLLSSSSSVIDALQYKLEGTTRLTRKRGLKLATALSL
SNKFVEGSHNSTVSLTTKNMEVSVATTTKAQIPILRMNFKQELNGNTKSKPTVSSSM
EFKYDFNSSMLYSTAKGAVDHKLSLESLTSYFSIESSTKGDVKGSVLSREYSGTIASE
ANTYLNSKSTRSSVKLQGTSKIDDIWNLEVKENFAGEATLQRIYSLWEHSTKNHLQL
EGLFFTNGEHTSKATLELSPWQMSALVQVHASQPSSFHDFPDLGQEVALNANTKNQ
KIRWKNEVRIHSGSFQSQVELSNDQEKAHLDIAGSLEGHLRFLKNIILPVYDKSLWDF
LKLDVTTSIGRRQHLRVSTAFVYTKNPNGYSFSIPVKVLADKFIIPGLKLNDLNSVLV
MPTFHVPFTDLQVPSCKLDFREIQIYKKLRTSSFALNLPTLPEVKFPEVDVLTKYSQPE
DSLIPFFEITVPESQLTVSQFTLPKSVSDGIAALDLNAVANKIADFELPTIIVPEQTIEIPS
IKFSVPAGIVIPSFQALTARFEVDSPVYNATWSASLKNKADYVETVLDSTCSSTVQFL
EYELNVLGTHKIEDGTLASKTKGTFAHRDFSAEYEEDGKYEGLQEWEGKAHLNIKS

PAFTDLHLRYQKDKKGISTSAASPAVGTVGMDMDEDDDFSKWNFYYSPQSSPDKKL
TIFKTELRVRESDEETQIKVNWEEEAASGLLTSLKDNVPKATGVLYDYVNKYHWEH
TGLTLREVSSKLRRNLQNNAEWVYQGAIRQIDDIDVRFQKAASGTTGTYQEWKDKA
QNLYQELLTQEGQASFQGLKDNVFDGLVRVTQEFHMKVKHLIDSLIDFLNFPRFQFP
GKPGIYTREELCTMFIREVGTVLSQVYSKVHNGSEILFSYFQDLVITLPFELRKHKLID
VISMYRELLKDLSKEAQEVFKAIQSLKTTEVLRNLQDLLQFIFQLIEDNIKQLKEMKF
TYLINYIQDEINTIFSDYIPYVFKLLKENLCLNLHKFNEFIQNELQEASQELQQIHQYIM
ALREEYFDPSIVGWTVKYYELEEKIVSLIKNLLVALKDFHSEYIVSASNFTSQLSSQVE
QFLHRNIQEYLSILTDPDGKGKEKIAELSATAQEIIKSQAIATKKIISDYHQQFRYKLQ
DFSDQLSDYYEKFIAESKRLIDLSIQNYHTFLIYITELLKKLQSTTVMNPYMKLAPGEL
TIIL

[0043] T cell epitopes typically are short amino acid sequences, e.g. about five to 15 amino acids in length (or 5-10 amino acids in length). A T cell epitope can elicit, stimulate, induce, promote, increase, enhance an immune or inflammatory T cell response *in vitro* (e.g., in solution, in solid phase, in tissue culture) or *in vivo*. Such T cell responses can be detected using various assays disclosed herein or known to the skilled artisan.

[0044] A protein or peptide of the present invention includes a protein or peptide comprising, consisting or consisting essentially of an amino acid sequence of ApoB100, or a sub-sequence, portion, homologue, variant or derivative thereof.

[0045] In different embodiments of the present invention, the protein or peptide, or sub-sequence, portion, homologue, variant or derivative thereof, comprises, consists, or consists essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof.

[0046] As described herein, proteins and peptides include homologues. A polynucleotide sequence or polypeptide sequence is a "homologue" of, or is "homologous" to, another sequence if the two sequences have substantial identity over a specified region and a functional activity of the sequences is preserved or conserved, at least in part (as used herein, the term 'homologous' does not infer evolutionary relatedness). Two polynucleotide sequences or polypeptide sequences are considered to have substantial identity if, when optimally aligned (with gaps permitted), they share at least about 60% sequence identity or greater (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, etc.

identify over a specific region), or if the sequences share defined functional motifs (e.g., epitopes). The percent identity can extend over the entire sequence length or a portion of the sequence. In particular aspects, the length of the sequence sharing the percent identity is 2, 3, 4, 5 or more contiguous amino acids, e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, etc. contiguous amino acids.

[0047] An "unrelated" or "non-homologous" sequence shares less than 50% identity.

[0048] The term "identity" and grammatical variations thereof, mean that two or more referenced entities are the same. Thus, where two proteins or peptides, or sub-sequences, portions, homologues, variants or derivatives thereof are identical, they have the same amino acid sequence. The identity can be over a defined area (region or domain) of the sequence. "Areas, regions or domains" of homology or identity mean that a portion of two or more referenced entities share homology or are the same.

[0049] The extent of identity between two sequences can be ascertained using a computer program and mathematical algorithm known in the art. Such algorithms that calculate percent sequence identity (homology) generally account for sequence gaps and mismatches over the comparison region or area. For example, a BLAST (*e.g.*, BLAST 2.0) search algorithm (see, *e.g.*, Altschul et al., *J. Mol. Biol.* 215:403 (1990), publicly available through NCBI) has exemplary search parameters as follows: Mismatch -2; gap open 5; gap extension 2. For polypeptide sequence comparisons, a BLASTP algorithm is typically used in combination with a scoring matrix, such as PAM100, PAM 250, BLOSUM 62 or BLOSUM 50. FASTA (e.g., FASTA2 and FASTA3) and SSEARCH sequence comparison programs are also used to quantitate the extent of identity (Pearson et al., *Proc. Natl. Acad. Sci.* USA 85:2444 (1988); Pearson, *Methods Mol Biol.* 132:185 (2000); and Smith et al., *J. Mol. Biol.* 147:195 (1981)). Programs for quantitating protein structural similarity using Delaunay-based topological mapping have also been developed (Bostick et al., *Biochem Biophys Res Commun.* 304:320 (2003)).

[0050] Thus in certain embodiments, the protein or peptide of the present invention comprises, consists, or consists essentially of homologue of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof.

[0051] Additional proteins or peptides, or sub-sequences, portions, homologues, variants or derivatives thereof can be based upon or derived from an ApoB100 amino acid sequence. For example, a protein or peptide of the present invention can comprise an amino acid sequence having 60% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%) sequence identity with a region of ApoB100. Thus, as disclosed herein, in particular embodiments, the proteins or peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof include those having at least partial sequence identity to one or more ApoB100 peptides, sub-sequences, portions, homologues, variants or derivatives thereof set forth as any one of the peptides set forth in in Table 1. The percent identity of such sequences can be as little as 60%, or can be greater (e.g., 60%, 65%, 70%, 75%, 75% 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, etc.). The percent identity can extend over the entire sequence length or a portion of the sequence. In particular aspects, the length of the sequence sharing the percent identity is 2, 3, 4, 5 or more contiguous amino acids, e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, etc. contiguous amino acids.

[0052] Thus, in accordance with the invention, there are also provided proteins or peptides or sub-sequences, portions, homologues, variants or derivatives thereof that exhibit sequence identity to a reference ApoB100 peptide, sub-sequence or portion, or modification thereof set forth as any one of the peptides set forth in in Table 1. In one embodiment, a protein or peptide of the present invention, or a sub-sequence, portion, homologue, variant or derivative thereof comprises, consists or consists essentially of a sequence at least 60% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) identical to a reference ApoB100 protein or peptide, or sub-sequence, portion, homologue, variant or derivative thereof as set forth as any one of the peptides set forth in in Table 1.

[0053] In another embodiment, proteins or peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof, include or consist of a an ApoB100 peptide, sub-sequence or portion thereof set forth as any one of the peptides set forth in in Table 1wherein the protein or peptide or sub-sequence, portion, homologue, variant or derivative thereof has one or more modifications, such as an amino acid addition to, deletion of, or substitution of any amino acid residue in any peptide set forth as any one of the peptides set forth in in Table 1. In particular aspects, a modified sequence is at least 80% or more, e.g., 80-85%, 85-90%, 90-95%, 95-100% identical, to a ApoB100 peptide, or sub-

sequence, portion, homologue or derivative thereof set forth as any one of the peptides set forth in in Table 1 or has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-25, 25-30, 30-50, 50-100, or more, additions to, deletions of, or substitutions.

[0054] Thus, in accordance with the invention, modified and variant forms of ApoB100 peptides, sub-sequences, portions, homologues or derivatives thereof are provided. Such forms, referred to as "modifications" or "variants" and grammatical variations thereof, mean an ApoB100 peptide, sub-sequence, portion, homologue or derivative thereof that deviates from a reference sequence. Such modifications may have greater or less activity or function than a reference ApoB100 peptide, sub-sequence or portion thereof, such as ability to elicit, stimulate, induce, promote, increase or enhance T cell response or immune or inflammatory response. Thus, proteins or peptides of the present invention, sub-sequences, portions, homologues, variants or derivatives thereof, include sequences having substantially the same, greater or less relative activity or function as a T cell epitope than a reference T cell epitope set forth as any one of the peptides set forth in in Table 1, for example, an ability to elicit, stimulate, induce, promote, increase or enhance a T response *in vitro* or *in vivo*.

[0055] Non-limiting examples of modifications include one or more amino acid substitutions (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-25, 25-30, 30-50, 50-100, or more residues), additions and insertions (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-25, 25-30, 30-50, 50-100, or more residues) and deletions (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-25, 25-30, 30-50, 50-100) of a reference ApoB100 peptide, sub-sequence, portion, homologue or derivative thereof. In particular embodiments, a modified or variant sequence retains at least part of a function or an activity of unmodified sequence, which can have less than, approximately the same, or greater, but at least a part of, a function or activity of a reference sequence, for example, the ability to elicit, stimulate, induce, promote, increase or enhance a T cell response *in vitro* or *in vivo*. Such T cell responses elicited include, for example, among others, induced, increased, enhanced, stimulated or activated expression or production of a cytokine (e.g., IL-1α, IL-2, IL-5, IL-6, IL-10, IL-17a, IFN-γ, TNF, GM-CSF).

[0056] An addition can be the covalent or non-covalent attachment of any type of molecule to the sequence. Specific examples of additions include glycosylation, acetylation, phosphorylation, amidation, formylation, ubiquitination, and derivatization by

protecting/blocking groups and any of numerous chemical modifications. Additional specific non-limiting examples of an addition is one or more additional amino acid residues. Accordingly, proteins or peptides of the present invention including T cell epitopes, sub-sequences, portions, homologues or derivatives thereof can be a part of or contained within a larger molecule, such as another peptide sequence, such as a fusion, heterologous domain or chimera with another ApoB100 sequence, or a non-ApoB100 peptide sequence. In particular embodiments, an addition is a fusion (chimeric) sequence or heterologous domain, an amino acid sequence having one or more molecules not normally present in a reference native (wild type) sequence covalently attached to the sequence.

[0057] The term "chimeric" and grammatical variations thereof, when used in reference to a sequence, means that the sequence contains one or more portions that are derived from, obtained or isolated from, or based upon other physical or chemical entities. For example, a chimera of two or more different proteins may have one part an ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof, and a second part of the chimera may be from a non-ApoB100 sequence.

[0058] Another particular example of a modified sequence having an amino acid addition is one in which a second heterologous sequence, i.e., heterologous functional domain is attached (covalent or non-covalent binding) that confers a distinct or complementary function. Heterologous functional domains are not restricted to amino acid residues. Thus, a heterologous functional domain can consist of any of a variety of different types of small or large functional moieties. Such moieties include nucleic acid, peptide, carbohydrate, lipid or small organic compounds, such as a drug (e.g., an antiviral), a metal (gold, silver), and radioisotope. For example, a tag such as T7 or polyhistidine can be attached in order to facilitate purification or detection of a T cell epitope. Thus, in other embodiments the invention provides a protein or peptide comprising an ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof and a heterologous domain, wherein the heterologous functional domain confers a distinct function, on the ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof. For example, a fusion protein or chimera may comprise an immunoglobulin fusion protein or a monoclonal antibody fusion protein. Such constructs containing an ApoB100 peptide, or sub-sequence, portion,

homologue, variant or derivative thereof and a heterologous domain are also referred to as chimeras.

[0059] Linkers, such as amino acid or peptidomimetic sequences may be inserted between the sequence and the addition (e.g., heterologous functional domain) so that the two entities maintain, at least in part, a distinct function or activity. Linkers may have one or more properties that include a flexible conformation, an inability to form an ordered secondary structure or a hydrophobic or charged character which could promote or interact with either domain. Amino acids typically found in flexible protein regions include Gly, Asn and Ser. Other near neutral amino acids, such as Thr and Ala, may also be used in the linker sequence. The length of the linker sequence may vary without significantly affecting a function or activity of the fusion protein (see, e.g., U.S. Patent No. 6,087,329). Linkers further include chemical moieties and conjugating agents, such as sulfo-succinimidyl derivatives (sulfo-SMCC, sulfo-SMPB), disuccinimidyl suberate (DSS), disuccinimidyl glutarate (DSG) and disuccinimidyl tartrate (DST).

[0060] Further non-limiting examples of additions are detectable labels. Thus, in another embodiment, the invention provides the presently described proteins or peptides, or sub-sequences, portions, homologues, variants or derivatives thereof that are detectably labelled. Specific examples of detectable labels include fluorophores, chromophores, radioactive isotopes (e.g., S^{35}, P^{32}, I^{125}), electron-dense reagents, enzymes, ligands and receptors. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert a substrate such as 3,3-',5,5-'-tetramethylbenzidine (TMB) to a blue pigment, which can be quantified.

[0061] Another non-limiting example of an addition is an insertion of an amino acid within a ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof sequence, sub-sequence, portions or modification thereof, set forth as any one of the peptides set forth in in Table 1. In particular embodiments, an insertion is of one or more amino acid residues inserted into an ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof, set forth as any one of the peptides set forth in in Table 1.

[0062] Specific non-limiting examples of substitutions include conservative and non-conservative amino acid substitutions. A "conservative substitution" is the replacement of one amino acid by a biologically, chemically or structurally similar residue. Biologically

similar means that the substitution does not destroy a biological activity. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine, or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, serine for threonine, and the like.

[0063] Modified and variant proteins and peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof also include one or more D-amino acids substituted for L-amino acids (and mixtures thereof), structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues and derivatized forms. Modifications include cyclic structures such as an end-to-end amide bond between the amino and carboxy-terminus of the molecule or intra- or inter-molecular disulphide bond. Proteins and peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof, may be modified in vitro or *in vivo*, e.g., post-translationally modified to include, for example, sugar residues, phosphate groups, ubiquitin, fatty acids, lipids, etc.

[0064] Specific non-limiting examples of modifications include at least one amino acid deletion from ApoB100 peptide sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. In particular embodiments, a peptide sub-sequence or portion is from about 1 to 14 amino acids in length, provided that said sub-sequence or portion is at least one amino acid less in length than the full-length ApoB100 peptide sequence set forth as any one of the peptides set forth in in Table 1, or a homologue, variant or derivative thereof. In additional particular embodiments, a peptide sub-sequence or portion is from about 1 to 5, 5 to 10, 10 to 14, amino acids in length, provided that said sub-sequence or portion is at least one amino acid less in length than the full-length ApoB100 peptide sequence set forth as any one of the peptides set forth in in Table 1, or a homologue, variant or derivative thereof.

[0065] As used herein, a sub-sequence of a protein or peptide includes or consists of one or more amino acids less than the full-length protein or peptide. The term "sub-sequence" means a fragment or part of the full-length molecule. A sub-sequence of a protein or peptide

has one or more amino acids less than the full-length protein or peptide (*e.g.* one or more internal or terminal amino acid deletions from either amino or carboxy-termini). Sub-sequences therefore can be any length up to the full-length native molecule, provided said length is at least one amino acid less than full-length native molecule.

[0066] Sub-sequences can vary in size. For example a sub-sequence of a protein or peptide can be as small as an epitope capable of binding an antibody (*i.e.* about five amino acids) up to a polypeptide that is one amino acid less than the entire length of a reference protein or peptide.

[0067] In various embodiments, a sub-sequence of the protein or peptides of the present invention is characterized as including or consisting of a sub-sequence of an ApoB100 peptide set forth as any one of the peptides set forth in in Table 1, or homologue, variant or derivative thereof.

[0068] As used herein, sub-sequences may also include or consist of one or more amino acid additions or deletions, wherein the sub-sequence does not comprise the full-length ApoB100 peptide set forth as any one of the peptides set forth in in Table 1, or a homologue, variant or derivative thereof. Accordingly, total sub-sequence lengths can be greater than the length of the full-length ApoB100 peptide set forth as any one of the peptides set forth in in Table 1, a homologue, variant or derivative thereof, for example, where a sub-sequence of anApoB100 peptide set forth as any one of the peptides set forth in in Table 1is fused or forms a chimera with another polypeptide.

[0069] Proteins or peptides of the present invention, or sub-sequences, portions, homologues, derivative thereof including variant or modified forms can be produced by any of a variety of standard protein purification or recombinant expression techniques. For example, a peptide, sub-sequence, portion, homologue, derivative or variant thereof can be produced by standard peptide synthesis techniques, such as solid-phase synthesis. A portion of the protein may contain an amino acid sequence such as a T7 tag or polyhistidine sequence to facilitate purification of expressed or synthesized protein. The protein may be expressed in a cell and purified. The protein may be expressed as a part of a larger protein (e.g., a fusion or chimera) by recombinant methods.

[0070] Proteins or peptides of the present invention, or sub-sequences, portions, homologues, derivative or variants thereof can be made using recombinant DNA technology

via cell expression or *in vitro* translation. Polypeptide sequences including modified forms can also be produced by chemical synthesis using methods known in the art, for example, an automated peptide synthesis apparatus (see, *e.g.*, Applied Biosystems, Foster City, CA).

[0071] The invention provides isolated and/or purified protein or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. In particular embodiments, the isolated and/or purified proteins or peptides, or sub-sequence, portion, homologue, variant or derivative thereof includes a T cell epitope.

[0072] The term "isolated," when used as a modifier of a composition (e.g., ApoB100 peptides, or sub-sequences, portions, homologues, variants or derivatives thereof, nucleic acids encoding same, etc.), means that the compositions are made by the hand of man or are separated, completely or at least in part, from their naturally occurring *in vivo* environment. Generally, isolated compositions are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein, nucleic acid, lipid, carbohydrate, cell membrane. The term "isolated" does not exclude alternative physical forms of the composition, such as fusions/chimeras, multimers/oligomers, modifications (e.g., phosphorylation, glycosylation, lipidation) or derivatized forms, or forms expressed in host cells produced by the hand of man.

[0073] An "isolated" composition (e.g., ApoB100 peptides, or sub-sequences, portions, homologues, variants or derivatives thereof) can also be "substantially pure" or "purified" when free of most or all of the materials with which it typically associates with in nature. Thus, an isolated ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof that also is substantially pure or purified does not include polypeptides or polynucleotides present among millions of other sequences, such as peptides of a peptide library or nucleic acids in a genomic or cDNA library, for example.

[0074] A "substantially pure" or "purified" composition can be combined with one or more other molecules. Thus, "substantially pure" or "purified" does not exclude combinations of compositions, such as combinations of ApoB100 peptides, or sub-sequences, portions, homologues, variants or derivatives thereof (e.g., multiple T cell epitopes), and combination of an ApoB100 peptide, or sub-sequence, portion, homologue, variant or derivative thereof with other antigens, agents, drugs or therapies.

[0075] The invention also provides nucleic acids encoding proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. Such nucleic acid sequences encode a peptide sequence at least 75% or more (e.g., 75%, 80%, 85%, 90%, 95%, etc.) identical to an ApoB100 amino acid sequence set forth as any one of the peptides set forth in in Table 1. In an additional embodiment, a nucleic acid encodes a sequence having a modification, such as one or more amino acid additions (insertions), deletions or substitutions of an ApoB100 amino acid sequence set forth as any one of the peptides set forth in in Table 1.

[0076] The terms "nucleic acid," "polynucleotide" and "polynucleoside" and the like refer to at least two or more ribo- or deoxy-ribonucleic acid base pairs (nucleotides/nucleosides) that are linked through a phosphoester bond or equivalent. Nucleic acids include polynucleotides and polynucleosides. Nucleic acids include single, double or triplex, circular or linear, molecules. Exemplary nucleic acids include but are not limited to: RNA, DNA, cDNA, genomic nucleic acid, naturally occurring and non naturally occurring nucleic acid, e.g., synthetic nucleic acid.

[0077] Nucleic acids can be of various lengths. Nucleic acid lengths typically range from about 20 bases to 20 Kilobases (Kb), or any numerical value or range within or encompassing such lengths, 10 bases to 10Kb, 1 to 5 Kb or less, 1000 to about 500 bases or less in length. Nucleic acids can also be shorter, for example, 100 to about 500 bases, or from about 12 to 25, 25 to 50, 50 to 100, 100 to 250, or about 250 to 500 bases in length, or any numerical value or range or value within or encompassing such lengths. In particular aspects, a nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000 bases, or any numerical value or range within or encompassing such lengths. Shorter nucleic acids are commonly referred to as "oligonucleotides" or "probes" of single- or double-stranded DNA. However, there is no upper limit to the length of such oligonucleotides.

[0078] Nucleic acid sequences further include nucleotide and nucleoside substitutions, additions and deletions, as well as derivatized forms and fusion/chimeric sequences (*e.g.*, encoding recombinant polypeptide). For example, due to the degeneracy of the genetic code, nucleic acids include sequences and sub-sequences degenerate with respect to nucleic acids

that encode an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, as well as variants and modifications thereof (e.g., substitutions, additions, insertions and deletions).

[0079] Nucleic acids can be produced using various standard cloning and chemical synthesis techniques. Techniques include, but are not limited to nucleic acid amplification, *e.g.,* polymerase chain reaction (PCR), with genomic DNA or cDNA targets using primers (e.g., a degenerate primer mixture) capable of annealing to the encoding sequence. Nucleic acids can also be produced by chemical synthesis (e.g., solid phase phosphoramidite synthesis) or transcription from a gene. The sequences produced can then be translated *in vitro*, or cloned into a plasmid and propagated and then expressed in a cell (e.g., a host cell such as eukaryote or mammalian cell, yeast or bacteria, in an animal or in a plant).

[0080] Nucleic acid may be inserted into a nucleic acid construct in which expression of the nucleic acid is influenced or regulated by an "expression control element." An "expression control element" refers to a nucleic acid sequence element that regulates or influences expression of a nucleic acid sequence to which it is operatively linked. Expression control elements include, as appropriate, promoters, enhancers, transcription terminators, gene silencers, a start codon (*e.g.*, ATG) in front of a protein-encoding gene, etc.

[0081] An expression control element operatively linked to a nucleic acid sequence controls transcription and, as appropriate, translation of the nucleic acid sequence. Expression control elements include elements that activate transcription constitutively, that are inducible (*i.e.*, require an external signal for activation), or derepressible (*i.e.*, require a signal to turn transcription off; when the signal is no longer present, transcription is activated or "derepressed"), or specific for cell-types or tissues (i.e., tissue-specific control elements).

[0082] Nucleic acid can also be inserted into a plasmid for propagation into a host cell and for subsequent genetic manipulation. A plasmid is a nucleic acid that can be propagated in a host cell, plasmids may optionally contain expression control elements in order to drive expression of the nucleic acid encoding an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof, in the host cell. A vector is used herein synonymously with a plasmid and may also include an expression control element for expression in a host cell (e.g., expression vector). Plasmids

and vectors generally contain at least an origin of replication for propagation in a cell and a promoter. Plasmids and vectors are therefore useful for genetic manipulation and expression of proteins or peptides of the present invention, or sub-sequences, portion, homologues, variants or derivatives thereof. Accordingly, vectors that include nucleic acids encoding or complementary to an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof are provided.

[0083] In accordance with the invention, there are provided particles (e.g., viral particles) and transformed host cells that express and/or are transformed with a nucleic acid that encodes and/or express an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof. Particles and transformed host cells include but are not limited to virions, and prokaryotic and eukaryotic cells such as bacteria, fungi (yeast), plant, insect, and animal (*e.g.,* mammalian, including primate and human, CHO cells and hybridomas) cells. For example, bacteria transformed with recombinant bacteriophage nucleic acid, plasmid nucleic acid or cosmid nucleic acid expression vectors; yeast transformed with recombinant yeast expression vectors; plant cell systems infected with recombinant virus expression vectors (*e.g.,* cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.,* Ti plasmid); insect cell systems infected with recombinant virus expression vectors (*e.g.,* baculovirus); and animal cell systems infected with recombinant virus expression vectors (*e.g.,* retroviruses, adenovirus, vaccinia virus), or transformed animal cell systems engineered for stable expression. The cells may be a primary cell isolate, cell culture (*e.g.,* passaged, established or immortalized cell line), or part of a plurality of cells, or a tissue or organ *ex vivo* or in a subject (*in vivo*). Transfected or transformed cells of the invention may include tolerogenic dendritic cells or other antigen presenting cells that may enhance the disease reducing activity of the ApoB100 protein or peptide of the invention.

[0084] The term "transformed" or "transfected" when used in reference to a cell (*e.g.,* a host cell) or organism, means a genetic change in a cell following incorporation of an exogenous molecule, for example, a protein or nucleic acid (*e.g.,* a transgene) into the cell. Thus, a "transfected" or "transformed" cell is a cell into which, or a progeny thereof in which

an exogenous molecule has been introduced by the hand of man, for example, by recombinant DNA techniques.

[0085] The nucleic acid or protein can be stably or transiently transfected or transformed (expressed) in the host cell and progeny thereof. The cell(s) can be propagated and the introduced protein expressed, or nucleic acid transcribed. A progeny of a transfected or transformed cell may not be identical to the parent cell, since there may be mutations that occur during replication.

[0086] Expression of an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof and nucleic acid encoding such peptides in particles or introduction into target cells (*e.g.,* host cells) can also be carried out by methods known in the art. Non-limiting examples include osmotic shock (*e.g.,* calcium phosphate), electroporation, microinjection, cell fusion, etc. Introduction of nucleic acid and polypeptide *in vitro*, *ex vivo* and *in vivo* can also be accomplished using other techniques. For example, a polymeric substance, such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers. A nucleic acid can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules, or poly (methylmethacrolate) microcapsules, respectively, or in a colloid system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

[0087] Liposomes for introducing various compositions into cells are known in the art and include, for example, phosphatidylcholine, phosphatidylserine, lipofectin and DOTAP (e.g., U.S. Patent Nos. 4,844,904, 5,000,959, 4,863,740, and 4,975,282; and GIBCO-BRL, Gaithersburg, MD). Piperazine based amphilic cationic lipids useful for gene therapy also are known (see, e.g., U.S. Patent No. 5,861,397). Cationic lipid systems also are known (see, e.g., U.S. Patent No. 5,459,127). Polymeric substances, microcapsules and colloidal

dispersion systems such as liposomes are collectively referred to herein as "vesicles." Accordingly, viral and non-viral vector means delivery into cells are included.

[0088] As disclosed herein, an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof may elicit a cellular or humoral immune response. In particular embodiments of the present invention, the proteins or peptides of the present invention may elicit, stimulate, induce, promote, increase or enhance an immune or inflammatory response. In certain embodiments, the proteins or peptides of the present invention may elicit, stimulate induce, promote, increase or enhance a T cell response.

[0089] However, the present inventors have found that an ApoB100 peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof may produce a pro-inflammatory or atherogenic response or alternatively an anti-inflammatory or protective response depending on the manner in which the peptide is administered. For example, changes in the type of adjuvant administered with the peptides, the timing of administration, the amount of peptide administered may all effect whether a peptide produces an atherogenic or protective response.

[0090] As used herein an atherogenic response refers to a inducing, promoting, increasing or enhancing the development, severity, or symptoms of an adverse cardiovascular event or cardiovascular disease, including for example, atherosclerosis. In certain embodiments, an atherogenic response comprises an increase in the occurrence or size of atherosclerosis lesions.

[0091] As used herein, a protective response refers to decreasing, reducing, inhibiting, suppressing or disrupting the development, severity, or symptoms of an adverse cardiovascular event or cardiovascular disease, including for example, atherosclerosis. In certain embodiments, an atherogenic response comprises a decrease in the occurrence or size of atherosclerosis lesions.

[0092] Thus in certain embodiments of the present invention, a protein or peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or

derivative thereof may produce an atherogenic response. In other embodiments, of the present invention, a protein or peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof may produce a protective response. Such responses can provide protection against (e.g., prophylaxis) an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis) or a secondary or subsequent occurrence of an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis). Such responses can also be effective in treatment (e.g., therapeutic) of an initial adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), or a secondary or subsequent adverse cardiovascular event or cardiovascular disease.

[0093] In particular embodiments, the proteins or peptides of the present invention may decrease, reduce, inhibit, suppress or disrupt an immune or inflammatory response. In still further embodiments, the proteins or peptides of the present invention may elicit, stimulate, induce, promote, increase or enhance an anti- immune or anti- inflammatory response.

[0094] The proteins or peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof can be employed in various methods, uses and compositions. In different embodiments, the methods, uses and compositions of the present invention comprise a protein or peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or sub-sequence, portion, homologue, variant or derivative thereof.

[0095] Such methods and uses include, for example, use, contact or administration of one or more proteins or peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives *in vitro* and *in vivo*. Such methods are applicable to providing a subject with protection vaccinate against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), and also are applicable to providing treatment to a subject for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[0096] In certain embodiments of the present invention, proteins or peptides of the present invention, or sub-sequences, portions, homologues, variants or derivatives thereof may be used as vaccine antigens to vaccinate against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), or one or more physiological conditions,

disorders, illness, diseases or symptoms caused by or associated with an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[0097] Thus, in accordance with one aspect of the present invention, there are provided methods for vaccination and immunization to protect against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[0098] In one embodiment, there is provided a method of vaccinating a subject against an adverse cardiovascular event or cardiovascular disease, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to vaccinate the subject against the adverse cardiovascular event or cardiovascular disease.

[0099] As used herein, the terms "vaccination", "vaccinate", "protection", "protect" and grammatical variations thereof, when used in reference to an adverse cardiovascular event or cardiovascular disease, means preventing an adverse cardiovascular event or cardiovascular disease, or reducing or decreasing susceptibility to an adverse cardiovascular event or cardiovascular disease, or reducing or decreasing severity of an adverse cardiovascular event or cardiovascular disease, or preventing or reducing one or more symptoms or pathologies caused by or associated with an adverse cardiovascular event or cardiovascular disease. In particular embodiments, the method of vaccinating a subject against an adverse cardiovascular event or cardiovascular disease of the present invention comprises a method of vaccinating a subject against atherosclerosis.

[00100] In another aspect of the present invention there are provided methods for treatment of an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[00101] Thus, in one embodiment of the present invention there is provided a method of treating a subject for an adverse cardiovascular event or cardiovascular disease, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to treat the subject for the adverse cardiovascular event or cardiovascular disease.

[00102] As will be understood by a person skilled in the art, treating a subject for an adverse cardiovascular event or cardiovascular disease may include decreasing, reducing, inhibiting, suppressing, limiting, controlling or eliminating an adverse cardiovascular event or cardiovascular disease. In other embodiments, a method of treating a subject for an adverse cardiovascular event or cardiovascular disease comprises reducing the frequency, severity, progression, or duration of the adverse cardiovascular event or cardiovascular disease in the subject. In yet another embodiment, a method of treating a subject for an adverse cardiovascular event or cardiovascular disease comprises maintaining the severity of an adverse cardiovascular event or cardiovascular disease in a subject by preventing an increase in the occurrence, frequency, severity, progression, or duration of the adverse cardiovascular event or cardiovascular disease in the subject. In still further embodiments, a method of treating a subject for an adverse cardiovascular event or cardiovascular disease comprises eliminating, reducing or maintaining the occurrence, frequency, severity, progression, or duration of physiological conditions, disorders, illnesses, diseases, symptoms or complications caused by or associated with the adverse cardiovascular event or cardiovascular disease.

[00103] In certain embodiments, the subject of the methods provided herein may have been previously had an adverse cardiovascular event or cardiovascular disease. Thus, in certain embodiments, the present methods may be used for treating or protecting a subject from a secondary or subsequent adverse cardiovascular event or cardiovascular disease. Thus in different embodiments, the presently described methods of vaccination and treatment may be used for prophylactic vaccination against or treatment of an adverse cardiovascular event or cardiovascular disease or can be used to vaccinate against or treatment of a secondary or subsequent occurrence of an adverse cardiovascular event or cardiovascular disease. In particular embodiments, the methods may be used to vaccinate against or treat an adverse symptom of the adverse cardiovascular event or cardiovascular disease.

[00104] In accordance with different embodiments of the present invention, the therapeutic and prophylactic methods of vaccinating against and treating a subject for an adverse cardiovascular event or cardiovascular disease include but are not limited to treatment of a subject having or at risk of having an adverse cardiovascular event or cardiovascular disease, treating a subject with an adverse cardiovascular event or

cardiovascular disease, and methods of protecting a subject from an adverse cardiovascular event or cardiovascular disease (e.g., provide the subject with protection against the development or incidence of an adverse cardiovascular event or cardiovascular disease), to decrease or reduce the probability of an adverse cardiovascular event or cardiovascular disease in a subject, to decrease or reduce susceptibility of a subject to an adverse cardiovascular event or cardiovascular disease and to inhibit or prevent an adverse cardiovascular event or cardiovascular disease in a subject. In particular embodiments of the methods described herein, one or more disorders, diseases, physiological conditions, pathologies and symptoms associated with or caused by an adverse cardiovascular event or cardiovascular disease will respond to vaccination or treatment.

[00105] Non-limiting examples of an adverse cardiovascular event or cardiovascular disease are atherosclerosis, coronary artery disease, peripheral artery disease, cerebrovascular disease, renal artery disease, stroke, myocardial infarction (heart attack), ischemic heart failure, transient ischemic attack or brain trauma, artherosclerotic plaque formation, foam cells or foam cell formation, or one or more adverse symptoms, disorders, illnesses, pathologies, diseases, or complications caused by or associated with an adverse cardiovascular event or cardiovascular disease such as atherosclerosis, coronary artery disease, peripheral artery disease, cerebrovascular disease, renal artery disease, stroke, myocardial infarction (heart attack), ischemic heart failure, transient ischemic attack or brain trauma), artherosclerotic plaque formation or foam cells or foam cell formation.

[00106] In certain embodiments of the presently described methods, two or more proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, may be administered to a subject. As will be understood by a skilled person two or more proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, may be administered as a combination composition, or administered separately, such as concurrently or in series or sequentially. Different proteins or peptides of the present invention, or a sub-sequence, portion, homologue, variant or derivative thereof, may be administered to a subject in the same amount, volume or concentration or different amounts, volumes or

concentrations. Thus in certain embodiments, the subject may be administered the same amount of two or more different proteins or peptides of the present invention, or a sub-sequence, portion, homologue, variant or derivative thereof. In other embodiments, the subject may be administered one a protein or peptide of the present invention, or a sub-sequence, portion, homologue, variant or derivative thereof, in a amount, volume or concentration greater than one or more other protein or peptide of the present invention, or a sub-sequence, portion, homologue, variant or derivative thereof, administered to the subject.

[00107] Methods of the invention include methods of vaccination or treatment that result in any therapeutic or beneficial effect. In various methods embodiments, an adverse cardiovascular event or cardiovascular disease is reduced, decreased, inhibited, limited, delayed or prevented, or a method decreases, reduces, inhibits, suppresses, prevents, controls or limits one or more adverse (e.g., physical) symptoms, disorders, illnesses, diseases or complications caused by or associated with adverse cardiovascular event or cardiovascular disease. In additional various particular embodiments, methods of the present invention include reducing, decreasing, inhibiting, delaying or preventing onset, progression, frequency, duration, severity, probability or susceptibility of one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with an adverse cardiovascular event or cardiovascular disease. In further various particular embodiments, methods of the present invention include improving, accelerating, facilitating, enhancing, augmenting, or hastening recovery of a subject from an adverse cardiovascular event or cardiovascular disease, or one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with an adverse cardiovascular event or cardiovascular disease. In yet additional various embodiments, methods of treatment include stabilizing an adverse cardiovascular event or cardiovascular disease, or an adverse symptom, disorder, illness, disease or complication caused by or associated with an adverse cardiovascular event or cardiovascular disease.

[00108] A therapeutic or beneficial effect of vaccination or treatment is therefore any objective or subjective measurable or detectable improvement or benefit provided to a particular subject. A therapeutic or beneficial effect can but need not be complete ablation of or protection from all or any particular adverse symptom, disorder, illness, disease or complication caused by or associated with adverse cardiovascular event or cardiovascular

disease Thus, a satisfactory clinical endpoint is achieved when there is an incremental improvement or a partial reduction in an adverse symptom, disorder, illness, disease or complication caused by or associated with an adverse cardiovascular event or cardiovascular disease or an inhibition, decrease, reduction, suppression, prevention, limit or control of worsening or progression of one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with an adverse cardiovascular event or cardiovascular disease over a short or long duration (hours, days, weeks, months, etc.).

[00109] A therapeutic or beneficial effect also includes reducing or eliminating the need, dosage frequency or amount of a second active such as another drug or other agent used for treating a subject having or at risk of having an adverse cardiovascular event or cardiovascular disease For example, reducing an amount of an adjunct therapy, for example, a reduction or decrease of a treatment for an adverse cardiovascular event or cardiovascular disease, or a vaccination or immunization protocol for an adverse cardiovascular event or cardiovascular disease is considered a beneficial effect. In addition, reducing or decreasing an amount of protein or peptide used for vaccination or immunization of a subject to provide protection to the subject is considered a beneficial effect.

[00110] In another aspect of the present invention there is provided a composition comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. In different embodiments, the composition of the present invention may comprise an adjuvant. In certain embodiment, the composition of the present invention may be a solid or a liquid. In particular embodiments, the composition of the present invention is a vaccine.

[00111] Methods and compositions of the invention include administration of a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, to a subject prior to development of an adverse cardiovascular event or cardiovascular disease, administration substantially contemporaneously with development of an adverse cardiovascular event or cardiovascular disease, and administration after development of an adverse cardiovascular event or cardiovascular disease. Methods and compositions of the invention also include

administration of a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, to a subject prior to, substantially contemporaneously with or following development of an adverse symptom, disorder, illness or disease caused by or associated with an adverse cardiovascular event or cardiovascular disease. A subject suffering from an adverse cardiovascular event or cardiovascular disease or an adverse symptom, disorder, illness or disease caused by or associated with an adverse cardiovascular event or cardiovascular disease have the adverse cardiovascular event, cardiovascular disease or symptom over a period of 1-5, 5-10, 10-20, 20-30, 30-50, 50-100 hours, days, months, or years.

[00112] Methods and compositions of the invention include administration of a protein or peptide comprising, consisting or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, to a subject alone or in combination with any compound, agent, drug, treatment or other therapeutic regimen or protocol having a desired therapeutic, beneficial, additive, synergistic or complementary activity or effect.

[00113] Exemplary combination compositions and treatments include multiple proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, such as T cell epitopes as described herein, and second actives, such as compounds, agents, drugs, treatments and therapies for the treatment of an adverse cardiovascular event or cardiovascular disease (e.g. statins, fibrate), as well as agents that assist, promote, stimulate or enhance efficacy. Such compounds, agents, drugs, treatments and therapies can be administered or performed prior to, substantially contemporaneously with or following any composition or method of the invention, for example, a therapeutic use or method of vaccinating or treating a subject for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), or a method of prophylactic vaccination or treatment of a subject for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[00114] Invention compositions comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set

forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, and methods described herein can be combined with any compound, agent, drug, treatment or other therapeutic regimen or protocol having a desired therapeutic, beneficial, additive, synergistic or complementary activity or effect. Exemplary combination compositions and treatments include multiple proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, such as T cell epitopes as described herein, and second actives, such as compounds, agents, drugs, treatments and therapies for the treatment of an adverse cardiovascular event or cardiovascular disease (e.g. statins, fibrate), as well as agents that assist, promote, stimulate or enhance efficacy. Such compounds, agents, drugs, treatments and therapies can be administered or performed prior to, substantially contemporaneously with or following any composition or method of the invention, for example, a therapeutic use or method of vaccinating or treating a subject for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), or a method of prophylactic vaccination or treatment of a subject for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis).

[00115] The invention therefore provides combinations of one or more proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, in combination with a second active, including but not limited to any compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition, such as a treatment or vaccination protocol set forth herein or known in the art. The compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition can be administered or performed prior to, substantially contemporaneously with or following administration of one or more proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, or a nucleic acid encoding all or a portion (e.g., a T cell epitope) of proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, to a subject. Specific non-limiting

examples of combination embodiments therefore include the foregoing or other compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition.

[00116] In invention methods, uses an compositions for which there is a desired outcome, such as a therapeutic or prophylactic method that provides a benefit from treatment, vaccination or immunization, a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, can be administered in a sufficient or effective amount.

[00117] As used herein, a "sufficient amount" or "effective amount" or an "amount sufficient" or an "amount effective" refers to an amount that provides, in single (e.g., primary) or multiple (e.g., booster) doses, alone or in combination with one or more other compounds, treatments, therapeutic regimens or agents (e.g., a drug), a long term or a short term detectable or measurable improvement in a given subject or any objective or subjective benefit to a given subject of any degree or for any time period or duration (e.g., for minutes, hours, days, months, years, or cured).

[00118] An amount sufficient or an amount effective can but need not be provided in a single administration and can but need not be achieved by a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, alone, optionally in a combination composition or method that includes a second active. In addition, an amount sufficient or an amount effective need not be sufficient or effective if given in single or multiple doses without a second or additional administration or dosage, since additional doses, amounts or duration above and beyond such doses, or additional antigens, compounds, drugs, agents, treatment or therapeutic regimens may be included in order to provide a given subject with a detectable or measurable improvement or benefit to the subject. For example, to increase, enhance, improve or optimize immunization and/or vaccination, after an initial or primary administration of one or more proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, to a subject, the subject can be administered one or more additional "boosters" of one or more proteins or peptides comprising, consisting of or

consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. Such subsequent "booster" administrations can be of the same or a different formulation, dose or concentration, route, etc.

[00119] An amount sufficient or an amount effective need not be therapeutically or prophylactically effective in each and every subject treated, nor a majority of subjects treated in a given group or population. An amount sufficient or an amount effective means sufficiency or effectiveness in a particular subject, not a group of subjects or the general population. As is typical for such methods, different subjects will exhibit varied responses to a method of the invention, such as immunization, vaccination and therapeutic treatments.

[00120] The term "subject" refers includes but is not limited to a subject at risk of an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), as well as a subject that has already developed an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis). Such subjects, include mammalian animals (mammals), such as a non human primate (apes, gibbons, gorillas, chimpanzees, orangutans, macaques), a domestic animal (dogs and cats), a farm animal (poultry such as chickens and ducks, horses, cows, goats, sheep, pigs), experimental animal (mouse, rat, rabbit, guinea pig) and humans. Subjects include animal disease models, for example, mouse and other animal models of an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis) known in the art.

[00121] Accordingly, subjects appropriate for vaccination or treatment include those having or at risk of an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), also referred to as subjects in need of treatment. Subjects in need of vaccination or treatment therefore include subjects that have been previously had an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis) or that have an ongoing adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis) or have developed one or more adverse symptoms caused by or associated with an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), regardless of the type, timing or degree of onset, progression, severity, frequency, duration of the symptoms.

[00122] Prophylactic uses and methods are therefore included. Target subjects for prophylaxis may be at increased risk (probability or susceptibility) of developing an adverse

cardiovascular event or cardiovascular disease (e.g. atherosclerosis). Such subjects are considered in need of treatment due to being at risk.

[00123] Subjects for prophylaxis need not be at increased risk but may be from the general population in which it is desired to vaccinate or immunize a subject against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), for example. Such a subject that is desired to be vaccinated or immunized against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis) can be administered a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. In another non-limiting example, a subject that is not specifically at risk for an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), but nevertheless desires protection against an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), can be administered a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof. Such subjects are also considered in need of treatment.

[00124] "Prophylaxis" and grammatical variations thereof mean a method in which contact, administration or *in vivo* delivery to a subject is prior to development of an adverse cardiovascular event or cardiovascular disease. In certain situations it may not be known that a subject has developed an adverse cardiovascular event or cardiovascular disease, but administration or *in vivo* delivery to a subject can be performed prior to manifestation of disease pathology or an associated adverse symptom, condition, complication, etc. caused by or associated with an adverse cardiovascular event or cardiovascular disease. In such case, a composition or method of the present invention can eliminate, prevent, inhibit, suppress, limit, decrease or reduce the probability of or susceptibility to an adverse cardiovascular event or cardiovascular, or an adverse symptom, condition or complication associated with or caused by an adverse cardiovascular event or cardiovascular disease.

[00125] "Prophylaxis" can also refer to a method in which contact, administration or *in vivo* delivery to a subject is prior to a secondary or subsequent exposure or infection. In such a situation, a subject may have had a prior adverse cardiovascular event or cardiovascular disease or prior adverse symptom, condition or complication associated with or caused by an

adverse cardiovascular event or cardiovascular disease. Vaccination or treatment by administration or *in vivo* delivery to such a subject, can be performed prior to a secondary or subsequent adverse cardiovascular event or cardiovascular disease. Such a method can eliminate, prevent, inhibit, suppress, limit, decrease or reduce the probability of or susceptibility towards a secondary or subsequent adverse cardiovascular event or cardiovascular disease, or an adverse symptom, condition or complication associated with or caused by or associated with a secondary or subsequent adverse cardiovascular event or cardiovascular disease.

[00126] Treatment of an adverse cardiovascular event or cardiovascular disease can be at any time during the adverse cardiovascular event or cardiovascular disease. A protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, can be administered as a combination (e.g., with a second active), or separately concurrently or in sequence (sequentially) in accordance with the methods described herein as a single or multiple dose e.g., one or more times hourly, daily, weekly, monthly or annually or between about 1 to 10 weeks, or for as long as appropriate, for example, to achieve a reduction in the onset, progression, severity, frequency, duration of one or more symptoms or complications associated with or caused by an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis), or an adverse symptom, condition or complication associated with or caused by an adverse cardiovascular event or cardiovascular disease (e.g. atherosclerosis). Thus, a method can be practiced one or more times (e.g., 1-10, 1-5 or 1-3 times) an hour, day, week, month, or year. The skilled artisan will know when it is appropriate to delay or discontinue administration. A non-limiting dosage schedule is 1-7 times per week, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more weeks, and any numerical value or range or value within such ranges.

[00127] Methods of the invention may be practiced by any mode of administration or delivery, or by any route, systemic, regional and local administration or delivery. Exemplary administration and delivery routes include intravenous (i.v.), intraperitoneal (i.p.), intrarterial, intramuscular, parenteral, subcutaneous, intra-pleural, topical, dermal, intradermal, transdermal, transmucosal, intra-cranial, intra-spinal, rectal, oral (alimentary), mucosal, inhalation, respiration, intranasal, intubation, intrapulmonary, intrapulmonary instillation,

buccal, sublingual, intravascular, intrathecal, intracavity, iontophoretic, intraocular, ophthalmic, optical, intraglandular, intraorgan, or intralymphatic.

[00128] Doses can be based upon current existing protocols, empirically determined, using animal disease models or optionally in human clinical trials. Initial study doses can be based upon animal studies, e.g. a mouse, and the amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, administered that is determined to be effective. Exemplary non-limiting amounts (doses) are in a range of about 0.1 mg/kg to about 100 mg/kg, and any numerical value or range or value within such ranges. Greater or lesser amounts (doses) can be administered, for example, 0.01-500 mg/kg, and any numerical value or range or value within such ranges. The dose can be adjusted according to the mass of a subject, and will generally be in a range from about 1-10 ug/kg, 10-25 ug/kg, 25-50 ug/kg, 50-100 ug/kg,100-500 ug/kg, 500-1,000 ug/kg, 1-5 mg/kg, 5-10 mg/kg, 10-20 mg/kg, 20-50 mg/kg, 50-100 mg/kg, 100-250 mg/kg, 250-500 mg/kg, or more, two, three, four, or more times per hour, day, week, month or annually. A typical range will be from about 0.3 mg/kg to about 50 mg/kg, 0-25 mg/kg, or 1.0-10 mg/kg, or any numerical value or range or value within such ranges.

[00129] Doses can vary and depend upon whether the treatment is prophylactic or therapeutic, whether a subject has previously had an adverse cardiovascular event or cardiovascular disease, the onset, progression, severity, frequency, duration probability of or susceptibility of the symptom, condition, pathology or complication, the vaccination protocol and compositions, the clinical endpoint desired, the occurrence of previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject and other factors that will be appreciated by the skilled artisan. The skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

[00130] The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by the status of the subject. For example, whether the subject has previously had an adverse cardiovascular event or cardiovascular disease, whether the subject is merely at risk of an adverse cardiovascular event or cardiovascular disease, exposure or infection, whether the subject has been previously vaccinated against an adverse

cardiovascular event or cardiovascular disease. The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by any adverse side effects, complications or other risk factors of the treatment or therapy.

[00131] In the methods of the invention, the route, dose, number and frequency of administrations, treatments, immunizations or vaccinations, and timing/intervals between treatment, immunization and vaccination, and disease development can be modified. In certain embodiments, a desirable vaccine of the present invention will elicit robust, long-lasting immunity against an adverse cardiovascular event or cardiovascular disease. Thus, in certain embodiments, invention methods, uses and compositions provide long-lasting immunity to an adverse cardiovascular event or cardiovascular disease such as atherosclerosis.

[00132] In certain embodiments of the present invention, a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, may be provided as pharmaceutical compositions.

[00133] As used herein the term "pharmaceutically acceptable" and "physiologically acceptable" mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, *in vivo* delivery or contact. Such formulations include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds (e.g., preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[00134] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes. Exemplary routes of administration for contact or *in vivo* delivery which a composition can optionally be formulated include

inhalation, respiration, intranasal, intubation, intrapulmonary instillation, oral, buccal, intrapulmonary, intradermal, topical, dermal, parenteral, sublingual, subcutaneous, intravascular, intrathecal, intraarticular, intracavity, transdermal, iontophoretic, intraocular, opthalmic, optical, intravenous (i.v.), intramuscular, intraglandular, intraorgan, or intralymphatic.

[00135] Formulations suitable for parenteral administration comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, saline, dextrose, fructose, ethanol, animal, vegetable or synthetic oils.

[00136] To increase an vaccination, a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, can be coupled to another protein such as ovalbumin or keyhole limpet hemocyanin (KLH), thyroglobulin or a toxin such as tetanus or cholera toxin. Invention proteins or peptides, or a sub-sequence, portion, homologue, variant or derivative thereof, can also be mixed with adjuvants. As demonstrated herein, in certain embodiments, the form of adjuvant with which the invention proteins or peptides are mixed may change whether the protein or peptide elicits an atherogenic or protective response in a subject.

[00137] Adjuvants include, for example: Oil (mineral or organic) emulsion adjuvants such as Freund's complete (CFA) and incomplete adjuvant (IFA) (WO 95/17210; WO 98/56414; WO 99/12565; WO 99/11241; and U.S. Patent No. 5,422,109); metal and metallic salts, such as aluminum and aluminum salts, such as aluminum phosphate or aluminum hydroxide, alum (hydrated potassium aluminum sulfate); bacterially derived compounds, such as Monophosphoryl lipid A and derivatives thereof (e.g., 3 De-O-acylated monophosphoryl lipid A, aka 3D-MPL or d3-MPL, to indicate that position 3 of the reducing end glucosamine is de-O-acylated, 3D-MPL consisting of the tri and tetra acyl congeners), and enterobacterial lipopolysaccharides (LPS); plant derived saponins and derivatives thereof, for example Quil A (isolated from the Quilaja Saponaria Molina tree, see, e.g., “Saponin adjuvants”, Archiv. fur die gesamte Virusforschung, Vol. 44, Springer Verlag, Berlin, p243-254; U.S. Patent No. 5,057,540), and fragments of Quil A which retain adjuvant activity without associated

toxicity, for example QS7 and QS21 (also known as QA7 and QA21), as described in WO96/33739, for example; surfactants such as, soya lecithin and oleic acid; sorbitan esters such as sorbitan trioleate; and polyvinylpyrrolidone; oligonucleotides such as CpG (WO 96/02555, and WO 98/16247), polyriboA and polyriboU; block copolymers; and immunostimulatory cytokines such as GM-CSF and IL-1, and Muramyl tripeptide (MTP). Additional examples of adjuvants are described, for example, in "Vaccine Design--the subunit and adjuvant approach" (Edited by Powell, M. F. and Newman, M. J.; 1995, Pharmaceutical Biotechnology (Plenum Press, New York and London, ISBN 0-306-44867-X) entitled "Compendium of vaccine adjuvants and excipients" by Powell, M. F. and Newman M.

[00138] Salts may be added to a composition of the present invention, the composition comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in Table 1 or a sub-sequence, portion, homologue, variant or derivative thereof, composition or formulation. Non-limiting examples of salts include acetate, benzoate, besylate, bitartate, bromide, carbonate, chloride, citrate, edetate, edisylate, estolate, fumarate, gluceptate, gluconate, hydrobromide, hydrochloride, iodide, lactate, lactobionate, malate, maleate, mandelate, mesylate, methyl bromide, methyl sulphate, mucate, napsylate, nitrate, pamoate (embonate, phosphate, diphosphate, salicylate and disalicylate, stearate, succinate, sulphate, tartrate, tosylate, triethiodide, valerate, aluminium, benzathine, calcium, ethylene diamine, lysine, magnesium, megluminie, potassium, procaine, sodium, tromethyamine or zinc.

[00139] Chelating agents may be added to a composition of the present invention, the composition comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in Table 1 or a sub-sequence, portion, homologue, variant or derivative thereof, composition or formulation. Non-limiting examples of chelating agents include ethylenediamine, ethylene glycol tetraacetic acid, 1,2-bis(o-aminophenoxy)ethane- N,N,N',N'-tetraacetic acid, Penicillamine, Deferasirox, Deferiprone, Deferoxamine, 2,3-Disulfanylpropan-1-ol, Dexrazoxane, Iron(II,III) hexacyanoferrate(II,III), (R)-5-(1,2-dithiolan-3-yl)pentanoic acid, 2,3-Dimercapto-1-propanesulfonic acid, Dimercaptosuccinic acid, or diethylene triamine pentaacetic acid.

[00140] Buffering agents may be added to a composition of the present invention, the composition comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in Table 1 or a sub-sequence, portion, homologue, variant or derivative thereof, composition or formulation. Non-limiting examples of buffering agents include phosphate, citrate, acetate, borate, TAPS, bicine, tris, tricine, TAPSO, HEPES, TES, MOPS, PIPES, cacodylate, SSC, MES or succinic acid.

[00141] Cosolvents may be added to a composition of the present invention, the composition comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, composition or formulation. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters.

[00142] Supplementary compounds (e.g., preservatives, antioxidants, antimicrobial agents including biocides and biostats such as antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions. Pharmaceutical compositions may therefore include preservatives, anti-oxidants and antimicrobial agents.

[00143] Preservatives can be used to inhibit microbial growth or increase stability of ingredients thereby prolonging the shelf life of the pharmaceutical formulation. Suitable preservatives are known in the art and include, for example, EDTA, EGTA, benzalkonium chloride or benzoic acid or benzoates, such as sodium benzoate. Antioxidants include, for example, ascorbic acid, vitamin A, vitamin E, tocopherols, and similar vitamins or provitamins.

[00144] An antimicrobial agent or compound directly or indirectly inhibits, reduces, delays, halts, eliminates, arrests, suppresses or prevents contamination by or growth, infectivity, replication, proliferation, reproduction, of a pathogenic or non- pathogenic

microbial organism. Classes of antimicrobials include antibacterial, antiviral, antifungal and antiparasitics. Antimicrobials include agents and compounds that kill or destroy (-cidal) or inhibit (-static) contamination by or growth, infectivity, replication, proliferation, reproduction of the microbial organism.

[00145] Exemplary antibacterials (antibiotics) include penicillins (e.g., penicillin G, ampicillin, methicillin, oxacillin, and amoxicillin), cephalosporins (e.g., cefadroxil, ceforanid, cefotaxime, and ceftriaxone), tetracyclines (e.g., doxycycline, chlortetracycline, minocycline, and tetracycline), aminoglycosides (e.g., amikacin, gentamycin, kanamycin, neomycin, streptomycin, netilmicin, paromomycin and tobramycin), macrolides (e.g., azithromycin, clarithromycin, and erythromycin), fluoroquinolones (e.g., ciprofloxacin, lomefloxacin, and norfloxacin), and other antibiotics including chloramphenicol, clindamycin, cycloserine, isoniazid, rifampin, vancomycin, aztreonam, clavulanic acid, imipenem, polymyxin, bacitracin, amphotericin and nystatin.

[00146] Particular non-limiting classes of anti-virals include reverse transcriptase inhibitors; protease inhibitors; thymidine kinase inhibitors; sugar or glycoprotein synthesis inhibitors; structural protein synthesis inhibitors; nucleoside analogues; and viral maturation inhibitors. Specific non-limiting examples of anti-virals include nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, zidovudine (AZT), stavudine (d4T), larnivudine (3TC), didanosine (DDI), zalcitabine (ddC), abacavir, acyclovir, penciclovir, ribavirin, valacyclovir, ganciclovir, 1,-D-ribofuranosyl-1,2,4-triazole-3 carboxamide, 9->2-hydroxy-ethoxy methylguanine, adamantanamine, 5-iodo-2′-deoxyuridine, trifluorothymidine, interferon and adenine arabinoside.

[00147] Pharmaceutical formulations and delivery systems appropriate for the compositions and methods of the invention are known in the art (see, *e.g.*, Remington: The Science and Practice of Pharmacy (2003) 20th ed., Mack Publishing Co., Easton, PA; Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing Co., Easton, PA; The Merck Index (1996) 12th ed., Merck Publishing Group, Whitehouse, NJ; Pharmaceutical Principles of Solid Dosage Forms (1993), Technonic Publishing Co., Inc., Lancaster, Pa.; Ansel ad Soklosa, Pharmaceutical Calculations (2001) 11th ed., Lippincott Williams & Wilkins, Baltimore, MD; and Poznansky et al., Drug Delivery Systems (1980), R. L. Juliano, ed., Oxford, N.Y., pp. 253-315).

[00148] A protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, along with any adjunct agent, compound drug, composition, whether active or inactive, etc., can be packaged in unit dosage form (capsules, tablets, troches, cachets, lozenges) for ease of administration and uniformity of dosage. A "unit dosage form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active ingredient optionally in association with a pharmaceutical carrier (excipient, diluent, vehicle or filling agent) which, when administered in one or more doses, is calculated to produce a desired effect (e.g., prophylactic or therapeutic effect). Unit dosage forms also include, for example, ampules and vials, which may include a composition in a freeze-dried or lyophilized state; a sterile liquid carrier, for example, can be added prior to administration or delivery *in vivo*. Unit dosage forms additionally include, for example, ampules and vials with liquid compositions disposed therein. Individual unit dosage forms can be included in multi-dose kits or containers. Pharmaceutical formulations can be packaged in single or multiple unit dosage form for ease of administration and uniformity of dosage.

[00149] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[00150] All applications, publications, patents and other references, GenBank citations and ATCC citations cited herein are incorporated by reference in their entirety. In case of conflict, the specification, including definitions, will control.

[00151] As used herein, the singular forms "a," "and," and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to a "protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof," or an "adverse cardiovascular event or cardiovascular disease" includes a plurality of proteins or peptides comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set

forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, or a plurality of adverse cardiovascular events or cardiovascular diseases and reference to an "activity or function" can include reference to one or more activities or functions of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof," including function as a T cell epitopes; eliciting, stimulating, inducing, promoting, increasing or enhancing an immune or inflammatory response; eliciting, stimulating, inducing, promoting, increasing or enhancing a T cell response; decreasing, reducing, inhibiting, suppressing or disrupting an immune or inflammatory response; eliciting, stimulating, inducing, promoting, increasing or enhancing an anti- immune or anti- inflammatory response, and so forth,

[00152] As used herein, numerical values are often presented in a range format throughout this document. The use of a range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the use of a range expressly includes all possible subranges, all individual numerical values within that range, and all numerical values or numerical ranges include integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, to illustrate, reference to a range of 90-100% includes 91-99%, 92-98%, 93-95%, 91-98%, 91-97%, 91-96%, 91-95%, 91-94%, 91-93%, and so forth. Reference to a range of 90-100%, includes 91%, 92%, 93%, 94%, 95%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth. Reference to a range of 1-5 fold therefore includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, fold, etc., as well as 1.1, 1.2, 1.3, 1.4, 1.5, fold, etc., 2.1, 2.2, 2.3, 2.4, 2.5, fold, etc., and so forth. Further, for example, reference to a series of ranges of 2-72 hours, 2-48 hours, 4-24 hours, 4-18 hours and 6-12 hours, includes ranges of 2-6 hours, 2, 12 hours, 2-18 hours, 2-24 hours, etc., and 4-27 hours, 4-48 hours, 4-6 hours, etc.

[00153] As also used herein a series of range formats are used throughout this document. The use of a series of ranges includes combinations of the upper and lower ranges to provide a range. Accordingly, a series of ranges include ranges which combine the values of the

boundaries of different ranges within the series. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a series of ranges such as 5-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100-150, and 150-171, includes ranges such as 5-20, 5-30, 5-40, 5-50, 5-75, 5-100, 5-150, 5-171, and 10-30, 10-40, 10-50, 10-75, 10-100, 10-150, 10-171, and 20-40, 20-50, 20-75, 20-100, 20-150, 20-171, and so forth.

[00154] The invention is generally disclosed herein using affirmative language to describe the numerous embodiments and aspects. The invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. Thus, even though the invention is generally not expressed herein in terms of what is not included, embodiments and aspects that expressly exclude compositions or method steps are nevertheless disclosed and included in the invention.

[00155] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the following examples are intended to illustrate but not limit the scope of invention described in the claims.

EXAMPLES

Example 1: Peptides Useful for Preventing and Treating Atherosclerosis in Humans

[00156] Peptide binding to MHC-II requires anchor residues in certain positions and thus can be predicted using computer algorithms. Affinity can be measured experimentally using a competition assay (1). Both are excellent screening tools. However, affinity must be measured separately for each MHC-II allele. Humans express molecules encoded in four different MHC-II loci, denominated HLA-DRB1, DRB3/4/5, DP and DQ. Allelic variation is large, and most people are heterozygous at each locus, thus expressing up to 8 relevant MHC-II alleles. CD4 T cell responses strictly require that the antigenic peptide binds to the relevant MHC-II.

[00157] To identify peptides that could potentially be used in human patients, 30 peptides were screened for binding to 26 HLA alleles by competition assay. Among the 30 peptides predicted to bind based on the computer algorithms described in (4), peptides were found that bound HLA alleles at 10 nM or better (see Tables 1-4 below).

Table 1: ApoB100 Peptide Binding to Class II HLA Alleles (SEQ ID Nos: 2-31)

Epitope			HLA class II bound (IC50 <1000 nM)			
Peptide	**Sequence**	**Pos**	**DP**	**DQ**	**DR**	**Total**
3563.0011	QLYSKFLLKAEPLAF	1926	6	5	14	25
3563.0027	QIHQYIMALREEYFD	4376	5	5	14	24
3563.0006	FLHYIFMENAFELPT	826	5	5	13	23
3563.0004	DKRLAAYLMLMRSPS	556	5	6	11	22
3563.0019	GKIDFLNNYALFLSP	3066	4	6	12	22
3563.0020	RGLKLATALSLSNKF	3391	5	4	13	22
3563.0010	HFSNVFRSVMAPFTM	1891	6	3	12	21
3563.0018	SLFFSAQPFEITAST	3036	5	6	10	21
3563.0005	TLTAFGFASADLIEI	676	4	6	10	20
3563.0008	VGSKLIVAMSSWLQK	1226	5	4	11	20
3563.0014	HVKHFVINLIGDFEV	2316	5	4	11	20
3563.0009	IKHIYAISSAALSAS	1836	4	2	13	19
3563.0022	YKKLRTSSFALNLPT	3771	6	1	12	19
3563.0028	KIVSLIKNLLVALKD	4406	5	3	11	19
3563.0007	VEFVTNMGIIIPDFA	881	3	5	10	18
3563.0017	LEVLNFDFQANAQLS	2801	3	4	11	18
3563.0025	KFTYLINYIQDEINT	4321	2	5	11	18
3563.0015	LIINWLQEALSSASL	2491	1	4	12	17
3563.0023	ILFSYFQDLVITLPF	4241	4	3	10	17
3563.0012	LSQLQTYMIQFDQYI	2171	3	4	9	16
3563.0021	EGHLRFLKNIILPVY	3666	3	2	11	16
3563.0024	QEVFKAIQSLKTTEV	4281	4	1	11	16
3563.0026	DEINTIFSDYIPYVF	4331	3	5	8	16
3563.0013	LHDLKIAIANIIDEI	2191	0	5	10	15
3563.0030	FLIYITELLKKLQST	4531	5	0	8	13
3563.0016	GKLYSILKIQSPLFT	2756	1	1	10	12
3563.0001	PALLALLALPALLLL	6	0	4	7	11
3563.0003	QELLDIANYLMEQIQ	461	1	4	6	11
3563.0002	LLIDVVTYLVALIPE	406	1	2	3	6
3563.0029	IDLSIQNYHTFLIYI	4521	0	1	3	4

Table 1 (Continued) (SEQ ID Nos: 32-34)

Peptide	**Sequence**
P101	FGKQGFFPDSVNKALY
P102	TLYALSHAVNSYFDVD
P103	LYYKEDKTSLSASAAS

Table 2: ApoB100 Peptide Binding to HLA-DP Alleles

Peptide	DPB1*02:01	DPB1*03:01	DPB1*04:01	DPB1*04:02	DPB1*05:01	DPB1*14:01
3563.0011	**32**	**66**	**9.4**	**6.3**	**83**	**51**
3563.0027	**80**	**7.1**	**817**	**51**	**122**	1819
3563.0006	**40**	1261	**32**	**4.4**	**333**	**696**
3563.0004	1677	**26**	**372**	**23**	**4.1**	**102**
3563.0019	**456**	1042	**181**	**27**	**424**	6514
3563.0020	3688	**19**	**389**	**23**	**9.8**	**183**
3563.0010	**25**	**8.0**	**6.4**	**10**	**94**	**799**
3563.0018	**11**	**679**	**6.0**	**7.0**	1478	**126**
3563.0005	1384	1123	**490**	**5.3**	**412**	**847**
3563.0008	**128**	**583**	**89**	**161**	**15**	1858
3563.0014	**58**	**984**	**7.0**	**148**	**818**	12236
3563.0009	**712**	**90**	1376	**59**	1329	**132**
3563.0022	**3.3**	**2.5**	**2.1**	**67**	**221**	**15**
3563.0028	**657**	**710**	**78**	**155**	**12**	1796
3563.0007	**12**	4896	**37**	**10**	6693	15604
3563.0017	**86**	3535	**191**	**586**	39381	-
3563.0025	**439**	2518	1162	**14**	2615	13722
3563.0015	16282	**827**	6187	2049	-	9553
3563.0023	**10**	**596**	**4.0**	**295**	3821	1596
3563.0012	**576**	7900	**951**	**622**	4124	4511
3563.0021	**61**	3012	**9.4**	**20**	4609	2069
3563.0024	**200**	1693	**16**	**93**	**369**	1779
3563.0026	**418**	7341	**819**	**116**	6904	15241
3563.0013	20759	8803	1733	1384	6520	7759
3563.0030	**96**	4558	**215**	**8.6**	**32**	**925**
3563.0016	9070	1620	2099	**419**	2379	7015
3563.0001	-	4979	14008	1626	6560	1911
3563.0003	1784	9942	2575	**182**	-	-
3563.0002	25784	25617	14481	**190**	-	8566
3563.0029	23235	4386	25047	15632	9158	20820

Table 3: ApoB100 Peptide Binding to HLA-DQ Alleles

Peptide	DQB1*02:01	DQB1*03:01	DQB1*03:02	DQB1*0402	DQB1*05:01	DQB1*06:02
3563.0011	**138**	**534**	**553**	**655**	**588**	2766
3563.0027	**3.4**	3391	**18**	**63**	**3.3**	**897**
3563.0006	**2.2**	3402	**162**	**97**	**39**	**113**
3563.0004	**67**	**333**	**1.1**	**133**	**53**	**13**
3563.0019	**85**	**524**	**5.7**	**462**	**62**	**495**
3563.0020	**154**	**71**	**21**	1604	2631	**245**
3563.0010	**76**	**88**	1496	2438	5568	**516**
3563.0018	**150**	**34**	**19**	**523**	**643**	**895**
3563.0005	**8.6**	**103**	**360**	**29**	**118**	**997**
3563.0008	**884**	**402**	**415**	2472	3452	**617**
3563.0014	**2.5**	4663	**137**	**273**	**3.7**	13609
3563.0009	3027	**8.5**	1159	2038	6950	**66**
3563.0022	6841	4896	3586	-	**273**	2647
3563.0028	**340**	1454	**256**	22890	**124**	1459
3563.0007	**64**	**152**	**249**	**322**	**228**	8396
3563.0017	**416**	**918**	**43**	1008	**597**	1688
3563.0025	**12**	**875**	**650**	**351**	**13**	-
3563.0015	**288**	**143**	**54**	1497	**416**	1954
3563.0023	**67**	3809	**967**	2229	**19**	12221
3563.0012	**74**	1253	**642**	**589**	**28**	43062
3563.0021	**144**	3157	1208	6570	**10**	6142
3563.0024	1332	1248	2458	2109	**76**	1375
3563.0026	**7.0**	**393**	**260**	**374**	**18**	4554
3563.0013	**17**	**60**	77	**246**	**691**	1830
3563.0030	4923	6408	1064	36023	4765	5905
3563.0016	**558**	6205	1277	7266	5995	1623
3563.0001	**605**	3628	**618**	**619**	**137**	24231
3563.0003	**98**	6647	**241**	**394**	**4.0**	2433
3563.0002	**667**	-	**5.5**	1990	3622	38822
3563.0029	1086	3340	1149	**988**	1116	14901

Table 4: ApoB100 Peptide Binding to HLA-DR Alleles

Peptide	DRB1*01:01	DRB1*03:01	DRB1*04:01	DRB1*04:05	DRB1*07:01	DRB1*09:01	DRB1*1101	DRB1*12:01	DRB1*13:02	DRB1*15:01
3563.0011	4.3	572	7.5	61	4.2	5.7	42	350	0.18	7.3
3563.0027	4.1	643	175	158	78	66	291	84	57	5.7
3563.0006	5.0	21669	5.5	19	16	40	759	784	7.4	8.7
3563.0004	2.7	906	7.1	50	381	74	763	3978	180	0.67
3563.0019	0.81	5090	190	341	41	75	27900	243	0.52	0.22
3563.0020	7.1	724	16	546	5.1	17	1200	198	1.1	3.2
3563.0010	0.17	13209	0.73	4.3	0.39	1.5	228	6092	9.0	5.0
3563.0018	8.4	21919	18	906	10	6.2	4079	3621	8.0	358
3563.0005	60	16434	159	333	33	28	6994	27156	6.1	25
3563.0008	11	9916	2.0	145	15	29	1270	1149	1.0	0.39
3563.0014	530	-	143	248	20	472	9441	947	0.44	1.4
3563.0009	0.83	2799	0.44	86	77	5.7	334	159	4.1	1.1
3563.0022	146	33683	106	703	5.9	368	8279	49	0.72	17
3563.0028	55	7731	17	843	25	320	1930	230	3.5	0.084
3563.0007	10	1521	372	340	0.50	10.0	2218	3055	0.065	397
3563.0017	46	883	55	664	1099	417	11005	1226	80	250
3563.0025	15	-	8.2	20	69	473	32637	1753	312	1.3
3563.0015	34	38318	13	211	48	131	13776	142	12	47
3563.0023	748	-	8.0	479	6.3	1118	7897	1467	110	204
3563.0012	157	20534	19	69	14	425	33128	3356	35	1.1
3563.0021	3.0	2777	11	16	8.2	19	1383	10	2.4	0.17
3563.0024	3.0	7391	3.8	18	8.4	46	5054	558	19	0.82
3563.0026	550	308	17	4079	1441	1802	22312	1337	1.3	16
3563.0013	16	30115	103	366	9.7	294	12607	457	0.007	6.7
3563.0030	2864	4362	516	1315	132	2657	175	6287	28	121
3563.0016	36	4813	90	105	87	416	2309	72	5.6	65
3563.0001	89	40962	789	2930	1042	643	25804	832	38	119
3563.0003	790	6666	2035	497	4106	2423	30558	1544	110	41
3563.0002	2985	6580	803	3626	13508	9889	-	5251	161	1943
3563.0029	3751	40662	15912	-	2004	1185	-	10426	11	74

Table 4 (continued)

Peptide	DRB3*01:01	DRB3*02:02	DRB4*01:01	DRB5*01:01
3563.0011	28	276	1.1	36
3563.0027	20	630	1.2	227
3563.0006	2.0	858	1.8	19
3563.0004	9938	1195	12	11
3563.0019	106	5.1	965	57
3563.0020	94	8.5	8.1	8.2
3563.0010	240	5.9	231	9.1
3563.0018	103	3568	85	58
3563.0005	99	18146	997	276
3563.0008	499	110	32	23
3563.0014	247	5.4	1.7	21062
3563.0009	535	3.4	8.1	18
3563.0022	113	4.3	96	590
3563.0028	12099	63	68	14
3563.0007	37	0.78	1.9	9286
3563.0017	243	857	94	694
3563.0025	964	21	45	771
3563.0015	352	364	12	481
3563.0023	71	430	135	939
3563.0012	1110	4665	29	826
3563.0021	7622	3.3	3.3	46
3563.0024	1401	76	37	24
3563.0026	5.1	60	92	8932
3563.0013	1508	4012	17	527
3563.0030	162	6465	101	20
3563.0016	8497	1571	2.0	106
3563.0001	17017	7171	96	1022
3563.0003	88	25886	162	11070
3563.0002	28	7205	1573	33088
3563.0029	9192	27024	534	11751

[00158] Although all of these peptides can be used to develop a vaccine against atherosclerosis in humans, their in vivo efficacy cannot be tested easily in animal models. However, two peptides were discovered that can be tested in mice transgenic for HLA-DRB1*01:01, because their sequences are identical in human and mouse ApoB100. These two peptides are true autoantigens, because their sequence is homologous to mouse ApoB100. This eliminates the need for making a mouse that is transgenic for human ApoB100, which would have to be fully characterized for atherosclerosis. These two peptides are 3563.0019 with the sequence GKIDFLNNYALFLSP (0.81 nM affinity to HLA-DRB1*01:01) (SEQ ID NO.: 6) and 3563.0018 with the sequence SLFFSAQPFEITAST (8.4 nM affinity to HLA-DRB1*01:01) (SEQ ID NO.: 9).

[00159] Atherosclerosis-susceptible mice such as Apoe-/- or Ldlr-/- will be made transgenic for human ApoB100 and for at least one of the human MHC class II alleles. Such mouse is useful for testing the peptides that bind this particular human MHC class II allele. We have available a transgenic mouse (2) that expresses human HLA-DRB1*01:01. This one of the more common alleles of human MHC-II. HLA-DRB1*01:01 is expressed in about 5-10% of the general population (3). SLFFSAQPFEITAST (SEQ ID NO.: 9) is particularly useful because it is sequence identical AND it binds mouse MHC-II ($I\text{-}A^b$).

[00160] A vaccination scheme may include peptide plus complete Freund's adjuvant (CFA) initially, followed by peptide in incomplete Freund's adjuvant (IFA) for booster immunizations, where 50μg of each peptide (diluted in PBS) are emulsified in equal volumes of CFA (BD Difco, Sparks, MD, USA) and injected into the subcutaneous inguinal area of Apoe-/- mice at 8 weeks of age. Repeated boosters with 25μg of each peptide emulsified in IFA (BD Difco, Sparks, MD, USA) are administered intraperitoneally at age 12, 16, 20 and 22 weeks.

References

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Example 2: P9

[00161] CD4 T cells in patients with atherosclerosis have been investigated before. T cell clones made from human atherosclerotic plaque respond to re-stimulation with oxLDL 1, but the antigenic peptide was not identified, the CD4 T cell phenotype was not analyzed other than noting that most clones produced IFN-γ, and thus disease relevance of this finding remains unclear. ApoB-specific CD4 T cell populations were identified by tetramer staining and sorting of CD4+ T cells in PBMCs from subjects with and without preclinical cardiovascular disease.

[00162] About 600 peptides (15-mers) derived from human ApoB were screened and tested for binding to the most common MHC-II alleles. Because CD4 T cells are strictly MHC-II restricted, it was imperative to know which peptides bind MHC-II. Since human MHC-II has 3 to 5 loci and hundreds of alleles, we tested which peptides bind to which alleles. Unlike C57BL/6 mice, which express only one allele, called I-Ab, each human expresses at least 6 alleles at the DR, DQ and DP loci. Some people can also express DRB3

and DRB4. DR is composed of a monomorphic α chain (DRA) and a polymorphic β chain (DRB1, DRB3 and DRB4), with different distributions in different ethnicities. The DR1, DQ and DP are all highly polymorphic. Binding to 28 commonly expressed alleles was tested: 7 DP alleles (DPB1*01:01, DPB1*02:01, DPB1*03:01, DPB1*04:01, DPB1*04:02, DPB1*05:01, DPB1*14:01), 6 DQ alleles (DQB1*02:01, DQB1*03:01, DQB1*03:02, DQB1*0402, DQB1*05:01, DQB1*06:02) and 15 DR alleles, 11 of them DRB1 alleles (DRB1*01:01, DRB1*03:01, DRB1*04:01, DRB1*04:05, DRB1*07:01, DRB1*08:02, DRB1*09:01, DRB1*1101, DRB1*12:01, DRB1*13:02, DRB1*15:01) and the remaining four DRB3, 4 and 5 (DRB3*01:01, DRB3*02:02, DRB4*01:01, DRB5*01:01). These alleles cover about 95% of all humans. Thirty human ApoB peptides were identified that bind broadly (many alleles) with an affinity of 1 μM or better. The distribution of alleles bound by each ApoB peptide ranges from 4 to 27. The number of DR, DQ, DR and DR1 alleles bound by each peptide is tabulated as "total" in Table 5. These are all candidate autoantigens relevant for atherosclerosis.

Table 5. Human ApoB peptides (one per row) binding MHC-II DP, DQ, DR, DR1 and total alleles.

DP	DQ	DR	DRB1	Total
7	5	15	11	27
5	5	15	11	25
5	5	14	10	24
5	6	12	10	23
6	6	11	8	23
4	6	13	9	23
5	4	14	10	23
6	3	13	9	22
4	6	11	8	21
5	4	12	8	21
5	4	12	9	21
7	1	13	9	21
4	2	14	10	20
5	3	12	9	20
2	5	12	8	19
3	5	10	7	18
3	4	11	7	18
4	2	12	9	18
3	4	10	8	17
1	4	12	8	17
4	3	10	6	17
4	1	12	9	17
0	5	11	9	16
3	5	8	5	16
5	0	9	6	14
1	1	11	9	13
0	4	7	6	11
1	4	6	4	11
1	2	3	2	6
0	1	3	2	4

[00163] One human ApoB-DRB1*0101 tetramer loaded with a human ApoB peptide called P9 (P9 tetramer for short) was generated and tested. P9 tetramer was labeled with PE and tested on frozen human PBMCs acquired in the Women's Interagency HIV Study (WIHS) study with and without CVD. Average cell viability was over 95%. The gating strategy for P9 tetramer+ CD4 T cells in PBMCs is shown in Figure 3. In a subject with cardiovascular disease, 0.42% of all CD4+ T cells in PBMCs were fount to bind P9 tetramer (Figure 4). A mismatched (not expressing DRB1*0101) donor was used as a negative control, and few cells bound the tetramer. As a second control, healthy DRB1*0101+ donors were used and less than 0.1% P9 tetramer+ cells were found (Figure 5). By back-gating, almost 80% of P9 tetramer+ cells were indeed CD4 T cells (Figure 6).

[00164] These data clearly establish that ApoB peptide-specific CD4 T cells can be identified by tetramer in frozen PBMCs from patients with subclinical cardiovascular disease (in this case, identified by carotid IMT). Using this technology, cells can be phenotyped for surface markers, transcription factors and cytokines. In preliminary data, enrichment for CD69, OX40 and PD-1 in P9-tetramer+ CD4 T cells was observed (Figure 7). OX40 and PD-1 are markers of exhaustion. These data support the hypothesis that CD4 T cell exhaustion may be present in humans with atherosclerosis. Accordingly, the P9 tetramer can be used as a biomarker to assess the phenotype (FACS, CYTOF, RNA-Seq) of ApoB-specific CD4 T cells in humans, and as a biomarker to assess success of vaccination with P9.

[00165] Tetramers for human ApoB-specific CD4 T cells allows assessment of activation (CD45RO, CD44, CD62L), polarization (T-bet, Gata3, Bcl6, RORγt, FoxP3), exhaustion (Ox40, ICOS1, CTLA4 and PD-1), proliferation (Ki-67) and apoptosis (annexin-V and active caspase). Both laser-based FACS and CYTOF can be used. The transcriptome of P9 tetramer+ CD4 T cells can be analyzed by RNA-Seq.

References (for Example 2)

1. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL and Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci U S A. 1995;92:3893-3897.

2. Li J, McArdle S, Gholami A, Kimura T, Wolf D, Gerhardt T, Miller J, Weber C and Ley K. CCR5+T-bet+FoxP3+ Effector CD4 T Cells Drive Atherosclerosis. Circ Res. 2016.

Example 3: Liver protective vaccine

[00166] Peptides (15-mers) from mouse ApoB100, the core protein of LDL, were screened for binding to I-Ab by computer prediction and confirmed by radiolabeled peptide competition. Three new peptides, P101, FGKQGFFPDSVNKALY, affinity 5.5 nM, P102, TLYALSHAVNSYFDVD, affinity 6.8 nM, and P103, LYYKEDKTSLSASAAS, affinity 95 nM, were tested in an atherosclerosis model (ApoE-/- mice on western diet, Figure 8). Immunization with each of the three peptides in CFA and 4 x IFA, but not with adjuvant alone or with irrelevant peptide (ovalbumin, sequence, affinity) showed reduced atherosclerotic plaque in the aortic root by serial sections and in the whole aorta by en face staining. There were no differences in body weight, HDL or LDL cholesterol or triglycerides. Flow cytometry showed that peptide immunization induced IL-10 in 10-15% of peritoneal CD4 T cells, some of which also expressed CCR5. Vaccination with ApoB100 peptides expanded peritoneal FoxP3+ regulatory CD4 T cells and more than tripled the number of CCR5+FoxP3+ cells. Similar trends were also seen in the mediastinal lymph node that drains the peritoneal cavity, but not in the inguinal lymph node. In conclusion, vaccination with MHC-II restricted autologous ApoB100 peptides induces Tregs and IL-10, which resulted in protection from atherosclerosis.

[00167] ApoE-/- mice on western diet develop hepatosteatosis, which is associated with elevated liver enzymes including ALT. As disclosed herein, vaccination with ApoB100 peptides reduced ALT to control levels, suggesting that it prevented or reversed the hepatosteatosis associated with western diet. This means that vaccinating against ApoB100 peptides may be beneficial even in subjects without atherosclerosis.

WHAT IS CLAIMED:

1. A protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof.

2. The protein or peptide of claim 1, wherein the protein or peptide modifies an immune or inflammatory response.

3. The protein or peptide of claim 1, wherein the protein or peptide modifies a T cell response.

4. The protein or peptide of claim 1 wherein the protein or peptide induces, promotes, increases or enhances an immune response.

5. The protein or peptide of claim 1 wherein the protein or peptide elicits, stimulates, induces, promotes, increases or enhances a T cell response.

6. The protein or peptide of claim 1 wherein the protein or peptide decreases, reduces, inhibits, suppresses or disrupts an immune or inflammatory response.

7. The protein or peptide of claim 1 wherein the protein or peptide elicits, stimulates, induces, promotes, increases or enhances an anti- immune or anti- inflammatory response.

8. The protein or peptide of claim 1 wherein the protein or peptide has prophylactic or therapeutic activity or function against an adverse cardiovascular event or cardiovascular disease or against liver disease, disorder or damage.

9. The protein or peptide of claim 1 wherein the protein or peptide has prophylactic or therapeutic activity or function against atherosclerosis.

10. The protein or peptide of claim 1 wherein the protein or peptide has a length from about 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, 50-75 or 75-100 amino acids.

11. The protein or peptide of claim 1, wherein the peptide is set forth in Table 1.

12. The protein or peptide of claim 1, wherein the peptide comprises SLFFSAQPFEITAST (p18).

13. The protein or peptide of claim 1, wherein the peptide comprises IKHIYAISSAALSAS (p9).

14. The protein or peptide of claim 1, further comprising a heterologous domain.

15. The protein or peptide of claim 1 to 14, wherein the protein or peptide has a function or activity distinct from wild-type full length apoB100.

16. The protein or peptide of claim 15, wherein the function or activity comprises protecting against or reducing the risk of atherosclerosis or protecting against liver disease, disorder or damage.

17. The protein or peptide of claim 15, wherein the function or activity comprises protecting against an adverse cardiovascular event or cardiovascular disease or reducing the risk of a subject to an adverse cardiovascular event or cardiovascular disease.

18. The protein or peptide of any one of claims 1 to 17 wherein the protein or peptide is isolated or purified.

19. A nucleic acid encoding the protein or peptide of any one of claims 1 to 18.

20. A cell expressing the protein or peptide of any one of claims 1 to 18.

21. The cell of claim 20, wherein the cell is a eukaryotic or prokaryotic cell.

22. The cell of claim 20, wherein the cell is a mammalian, insect, fungal or bacterium cell.

23. The cell of claim 20, wherein the cell is a dendritic cell.

24. A composition comprising the protein or peptide of any one of claims 1 to 17.

25. A pharmaceutical composition comprising the protein or peptide of any one of claims 1 to 18 and a pharmaceutically acceptable carrier or excipient.

26. The composition of claim 24 or 25, further comprising an adjuvant.

27. The composition of any of claims 24 to 25, further comprising a statin.

28. The composition of any of claims 24 to 27, wherein the composition is a solid or a liquid.

29. The composition of any of claims 24 to 28, wherein the composition is a microparticle or a nanoparticle.

30. The composition of any one of claims 24 to 29, wherein the composition is a vaccine.

31. The composition of any one of claims 24 to 30, wherein the composition is sterile.

32. The composition of any one of claims 24 to 31, wherein the composition further comprises a salt, a chelating agent, a buffering agent, or an anti-microbial agent.

33. A method of vaccinating a subject against an adverse cardiovascular event or

cardiovascular disease or liver disease, disorder or damage, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to vaccinate the subject against the adverse cardiovascular event or cardiovascular disease.

34. A method of providing a subject with protection against an adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage, comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to provide the subject with protection against the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage.

35. A method of reducing the risk of an adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage in a subject, comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to reduce the risk of the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage in the subject.

36. The method of any one of claims 33 to 35, wherein the method comprises prophylactic vaccination, protection or risk reduction against the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage.

37. The method of any one of claims 33 to 35, wherein the method comprises vaccinating, protecting or reducing risk of the subject against an adverse symptom of the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage.

38. The method of any one of claims 33 to 35, wherein the method comprises vaccinating, protecting or reducing risk of the subject against atherosclerosis.

39. A method of treating a subject for an adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage, the method comprising administering to the subject an amount of protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, sufficient to treat the subject for the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage.

40. The method of claim 39, wherein the adverse cardiovascular event or cardiovascular disease comprises coronary artery disease, peripheral artery disease, cerebrovascular disease, renal artery disease, stroke, myocardial infarction (heart attack), ischemic heart failure, transient ischemic attack or brain trauma.

41. The method of claim 39, wherein the method reduces an adverse symptom of the adverse cardiovascular event or cardiovascular disease or liver disease, disorder or damage

42. The method of any one of claims 39 to 41, wherein the method comprises treating atherosclerosis.

43. The method of any one of claims 39 to 42, wherein the method comprises inducing plaque regression.

44. The method of any one of claims 33 to 43, further comprising administering a statin.

45. The method of any one of claims 33 to 44, wherein the subject is a mammal.

46. The method of any one of claims 33 to 45, wherein the subject is human.

47. The method of any one of claims 33 to 46, wherein the peptide comprises SLFFSAQPFEITAST (p18).

48. The method of any one of claims 33 to 46, wherein the peptide comprises IKHIYAISSAALSAS.

49. A method of determining whether a subject has, has had, is at risk of having or is in need of treatment for an adverse cardiovascular event or cardiovascular disease, the method comprising:

contacting a biological sample of the subject with an agent that comprises a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof, and

detecting binding of immune cells in the biological sample to the agent, wherein detection of binding of immune cells in the biological sample to the agent indicates that the subject has, has had, is at risk of having or is need of treatment for an adverse cardiovascular event or cardiovascular disease.

50. A method of determining the efficacy of the method of any one of claims 33 to 47, the method comprising:

a) contacting a biological sample of the subject prior to performance of the method of any one of claims 33 to 47 with an agent that comprises a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof;

b) detecting binding of immune cells in the biological sample to the agent;

c) performing the method of any one of claims 33 to 47;

d) contacting a biological sample of the subject after performance of the method of any one of claims 33 to 47 with the agent;

e) detecting binding of immune cells in the biological sample of the subject to the agent after performance of the method of any one of claims 33 to 47; and

f) comparing the amount of binding of immune cells to the agent after performance of the method of any one of claims 33 to 47 to the amount of binding of immune cells in the biological sample to the agent prior to performance of the method of any one of claims 33 to 47,

wherein an increase in the binding of immune cells to the agent after performance of the method of any one of claims 33 to 47 when compared to the biological sample of the subject prior to performance of the method of any one of claims 33 to 47 indicates that there is efficacy in the method of any one of claims 33 to 47.

51. The method of claim 49 or 50, wherein the agent is a multimer comprising a protein or peptide comprising, consisting of or consisting essentially of an amino acid sequence set forth as any one of the peptides set forth in in Table 1, or a sub-sequence, portion, homologue, variant or derivative thereof and appropriate MHC-II molecule.

52. The method of claim 51, wherein the multimer comprises a MHC-II molecule that is DRB1*01:01, DRB1*01:02, DRB1*03:01, DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05, DRB1*07:01, DRB1*09:01, DRB1*10:01, DRB1*11:01, DRB1*11:04, DRB1*12:01, DRB1*13:01, DRB1*13:02, DRB1*14:01, DRB1*15:01, DRB3*01:01, DRB3*02:02, DRB4*01:01, DRB5*01:01, DPB1*04:01, DPB1*05:01, or DQB1*06:02.

53. The method of claim 51 or claim 52, wherein the multimer is a tetramer.

54. The method of any one of claims 49 to 53, wherein the agent comprises a protein or peptide comprising, consisting of or consisting essentially of IKHIYAISSAALSAS.

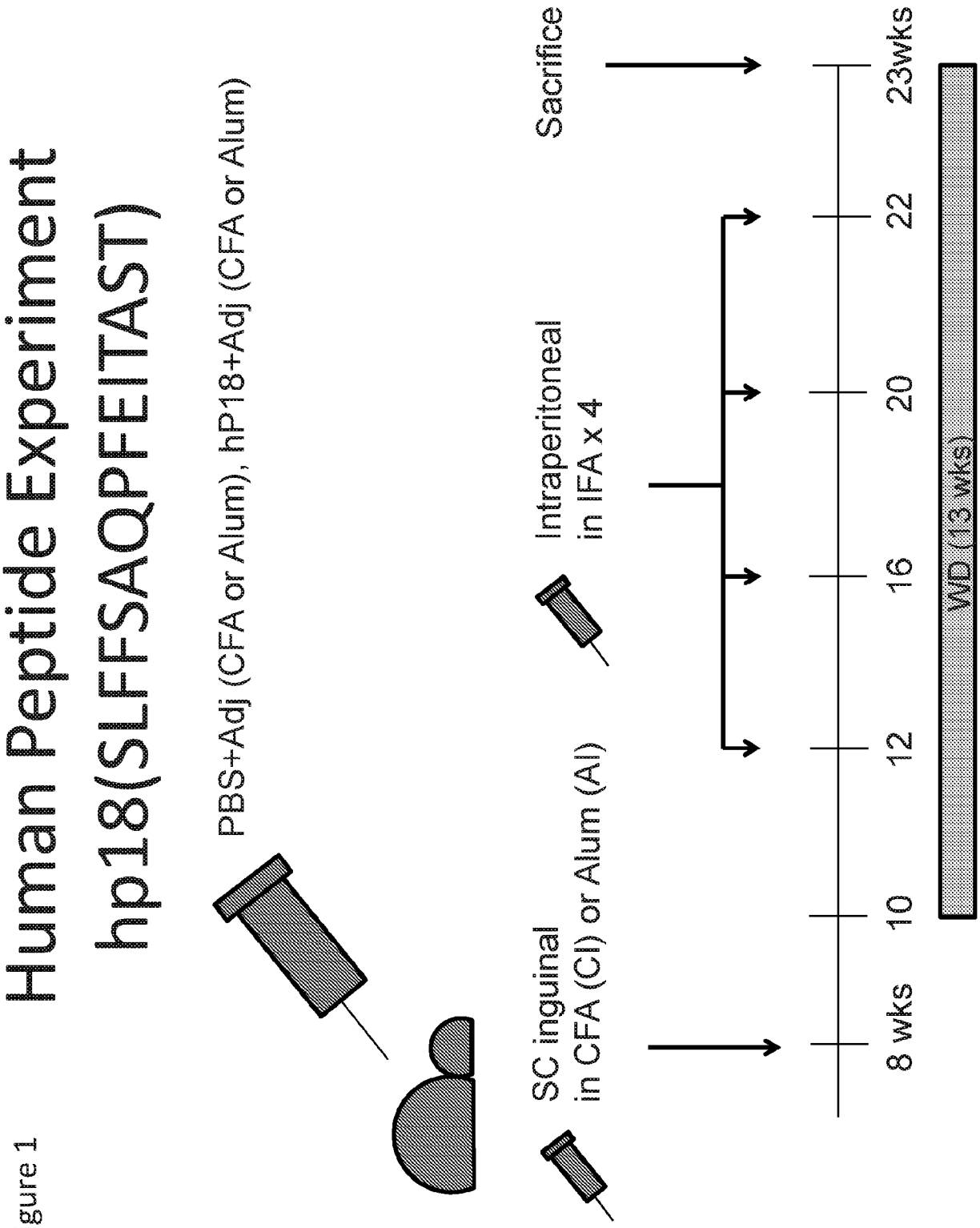
55. The method of claim 54, wherein the agent comprises a MHC-II molecule that is DPB1*02:01, DPB1*03:01, DPB1*04:02, DPB1*14:01, DQB1*02:01, DQB1*03:01, DQB1*06:02, DRB1*01:01, DRB1*04:01, DRB1*04:05, DRB1*07:01, DRB1*08:02, DRB1*09:01, DRB1*1101, DRB1*12:01, DRB1*13:02, DRB1*15:01, DRB3*01:01, DRB3*02:02, DRB4*01:01 or DRB5*01:01.

56. The method of any one of claims 49 to 55, wherein the method comprises detecting immune cell activity in the biological sample.

57. The method of claim 56, wherein the method comprises detecting the amount of immune cell activation, immune cell exhaustion, immune cell polarization or apoptosis of immune cells in the biological sample.

58. The method of claim 57, wherein the method comprises detecting the amount of T cell activation, T cell exhaustion, T cell polarization or apoptosis of T cells in the biological sample.

Figure 1


Human Peptide Experiment
hp18(SLFFSAQPFEITAST)
PBS+Adj (CFA or Alum), hP18+Adj (CFA or Alum)
SC inguinal
in CFA (CI) or Alum (AI)
Intraperitoneal
in IFA x 4
Sacrifice
8 wks
10
12
16
20
22
23wks
WD (13 wks)

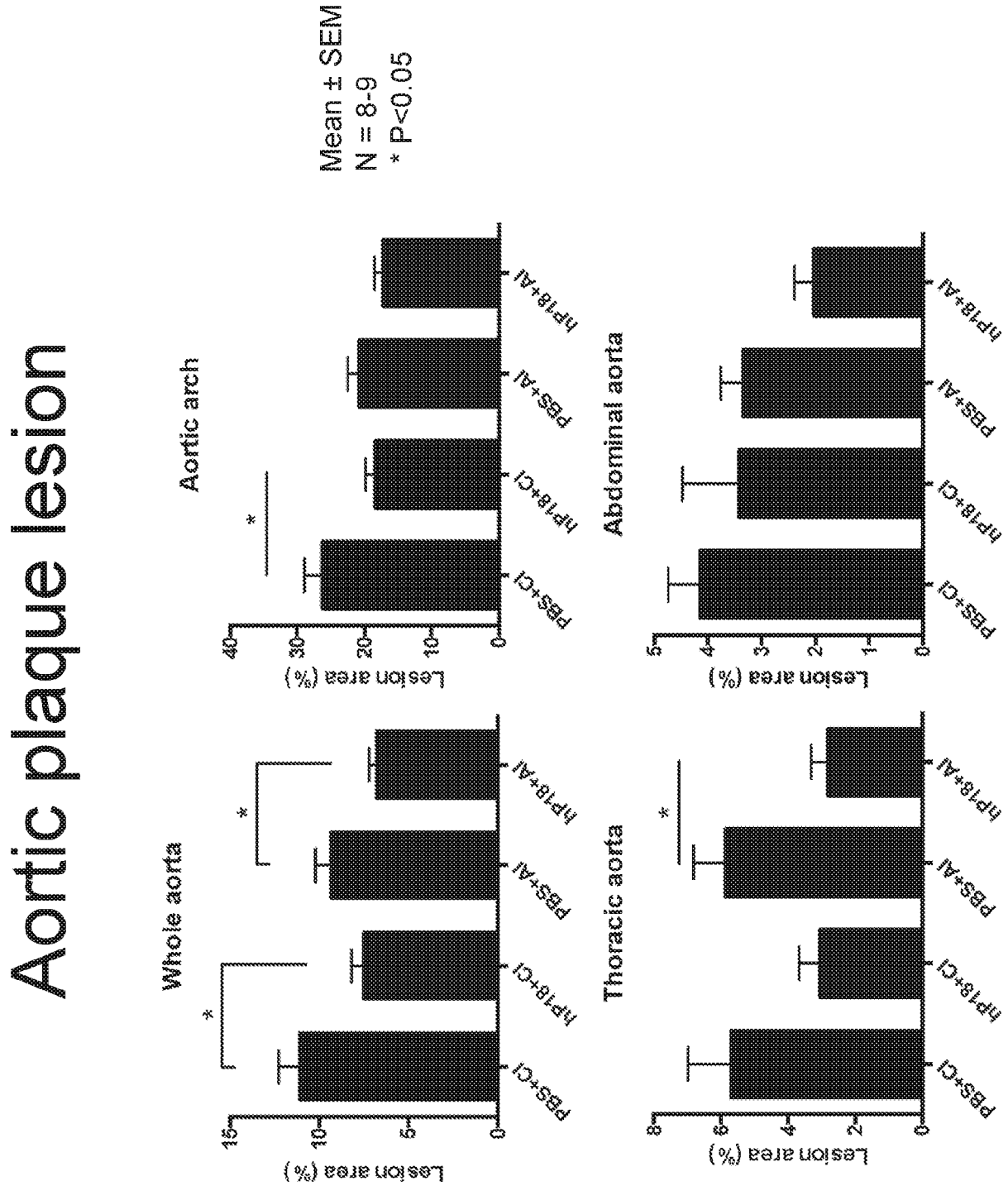

Aortic plaque lesion
Mean ± SEM
N = 8-9
* P<0.05
Aortic arch
Lesion area (%)
0
10
20
30
40
*
PBS+Cl
hPrl8+Cl
PBS+Al
hPrl8+Al
Whole aorta
Lesion area (%)
0
5
10
15
*
*
Abdominal aorta
Lesion area (%)
0
1
2
3
4
5
Thoracic aorta
Lesion area (%)
0
2
4
6
8
*

Figure 2

Figure 3

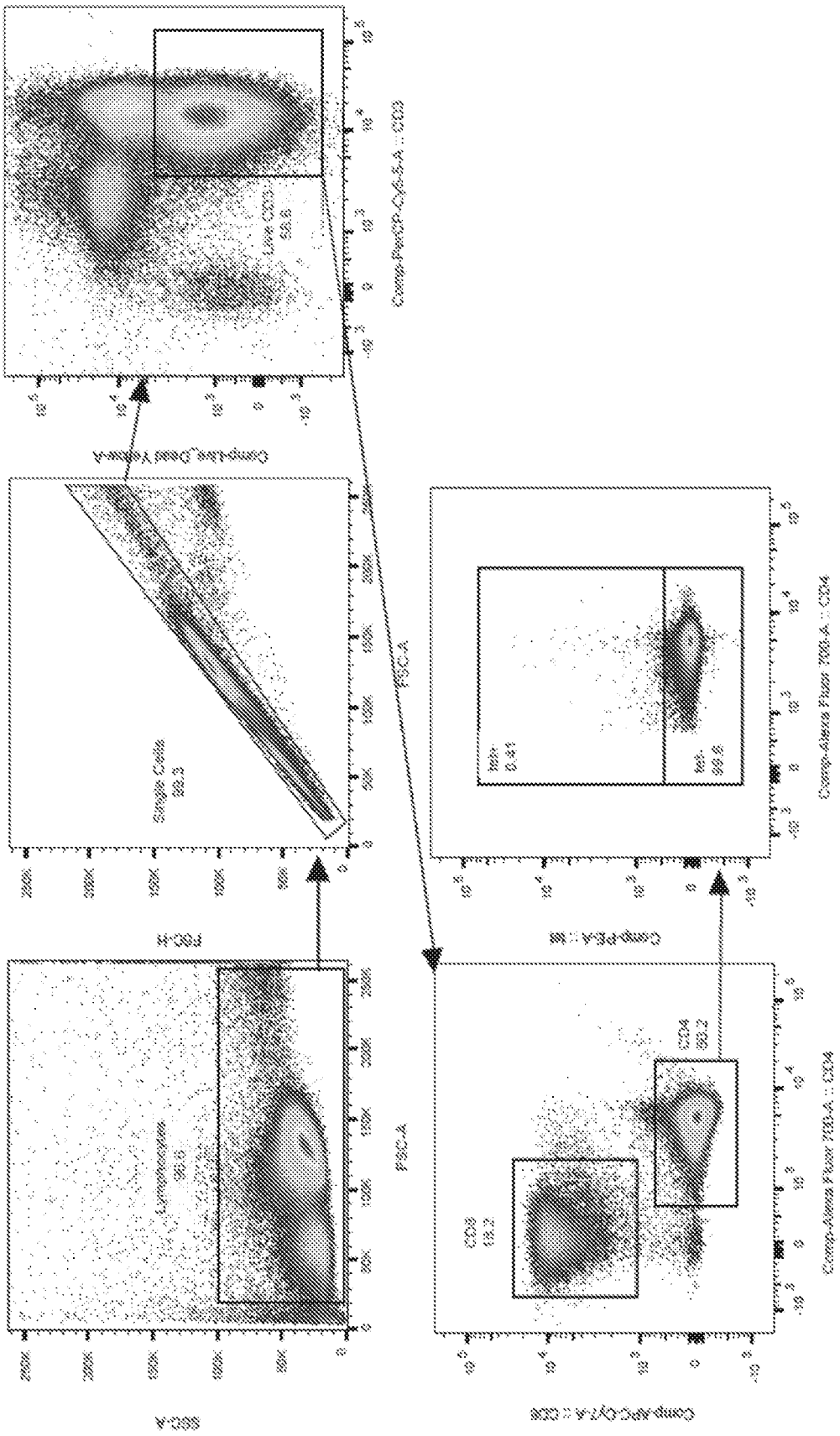

Lymphocytes
68.6
SSC-A
FSC-A
Singlets
98.3
FSC-H
FSC-A
Live CD3
59.6
Comp-PerCP-Cy5-5-A :: CD3
Comp-PE-A :: IgG
Comp-Alexa Fluor 700-A :: CD4
CD8
18.2
CD4
59.3
Comp-APC-Cy7-A :: CD8
Comp-Alexa Fluor 700-A :: CD4

Figure 4

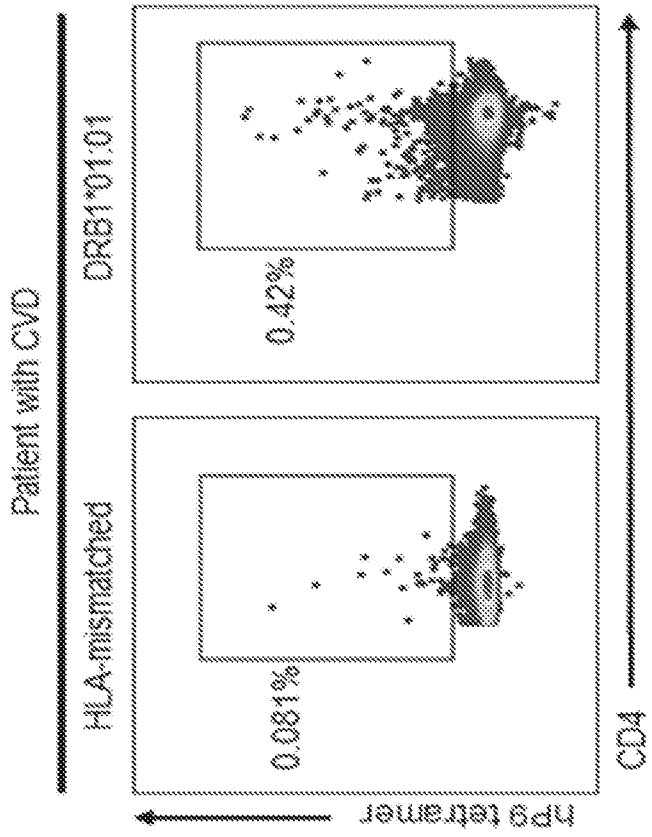
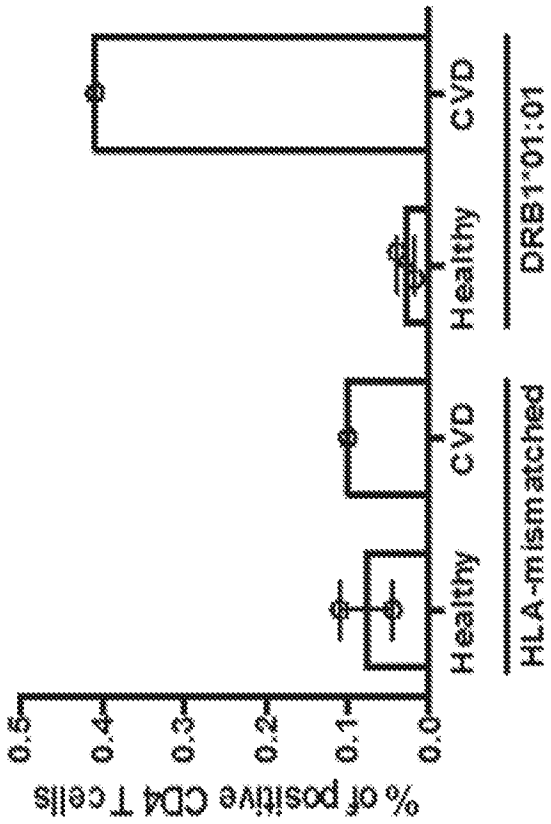


Figure 5



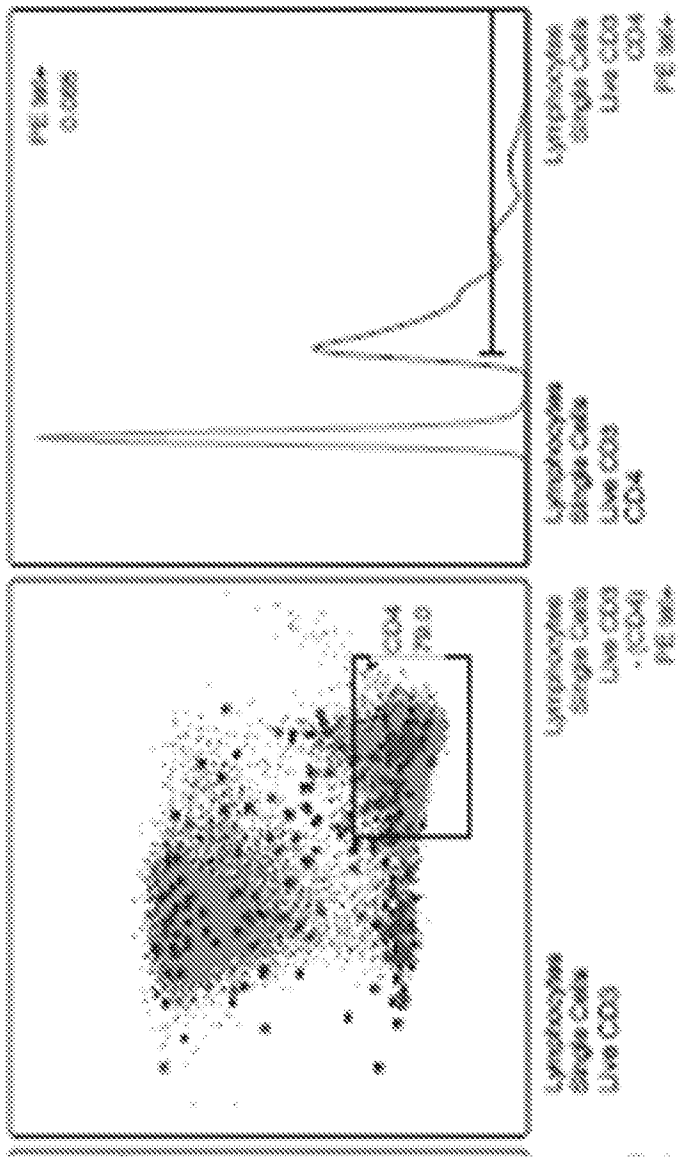


Figure 6

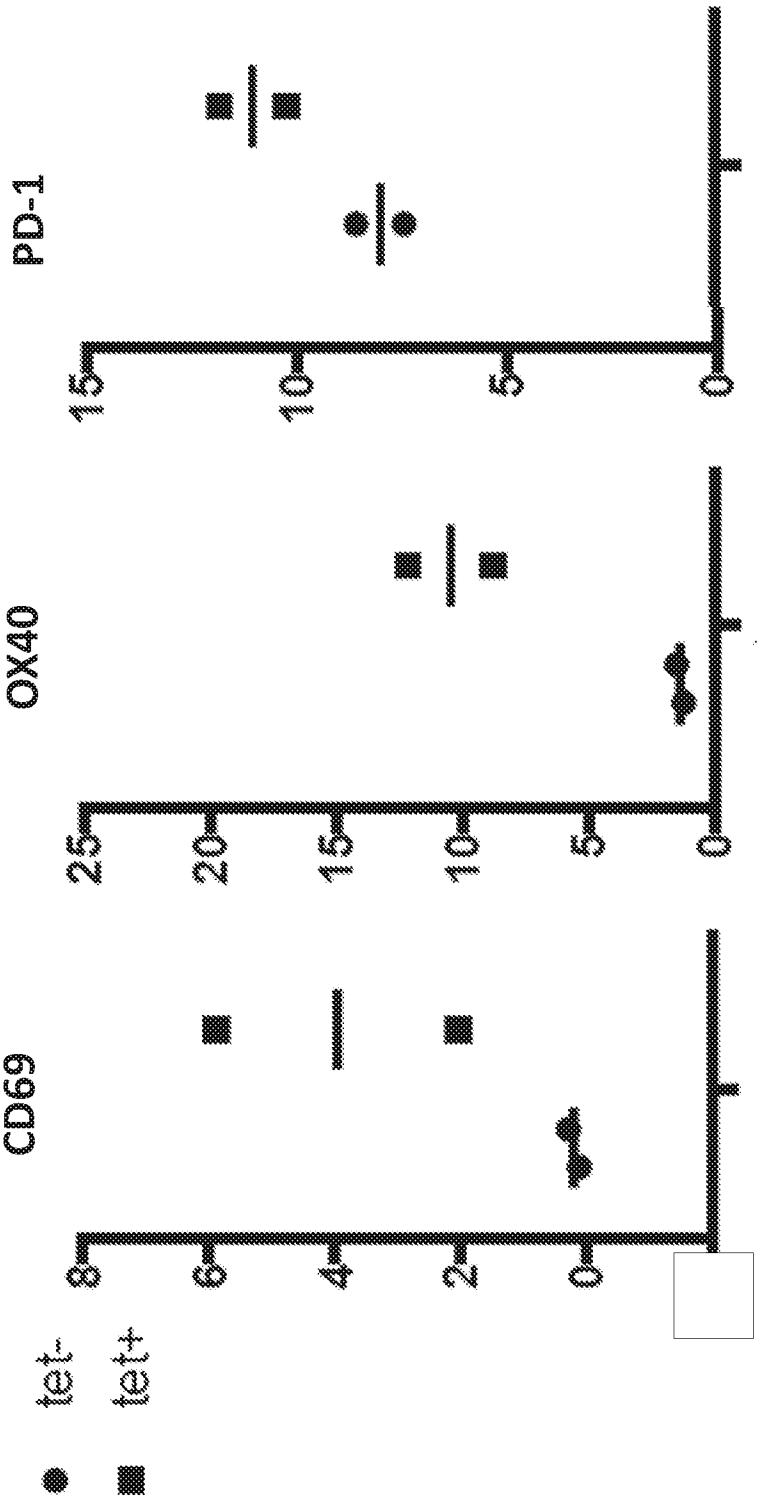
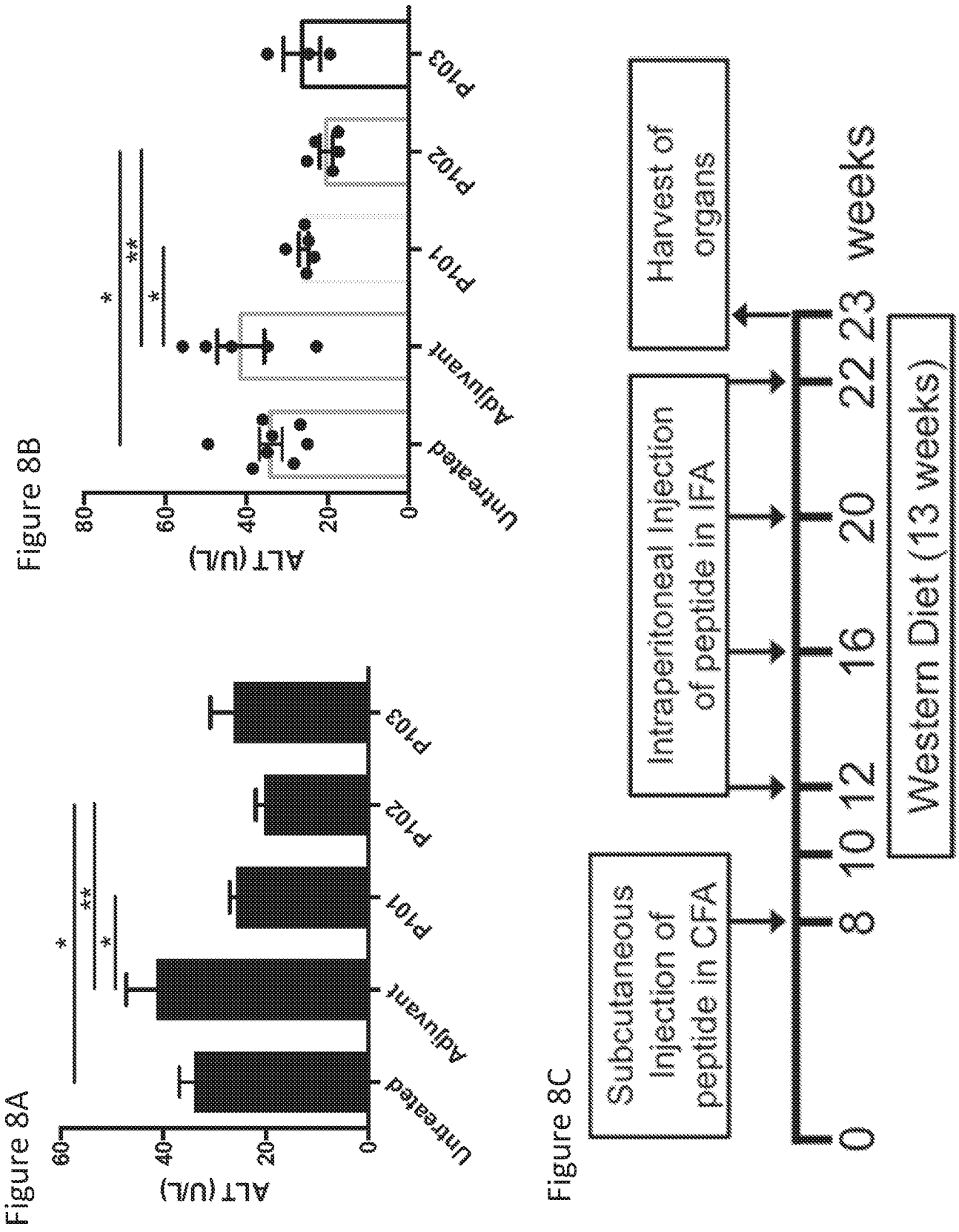


Figure 7


Figure 8A
ALT (U/L)
60
40
20
0
Untreated
Adjuvant
P101
P102
P103
*
**
*
Figure 8B
ALT (U/L)
80
60
40
20
0
Untreated
Adjuvant
P101
P102
P103
*
**
*
Figure 8C
Subcutaneous Injection of peptide in CFA
Intraperitoneal Injection of peptide in IFA
Harvest of organs
0
8
10
12
16
20
22
23
weeks
Western Diet (13 weeks)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2016/032981

A. CLASSIFICATION OF SUBJECT MATTER
(see extra sheet)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
A61K38/17, 39/385, 39/39, 39/395, A61P 9/10, C07K 14/775

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
RUPTO, USPTO, WIPO, Espacenet, Eapatis, DWPI, ARIPO, OAPI, PubMed, EBI-Proteins

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	DATABSE USPTO Proteins: AHD84252, 23.12.2013	1-12, 19, 20-27 13-17, 33-42, 49-52
Y	WO 2005/021029 A2 (AGADJANYAN MICHAEL G et al.) 10.03.2005, claims	20-23
Y	US 2013/302362 A1 (SHAN PREDIMAN K et al.) 14.11.2013, abstract, paragraph [0076].	24-26
Y	ISRAEL F. CHARO et al. Anti-inflammatory therapeutics for the treatment of atherosclerosis. Nat Rev Drug Discov., 2011 May; 10(5): 365-376. doi;10.1038/nrd3444, p.1-25	27
A	EP 2295464 A1 (FORSKARPATENT I SYD AB et al.) 16.03.2011, abstract, claims	1-17, 19-27, 33-42, 49-52

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2016 (19.07.2016)	25 August 2016 (25.08.2016)

Name and mailing address of the ISA/RU:	Authorized officer
Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	P.Dzharmakyun Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2016/032981

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18, 28-32, 43-48, 53-58
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.
PCT/US 2016/032981

A61K 38/17 *(2006.01)*
A61K 39/385 *(2006.01)*
A61K 39/39 *(2006.01)*
A61K 39/395*(2006.01)*
A61P 9/10 *(2006.01)*
C07K 14/775 *(2006.01)*