Modified apolipoproteins with a targeting body for lipid nanoparticles

Field of the invention

The invention relates to the field of modified apolipoproteins that find use in the treatment or prevention of disorders. More specifically the invention relates to apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative with a targeting body. The invention further relates to lipid nanoparticles comprising the modified apolipoproteins and methods of making such. Lastly the invention relates to methods of treatment using the modified apolipoproteins or lipid nanoparticles comprising such.

Introduction

Many promising therapeutics suffer from the fact that they either do not, or poorly, reach the intended target site, or present undesired off-target effects. Additionally, many therapies may trigger unwanted immune responses, resulting in degradation of the therapeutic or undesired inflammatory reactions. Therefore, there is a continuous need to improve or provide alternative ways for targeting of therapeutics and to evade immune responses while delivering the therapeutic to the intended target.

These, among other, problems are addressed by the products and methods as defined in the appended claims.

Summary

The present disclosure is based on the inventors' finding that apolipoproteins, apolipoprotein derivatives, apolipoprotein mimetics or apolipoprotein mimetic derivatives may be modified to target specific cells, tissues or organs, by attaching them to a targeting body, wherein the targeting body is capable of binding a molecule on the cell surface of a target cell, and that such modified apolipoproteins can be used as a carrier for therapeutic agents, either when being used as such, meaning not as part of a lipid nanoparticle, or when as part of a lipid nanoparticle, preferably a spherical lipid nanoparticle.

Moreover, present inventors unexpectedly found that attaching a targeting body to an apolipoprotein, apolipoprotein derivative, an apolipoprotein mimetic or apolipoprotein mimetic derivative allows to easily incorporate said targeting body in a lipid nanoparticle comprising an outer layer and a core, preferably a spherical lipid nanoparticle, and to expose said targeting body to the environment surrounding said lipid nanoparticle. In

this way the modified apolipoprotein can be targeted to cells, tissues or organs it would otherwise not or insufficiently reach, or it could be used to reduce off-target effects.

In a first aspect, the disclosure relates to a modified apolipoprotein comprising an apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative attached to a targeting body, wherein the targeting body is a molecule capable of binding a molecule on the cell surface of a target cell.

In a second aspect, the disclosure relates to a lipid nanoparticle comprising an outer layer and a core, wherein the outer layer comprises:

- a phospholipid;
- a sterol; and
- the modified apolipoprotein as disclosed herein; and wherein the core comprises at least one core component selected from:

a lipid, a cationic lipid, or a polyvalent molecule.

The disclosure also relates to a lipid nanoparticle comprising an outer layer and a core, wherein the outer layer comprises:

- a phospholipid;
- a sterol; and
- the modified apolipoprotein as disclosed herein; and

wherein the core comprises a nucleic acid and a cationic or ionizable cationic lipid.

In a third aspect, the disclosure relates to a method of manufacturing a lipid nanoparticle as disclosed herein, the method comprising the steps of:

a1) expressing and isolating a modified apolipoprotein as disclosed herein to obtain an isolated modified apolipoprotein; and/or

a2) chemically conjugating a targeting body to an apolipoprotein or apolipoprotein mimetic to obtain a modified apolipoprotein and isolating the modified apolipoprotein;

b) combining the isolated modified apolipoprotein obtained in step a1 and/or step a2 with phospholipids, sterols and optionally lipids to obtain a lipid nanoparticle.

In a fourth aspect, the disclosure relates to a method for producing a lipid nanoparticle, comprising the step of:

a) rapid mixing of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce lipid nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a cationic lipid or ionizable cationic lipid, wherein the aqueous buffer has a pH of 5.0 or lower; and

b) rapid mixing of the lipid nanoparticles with one or more modified apolipoprotein as taught herein;

to produce the lipid nanoparticle at a pH between 5.5 and 8.0, preferably pH between 6.0 and 8.0.

In a fifth aspect, the disclosure relates to a modified apolipoprotein according to the first aspect or the lipid nanoparticle according to the second aspect, or the lipid nanoparticle obtained or obtainable by the method of the third or fourth aspect of the disclosure for use as a medicament.

The disclosure also relates to a modified apolipoprotein as defined herein or the lipid nanoparticle as defined herein, or the lipid nanoparticle obtained or obtainable by the method as defined herein for use in the treatment or prevention of an immune related disorder such as transplantation rejection, graft-versus-host disease (GVH), atherosclerosis, infection, inflammation, auto-immunity, allergy, cancer, a genetic disorder, a metabolic disorder, a neurological disorder or tissue trauma.

In a sixth aspect, the disclosure relates to use of the modified apolipoprotein according to the first aspect or the lipid nanoparticle according to the second aspect, or the lipid nanoparticle obtained or obtainable by the method of of the third or fourth aspect of the disclosure in the manufacture of a medicament for the treatment or prevention of an immune related disorder such as transplantation rejection, graft-versus-host disease (GVH), atherosclerosis, infection, inflammation, auto-immunity, allergy, cancer, a genetic disorder, a metabolic disorder, a neurological disorder or tissue trauma.

The disclosure also relates to the use of a modified apolipoprotein as defined herein, or the lipid nanoparticle obtained or obtainable by the method as defined herein in delivering a compound to a target, preferably wherein the target is a cell, tissue, and/or organ, even more preferably wherein the target is a lymphoid cell, a myeloid cell, a tumor cell, an endothelial cell, a hematopoietic stem and progenitor cell (HSPC), a hematopoietic stem cell (HSC), a multipotent progenitor (MPP), a common myeloid progenitor cell (CMP), or wherein the targeting body binds a bacterial, viral, fungal or parasitic protein or antigen,

preferably wherein the lymphoid or myeloid cell is selected from: a monocyte, a macrophage, an M1-like macrophage, an M2-like macrophage, an eosinophil, a basophil, a mast cell, an NK cell, a B cell, a plasma cell, a regulatory T cell, a hematopoietic stem cell, a T helper cell such as Th1, Th2, Th17 or Th22, a dendritic

cell, such as a plasmacytoid dendritic cell, a conventional DC 1 or a conventional DC 2, or a tumor-associated macrophage.

In a seventh aspect, the disclosure relates to use of the modified apolipoprotein according to the first aspect or the lipid nanoparticle according to the second aspect, or the lipid nanoparticle obtained or obtainable by the method of of the third or fourth aspect of the disclosure in the manufacture of a medicament for delivering a compound to a target, preferably wherein the target is a cell, tissue, and/or organ, even more preferably wherein the target is a lymphoid cell, a myeloid cell, a tumor cell, an endothelial cell, a hematopoietic stem and progenitor cell (HSPC), a hematopoietic stem cell (HSC), a multipotent progenitor (MPP), a common myeloid progenitor cell (CMP), or wherein the targeting body binds a bacterial, viral, fungal or parasitic protein or antigen,

preferably wherein the lymphoid or myeloid cell is selected from: a monocyte, a macrophage, an M1-like macrophage, an M2-like macrophage, an eosinophil, a basophil, a mast cell, an NK cell, a B cell, a plasma cell, a regulatory T cell, a hematopoietic stem cell, a T helper cell such as Th1, Th2, Th17 or Th22, a dendritic cell, such as a plasmacytoid dendritic cell, a conventional DC 1 or a conventional DC 2, or a tumor-associated macrophage.

The disclosure also relates to an *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein, with a cell.

In an eighth aspect, the disclosure relates to an *in vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein, with a cell.

In a ninth aspect, the disclosure relates to use of the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein, in the manufacture of a medicament for *in vivo* delivery of a nucleic acid to a subject.

The disclosure also relates to a method for the *in vivo* delivery of a nucleic acid, the method comprising administering the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein, to a subject.

The disclosure also relates to a method for treating a disease or disorder in a subject in need thereof, the method comprising administering a therapeutically effective amount of the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein, to the subject.

In a tenth aspect, the disclosure relates to use of the lipid nanoparticle as disclosed herein, or the lipid nanoparticle obtained or obtainable by the method as disclosed herein in the manufacture of a medicament for treating a disease or disorder.

Brief description of the Figures

Fig. 1 depicts an embodiment of the invention, where a modified apolipoprotein (in this case an apolipoprotein-rerouter fusion protein and more particularly an apolipoprotein-nanobody (VHH) fusion protein) is included in a lipid nanoparticle, such as a spherical lipid nanoparticle. The apolipoprotein nanoparticle's (aNP) main constituents are lipid (e.g. phospholipid), sterol (e.g. cholesterol) and (modified) apolipoprotein.

Fig. 2 is a schematic overview of apolipoprotein (e.g. apolipoprotein A1 (apoA1))-rerouter fusion protein nanoparticle technology. (A) Targeting or rerouting protein is a molecule capable of binding a molecule (on the cell surface) of a target cell and can include an antibody (fragment), protein ligand, peptide, peptidomimetic, or sugar polymer. (B) Apolipoprotein-rerouter fusion protein is a modified apolipoprotein comprising an apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative attached to a targeting body, and more particularly to a rerouting peptide or protein. (C) Apoliprotein-rerouter fusion proteins are readily and stably incorporated in lipid nanoparticles, such as spherical lipid nanoparticles, comprising (phospho)lipids, sterols. In another example, apolipoprotein-rerouter fusion proteins are readily and stably incorporated in spherical lipid nanoparticles comprising (phospho)lipids, sterols, ionizable cationic lipids, and therapeutic payloads including nucleic acids such as small interfering RNA or messenger RNA.

Fig. 3 shows the expression of VHHCD8-apolipoprotein A1 (apoA1) fusion protein in Clearcoli cells. Minor protein contaminants are present after IMAC purification [lane E1]. The most prominent band corresponds to the fusion protein with a molecular weight of 43.3 kDa (rectangle).

Fig. 4 shows the mean fluorescence intensity (MFI) of fluorescently labelled VHHCD8-apoA1 and apoA1 in mouse splenocytes (upper panel: CD3+ T cells from splenocytes; lower panel: all cells from the spleen).

CLAIMS

1. A modified apolipoprotein comprising an apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative attached to a targeting body, wherein the targeting body is a molecule capable of binding a molecule on the cell surface of a target cell.

2. The modified apolipoprotein according to claim 1, wherein the targeting body is selected from:

- an antibody or antigen binding fragment of an antibody,
- a protein ligand, a protein binding domain, or a protein binding fragment thereof,
- a peptide,
- a peptidometic, or
- a sugar polymer.

3. The modified apolipoprotein according to claim 2, wherein the antibody or antigen binding fragment thereof is selected from a Fab, a Fab2, a scFv, a scFv-Fc, a dAb-Fc, a free light chain antibody, a half antibody, a bispecific Fab2, a Fab3, a trispecific Fab3 a diabody, a bispecific diabody, a triabody, a trispecific triabody, a minibody, an IgG, an IgNAR, a monovalent IgG, a VhH, a nanobody, or a VNAR, or an antigen binding fragment thereof, preferably a camelid or shark VhH or derivative thereof or antigen binding fragment thereof.

4. The modified apolipoprotein according to any one of the preceding claims, wherein the targeting body binds a protein on the surface of a lymphoid cell, a myeloid cell, a tumor cell, an endothelial cell, a hematopoietic stem and progenitor cell (HSPC), a hematopoietic stem cell (HSC), a multipotent progenitor (MPP), a common myeloid progenitor cell (CMP), or wherein the targeting body binds a bacterial, viral, fungal or parasitic protein or antigen,

preferably wherein the lymphoid or myeloid cell is selected from a monocyte, a macrophage, an M1-like macrophage, an M2-like macrophage, an eosinophil, a basophil, a mast cell, an NK cell, a B cell, a plasma cell, a regulatory T cell, a hematopoietic stem cell, a granulocyte, a T helper cell such as Th1, Th2, Th17 or Th22, a dendritic cell, such as a plasmacytoid dendritic cell, a conventional DC 1 or a conventional DC 2, or a tumor-associated macrophage.

5. The modified apolipoprotein according to any one of the preceding claims, wherein the targeting body binds a protein selected from CD1a, CD1b, CD1c, CD1d, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD15u, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79α, CD79β, CD80, CD81, CD82, CD83, CDw84, CD85, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD114, CD115, CD116, CD117, CD118, CD119, CD120a, CD120b, CD121a, CDw121b, CD122, CD123, CD124, CD125, CD126, CD127, CDw128, CD129, CD130, CDw131, CD132, CD133, CD134, CD135, CDw136, CDw137, CD138, CD139, CD140a, CD140b, CD141, CD142, CD143, CD144, CD145, CD146, CD147, CD148, CD150, CD151, CD152, CD153, CD154, CD155, CD156a, CD156b, CD157, CD158, CD158a, CD158b, CD159a, CD160, CD161, CD162, CD162R, CD163, CD164, CD165, CD166, CD167a, CD168, CD169, CD170, CD171, CD172a, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD183, CD184, CD195, CDw197, CD200, CD201, CD202b, CD203c, CD204, CD205, CD206, CD207 (CLEC4K), CD208, CD209 (CLEC4L), CDw210, CD212, CD213a1, CD213a2, CDw217, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD236, CD236R, CD238, CD239, CD240CE, CD240D, CD241, CD242, CD243, CD244, CD245, CD246, CD247, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, CCR9, CLEC1A, CLEC1B, CLEC2A, CLEC2B, CLEC3A, CLEC3B, CLEC4A, CLEC4C (CD303), CLEC4D,CLEC4J CLEC4E, CLEC4F, CLEC4G, ASGR1 (CLEC4H1), ASGR2 (CLEC4H2), FCER2 (), CLEC4M, CLEC5A, CLEC6A, CLEC7A, OLR1 (CLEC8A), CLEC9A, CLEC10A, CLEC11A, CLEC12A, CLEC12B, CD302 (CLEC13A), LY75 (CLEC13B), $PLA2R_1$ (CLEC13C), MRC1 (CLEC13D), MRC2 (CLEC13E), CLEC14A, CLEC16A, CLEC17A, KLRA1, KLRB1 (CLEC5B), KLRC1, KLRC2, KLRC3, KLRC4, KLRD1, KLRF1 (CLEC5C), KLRG1 (CLEC15A), KLRG2 (CLEC15B), KLRK1, AGC1, ATRNL1, BCAN, CD248, CHODL, CL-K1-Ia, CL-K1-Ib, CL-

K1-Ic, CLECSF5, COLEC10, COLEC11, COLEC12, CSPG3, FCER2, FREM1, HBXBP, LAYN, LOC348174, LOC728276, MAFA, MBL2, MGC34761, MICL, MRC1L1, PAP, PKD1, PKD1L2, PRG2, PRG3, REG1A, REG1B, REG3A, REG3G, REG4, SELE, SELL, SELP, SFTPA1, SFTPA2, SFTPA2B, SFTPD, SRCL, THBD, VCAN, Alphafetoprotein (AFP), Carcinoembryonic antigen (CEA), CA-125, MUC-1, Epithelial tumor antigen (ETA), Tyrosinase, and melanoma-associated antigen (MAGE).

preferably selected from CD14, CD11b, CD357 (GITR), CD193, CD123, CD117, CD56, CD19, CD38, CD25, CD133, CXCR3, CCR3, CD196, CCR10, CD64, CD206, CLEC4C (CD303), CLEC9A, CD1c, CD163, Alphafetoprotein (AFP), Carcinoembryonic antigen (CEA), CA-125, MUC-1, Epithelial tumor antigen (ETA), Tyrosinase, and melanoma-associated antigen (MAGE).

6. The modified apolipoprotein according to any one of the previous claims, wherein the apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative is selected from apoA1, apoA-1 Milano, apoA2, apoA4, apoA5, apoB, apoB48, apoB100, apoC-I, apoC-II, apoC-III, apoC-IV, apoD, apoE, apoF, apoH, apoL, apoL1, apoL2, apoL3, apoL4, apoL5, apoL6, apoLD1, apoO, apoOL and apoM, or a combination thereof, or a mimetic or derivative thereof,

preferably selected from apoA1, apoA2, apoA4, apoA5, apoB48, apoB100, apoC-I, apoC-II, apoC-III, apoC-IV, apoD, apoE, apoF, apoH, apoL and apoM or a mimetic or derivative thereof,

more preferably selected from apoA1, apoA2, apoA4, apoA5, apoB100, apoC-I, apoC-II, apoC-III, apoC-IV and apoE or a mimetic or derivative thereof,

even more preferably selected from apoA1, apoA4, apoA5, apoB100, apoC-III and apoE or a mimetic or derivative thereof,

most preferably selected from apoA1, apoB100 and apoE or a mimetic or derivative thereof.

7. A lipid nanoparticle comprising an outer layer and a core, wherein the outer layer comprises:

- a phospholipid;
- a sterol; and
- the modified apolipoprotein as defined in any one of the preceding claims; and wherein the core comprises at least one core component selected from:

a lipid, a cationic lipid, or a polyvalent molecule.

8. The lipid nanoparticle according to claim 7, wherein the nanoparticle further comprises a payload, preferably wherein the payload is comprised in the core, the phospholipid layer or wherein the payload is bound to a component of the outer layer.

9. The lipid nanoparticle according to claim 8, wherein the payload is selected from a nucleic acid or a nucleic acid analog, a therapeutic, a biologic, a cytokine, a chemokine, a hormone, a growth factor, or combinations thereof.

10. The lipid nanoparticle according to claim 9, wherein the lipid nanoparticle comprises a nucleic acid and a cationic or ionizable cationic lipid.

11. The lipid nanoparticle according to claim 10, wherein the nucleic acid and the cationic or ionizable cationic lipid are comprised by the core, and the modified apolipoprotein and the phospholipid are comprised by the outer layer.

12. The lipid nanoparticle according to claim 10 or 11, wherein the nanoparticle core further comprises a filler, preferably a filler selected from a triacylglyceride and a cholesterol acyl ester, or combinations thereof, such as wherein the triacylglyceride is tricaprylin and/or wherein the cholesterol acyl ester is cholesteryl caprylate and/or cholesteryl oleate.

13. The lipid nanoparticle according to any one of claims 9 to 12, wherein the nucleic acid is RNA, DNA or a nucleic acid analogue.

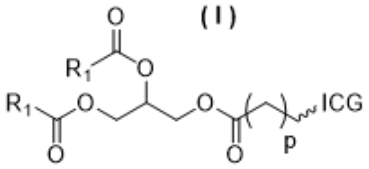
14. The lipid nanoparticle according to claim 13, wherein the RNA is microRNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), transfer RNA (tRNA), tRNA-derived small RNA (tsRNA), small regulatory RNA (srRNA), messenger RNA (mRNA), modified mRNA, ribosomal RNA (rRNA), long non-coding RNA (lncRNA), or guide RNA (gRNA), or combinations thereof and/or modifications thereof.

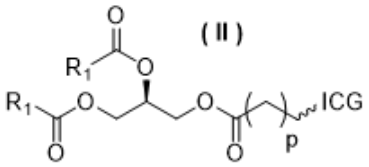
15. The lipid nanoparticle according to claim 13, wherein the DNA is single stranded or double stranded DNA.

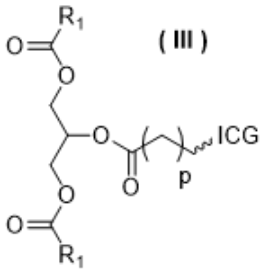
16. The lipid nanoparticle according to any one of claims 10 to 15, wherein the cationic or ionizable cationic lipid is selected from an ionizable cationic ester of a long

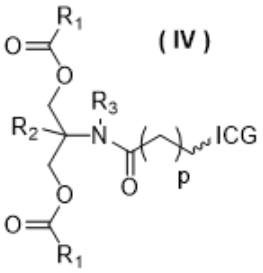
chain alcohol, an ionizable cationic ester of a diglyceride or an ionizable cationic ester of a sterol or combinations thereof.

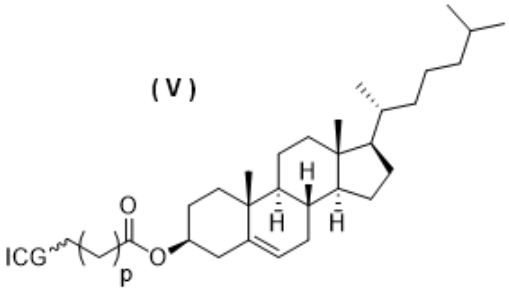
17. The lipid nanoparticle according to any one of claims 10 to 16, wherein the ionizable cationic lipid is a molecule according to any one of Formulae (I), (II), (III), (IV) or (V)

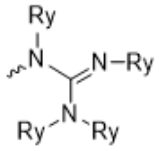










wherein ICG is  or , wherein the wavy line indicates the point of attachment to the compound of formulae (I), (II), (III), (IV) or (V);

p is an integer selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11;

each R_1 is independently selected from the group consisting of linear or branched C1-C19 alkyl, linear or branched C1-C19 alkenyl, aryl, arylene-alkyl and alkylene-aryl group, wherein said alkyl or alkenyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

R_2 is selected from the group consisting of hydrogen, methyl, ethyl and a $-CH_2-O-C(O)-R_{1a}$;

R_3 is selected from the group consisting of hydrogen, aryl, arylene-alkyl, alkylene-aryl and linear C1-C6 alkyl group;

R_{1a} is selected from the group consisting of linear or branched C1-C19 alkyl, linear or branched C1-C19 alkenyl, aryl, arylene-alkyl and alkylene-aryl group, wherein said alkyl or alkenyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

each R_x is independently selected from the group consisting of methyl, ethyl, propyl and $-CH_2-CH_2-OH$;

each R_y group is independently selected from the group consisting of hydrogen, linear or branched C1-C18 alkyl, aryl, arylene-alkyl or alkylene-aryl group, wherein said alkyl group optionally contains up to 5 heteroatoms, independently selected from O and N;

or rotamers, tautomers stereoisomers or regioisomers thereof.

18. The lipid nanoparticle according to any one of claims 10 to 16, wherein:

the amount of the modified apolipoprotein (in case of two or more modified apolipoproteins, cumulatively) ranges from 0.08 to 2.0 mol%, such as from 0.10 to 2.0 mol%; and/or

the amount of phospholipid ranges from 5 to 90 mol%, such as from 15 to 90 mol%; and/or

the amount of sterol ranges from 2.5 to 65 mol%, such as from 2.5 to 50 mol%; and/or

the amount of cationic or ionizable cationic lipid ranges from 5.0 to 80 mol%, such as from 8.0 to 80 mol%,

wherein the molar percentage is based solely on the combined amounts of the modified apolipoprotein, phospholipids, sterols and cationic or ionizable cationic lipids in the lipid nanoparticle.

19. The apolipoprotein lipid nanoparticle according to any one of claims 10 to 18, wherein:

the amount of the modified apolipoprotein (in case of two or more modified apolipoproteins, cumulatively) ranges from 0.1 to 90 weight%;

the amount of nucleic acid ranges from 0.01 to 90 weight%;

the amount of phospholipid ranges from 0.1 to 95 weight%;

the amount of sterol ranges from 0.1 to 95 weight%; and/or

the amount of cationic and/or ionizable cationic lipid ranges from 0.1 to 95 weight%,

wherein these weight percentages are based on the combined amounts of the modified apolipoprotein, the nucleic acid, the phospholipid, the sterol and the cationic and/or ionizable cationic lipid.

20. The lipid nanoparticle according to any one of claims 10 to 19, wherein the ratio of the modified apolipoprotein (in case of two or more modified apolipoproteins, cumulatively) to phospholipid based on percentage molar weight is between 1:25 and 1:400, more preferably between 1:50 and 1:200, even more preferably between 1:75 and 1:150.

21. The lipid nanoparticle according to any one of claims 10 to 20, wherein the ratio of the modified apolipoprotein (in case of two or more modified apolipoproteins, cumulatively) to phospholipid based on weight is from 2:1 to 1:10, more preferably from 1:1 to 1:5, even more preferably from 1:1.5 to 1:4.

22. Method of manufacturing a lipid nanoparticle as defined in any one of claims 7 to 9, the method comprising the steps of:

a1) expressing and isolating a modified apolipoprotein as defined in any one of claims 1-5 to obtain an isolated modified apolipoprotein; and/or

a2) chemically conjugating a targeting body to an apolipoprotein or apolipoprotein mimetic to obtain a modified apolipoprotein and isolating the modified apolipoprotein;

b) combining the isolated modified apolipoprotein obtained in step a1 and/or step a2 with phospholipids, sterols and optionally lipids to obtain a lipid nanoparticle.

23. The method of claim 22 wherein step b) comprises:

b1) mixing, preferably rapid mixing, of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a core component, and optionally a lipid; and wherein the aqueous buffer has a pH of 5.5 or lower, preferably 5.0 or lower; and

b2) mixing, preferably rapid mixing, of lipid nanoparticles with the modified apolipoprotein to produce the nanoparticle at a pH between 5.5 and 9.0, preferably at a pH between 6.0 and 8.0, more preferably at a pH between 6.5 and 8.0.

24. Method for producing a lipid nanoparticle, comprising the step of:

a) rapid mixing of lipid components in organic solvent with a nucleic acid in an aqueous buffer to produce lipid nanoparticles, wherein the lipid components comprise a phospholipid, a sterol, a cationic lipid or ionizable cationic lipid, wherein the aqueous buffer has a pH of 5.0 or lower; and

b) rapid mixing of the lipid nanoparticles with one or more modified apolipoprotein as defined in any one of claims 1 to 6;

to produce the lipid nanoparticle at a pH between 5.5 and 8.0, preferably pH between 6.0 and 8.0.

25. The modified apolipoprotein according to any one of claims 1 to 6 or the lipid nanoparticle according to any one of claims 7 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24 for use as a medicament.

26. Use of the modified apolipoprotein according to any one of claims 1 to 6 or the lipid nanoparticle according to any one of claims 7 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24 in the manufacture of a medicament for the treatment or prevention of an immune related disorder such as transplantation rejection, graft-versus-host disease (GVH), atherosclerosis, infection, inflammation, auto-immunity, allergy, cancer, a genetic disorder, a metabolic disorder, a neurological disorder or tissue trauma.

27. Use of the modified apolipoprotein according to any one of claims 1 to 6 or the lipid nanoparticle according to any one of claims 7 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24 in the manufacture of a medicament for delivering a compound to a target, preferably wherein the target is a cell, tissue, and/or organ, even more preferably wherein the target is a lymphoid cell, a myeloid cell, a tumor cell, an endothelial cell, a hematopoietic stem and progenitor cell (HSPC), a hematopoietic stem cell (HSC), a multipotent progenitor (MPP), a common myeloid progenitor cell (CMP), or wherein the targeting body binds a bacterial, viral, fungal or parasitic protein or antigen,

preferably wherein the lymphoid or myeloid cell is selected from: a monocyte, a macrophage, an M1-like macrophage, an M2-like macrophage, an eosinophil, a basophil, a mast cell, an NK cell, a B cell, a plasma cell, a regulatory T cell, a hematopoietic stem cell, a T helper cell such as Th1, Th2, Th17 or Th22, a dendritic cell, such as a plasmacytoid dendritic cell, a conventional DC 1 or a conventional DC 2, or a tumor-associated macrophage.

28. An *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the lipid nanoparticle according to any one of claims 9 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24, with a cell.

29. Use of the lipid nanoparticle according to any one of claims 9 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24, in the manufacture of a medicament for *in vivo* delivery of a nucleic acid to a subject.

30. Use of the lipid nanoparticle according to any one of claims 9 to 21, or the lipid nanoparticle obtained or obtainable by the method of any one of claims 22 to 24 in the manufacture of a medicament for treating a disease or disorder.