APOLIPOPROTEIN FUSION PROTEINS FOR CELL-SPECIFIC IMMUNE REGULATION

FIELD OF THE INVENTION

The invention relates to the field of fusion proteins and more particularly fusion proteins that find use in the treatment of immune related disorders. The invention further relates to lipid nanoparticles comprising the fusion proteins and methods of making such. Lastly the invention relates to methods of treatment using the fusion proteins or lipid nanoparticles.

BACKGROUND OF THE INVENTION

Many promising therapeutics are hampered by poor circulation time due to rapid clearance of the therapeutic from the body. For example, it was found that cytokines may hold very promising uses in many immunological applications. However due to the very short half-life in the body, either no effect can be exerted on the intended target, or toxic amounts are needed to achieve an effect. Therefore, there is a need for improved methods to safely reduce the circulation half-life of therapeutics.

Additionally, 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. Therefore, there is a further need to improve targeting of therapeutics.

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 apolipoprotein can be used as a carrier for therapeutic agents, and that apolipoproteins may further be modified to target specific cells, tissues or organs. The inventors found that fusion proteins of immunomodulatory biomolecules, such as cytokines, with apolipoproteins or apolipoprotein mimetics demonstrate strongly increased half-life in blood, thereby opening up the possibility to use immunomodulatory biomolecules, such as cytokines, in a therapeutical manner without requiring toxic concentrations to be administered. Further it was realized that apolipoproteins or mimetics thereof allow targeting of immunomodulatory biomolecules, such as cytokines, when fused together.

It was further realized that it is possible to direct a fusion protein or apolipoprotein (or mimetic thereof) to an intended target by linking it to a rerouting molecule. Moreover, present inventors unexpectedly found that the fusion of an immunomodulatory biomolecule and/or a rerouting molecule to an apolipoprotein or an apolipoprotein mimetic allows to

easily incorporate said immunomodulatory biomolecule and/or a rerouting molecule in a lipid nanoparticle and to expose said immunomodulatory biomolecule and/or a rerouting molecule to the environment surrounding said lipid nanoparticle. In this way the apolipoprotein (or mimetic thereof) or fusion protein 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.

The fusion proteins may be used as such, meaning not as part of a lipoprotein or lipid nanoparticle. In such way the fusion protein may serve as a carrier to deliver an immunomodulatory biomolecule to a target site. Alternatively, the fusion protein may be used to prepare a lipid nanoparticle.

The disclosure provides an apolipoprotein lipid nanoparticle comprising

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and an immunomodulatory biomolecule; and

phospholipids;

wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response.

A first aspect of the disclosure provides an apolipoprotein lipid nanoparticle comprising

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and a rerouting molecule; and

phospholipids;

wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity.

The disclosure provides an apolipoprotein lipid nanoparticle comprising

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic, an immunomodulatory biomolecule, and a rerouting molecule; and

phospholipids;

wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response; and

wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity.

The disclosure provides an apolipoprotein lipid nanoparticle comprising

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and an immunomodulatory biomolecule; wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response;

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and a rerouting molecule; wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity; and

phospholipids.

The disclosure provides a method of manufacturing an apolipoprotein lipid nanoparticle as described herein, the method comprising the steps of:

a1) expressing and isolating one or more apolipoprotein fusion proteins to obtain one or more isolated apolipoprotein fusion proteins,

wherein the one or more apolipoprotein fusion proteins are selected from the group consisting of: an apolipoprotein or apolipoprotein mimetic fused to an immunomodulatory biomolecule;

an apolipoprotein or apolipoprotein mimetic fused to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic fused to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and/or

a2) chemically conjugating one or more apolipoproteins or apolipoprotein mimetics and isolating the one or more conjugated apolipoproteins to obtain one or more isolated conjugated apolipoproteins,

wherein the one or more conjugated apolipoproteins are selected from the group consisting of: an apolipoprotein or apolipoprotein mimetic conjugated to an immunomodulatory biomolecule;

an apolipoprotein or apolipoprotein mimetic conjugated to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic conjugated to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and

b) combining the one or more isolated apolipoprotein fusion proteins obtained in step a1 and/or the one or more isolated conjugated apolipoproteins obtained in step a2 with phospholipids, and optionally sterols and/or lipids, to obtain an apolipoprotein lipid nanoparticle.

A further aspect of the disclosure provides a method of manufacturing an apolipoprotein lipid nanoparticle as described herein, the method comprising the steps of:

a1) expressing and isolating one or more apolipoprotein fusion proteins to obtain one or more isolated apolipoprotein fusion proteins,

wherein the one or more apolipoprotein fusion proteins are selected from the group consisting of:

an apolipoprotein or apolipoprotein mimetic fused to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic fused to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and/or

a2) chemically conjugating one or more apolipoproteins or apolipoprotein mimetics and isolating the one or more conjugated apolipoproteins to obtain one or more isolated conjugated apolipoproteins,

wherein the one or more conjugated apolipoproteins are selected from the group consisting of:

an apolipoprotein or apolipoprotein mimetic conjugated to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic conjugated to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and

b) combining the one or more isolated apolipoprotein fusion proteins obtained in step a1 and/or the one or more isolated conjugated apolipoproteins obtained in step a2 with phospholipids, .and optionally sterols and/or lipids, to obtain an apolipoprotein lipid nanoparticle

The disclosure provides an apolipoprotein lipid nanoparticle obtained by or obtainable by the method as taught herein.

A further aspect of the disclosure provides a pharmaceutical composition comprising the apolipoprotein lipid nanoparticle as taught herein, and a pharmaceutically acceptable carrier.

A further aspect of the disclosure provides the apolipoprotein lipid nanoparticle as taught herein or the pharmaceutical composition as taught herein for use as a medicament.

The disclosure provides use of the apolipoprotein lipid nanoparticle as taught herein or the pharmaceutical composition as taught herein in the manufacture of a medicament for the treatment of an immune related disorder.

The disclosure provides the apolipoprotein lipid nanoparticle as taught herein or the pharmaceutical composition as taught herein for use in targeting said immunomodulatory biomolecule to a target cell, preferably a myeloid cell.

The disclosure provides the use of the apolipoprotein lipid nanoparticle as taught herein for delivering an immunomodulatory biomolecule to a target, preferably wherein the target is a cell, tissue, and/or organ.

The disclosure provides a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and an immunomodulatory biomolecule, wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response, for use in targeting said immunomodulatory biomolecule to a myeloid cell.

A further aspect of the disclosure provides a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and a rerouting molecule, wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity.

The disclosure provides a nucleic acid encoding the fusion protein comprising the apolipoprotein or an apolipoprotein mimetic and the rerouting molecule as taught herein.

The disclosure provides a pharmaceutical composition comprising the fusion protein as taught herein or the nucleic acid as taught herein, and a pharmaceutically acceptable carrier.

The disclosure provides the fusion protein as taught herein, the nucleic acid as taught herein or the pharmaceutical composition as taught herein for use as a medicament.

The disclosure provides the fusion protein as taught herein, the nucleic acid as taught herein or the pharmaceutical composition as taught herein for use in the treatment of an immune related disorder, preferably wherein the immune related

disorder is an immune related disorder selected from the group consisting of cancer, inflammation, an infectious disease, an autoimmune disorder, allergy, organ transplant rejection, and graft-versus-host disease (GVH).

The disclosure also provides the fusion protein as taught herein, the nucleic acid encoding the fusion protein as taught herein or the pharmaceutical composition as taught herein when comprising an immunomodulatory biomolecule, wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response, for use in targeting said immunomodulatory biomolecule to a target cell.

As supported by the example section, present inventors found that a fusion protein of an apolipoprotein or an apolipoprotein mimetic, preferably ApoA1, with IL-4 allows targeting IL-4 to the myeloid compartment. To their surprise, present inventors found that IL-4 can simultaneously reduce inflammation and induce trained immunity, particularly when targeted to the myeloid compartment. Therefore, the present inventors concluded that fusion proteins of an apolipoprotein or an apolipoprotein mimetic, preferably ApoA1, with IL-4 can be used to prevent immune related disorders by promoting trained immunity. Furthermore, present inventors found that IL-4's pharmacokinetic profile and bioavailability to innate immune cells can be further improved by integrating said fusion proteins into myeloid cell-avid lipid nanoparticles. Incorporation of such IL-4 fusion protein into a lipid nanoparticle does not hamper its ability to target to the myeloid compartment.

Accordingly, the disclosure also provides a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4).

The disclosure provides a nucleic acid encoding the fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4) as taught herein.

The disclosure provides a pharmaceutical composition comprising the fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4) as taught herein or the nucleic acid encoding said fusion protein as taught herein, and a pharmaceutically acceptable carrier.

The disclosure provides the fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4) as taught herein, the nucleic acid encoding such fusion protein as taught herein or the pharmaceutical composition comprising such fusion protein or nucleic acid for use as a medicament.

The disclosure provides the fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4) as taught herein, the nucleic acid encoding such fusion protein as taught herein or the pharmaceutical composition comprising such fusion protein or nucleic acid for use in the treatment of an

immune related disorder, preferably wherein the immune related disorder is a state of hyperinflammation followed by immune paralysis, preferably wherein the hyperinflammation and/or the immune paralysis is caused by an infectious disease such as COVID-19, by sepsis, myocardial infarction, stroke, cancer, or multiple sclerosis.

The disclosure provides the fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and interleukin-4 (IL-4) as taught herein, the nucleic acid encoding such fusion protein as taught herein or the pharmaceutical composition comprising such fusion protein or nucleic acid for use in targeting IL-4 to a target cell, preferably a myeloid cell.

The disclosure provides a fusion protein comprising a myeloid-targeting molecule and IL-4, wherein the myeloid-targeting molecule is capable of targeting the IL-4 to a myeloid cell.

The disclosure provides a nucleic acid encoding the fusion protein comprising a myeloid-targeting molecule and IL-4 as taught herein.

The disclosure provides a nucleic acid comprising a nucleic acid sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO. 44 or comprising a nucleic acid sequence encoding a polypeptide having a sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO. 43 and further comprising means for targeted expression in a myeloid cell, wherein said mean are selected from:

- a promoter for selective or inducible expression in said myeloid cell operatively linked to said nucleic acid; or
- a viral expression vector comprising said nucleic acid capable of stably expressing said nucleic acid in said myeloid cell; or
- a lipid nanoparticle comprising one or more apolipoproteins, phospholipids, said nucleic acid, and optionally sterol.

The disclosure provides a pharmaceutical composition comprising the fusion protein comprising a myeloid-targeting molecule and IL-4 as taught herein or the nucleic acid encoding said fusion protein as taught herein or the nucleic acid comprising means for targeted expression in a myeloid cell as taught herein, and a pharmaceutically acceptable carrier.

The disclosure provides the fusion protein comprising a myeloid-targeting molecule and IL-4 as taught herein or the nucleic acid encoding said fusion protein as taught herein or the nucleic acid comprising means for targeted expression in a myeloid cell as

taught herein, or the pharmaceutical composition comprising said fusion protein or nucleic acid as taught herein for use as a medicament.

The disclosure provides the fusion protein comprising a myeloid-targeting molecule and IL-4 as taught herein or the nucleic acid encoding said fusion protein as taught herein or the nucleic acid comprising means for targeted expression in a myeloid cell as taught herein, or the pharmaceutical composition comprising said fusion protein or nucleic acid as taught herein for use in the treatment of an immune related disorder.

The disclosure provides in vivo, in vitro or ex vivo use of IL-4 in stimulating or promoting trained immunity in a cell, organ, tissue or an organism.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 depicts a schematic overview of the optional assembly of an exemplary apolipoprotein fusion in a lipid nanoparticle (sphere or disk) and subsequent binding to a target cell.

Fig. 2 depicts a schematic overview of different envisioned apolipoproteins and subsequent assembly in lipid nanoparticles. Depicted are the following fusion proteins (schematically): top left depicts both an apolipoprotein fused to an immunomodulatory biomolecule, and an apolipoprotein fused to a rerouting molecule; bottom left depicts an apolipoprotein fused to an immunomodulatory biomolecule; top right depicts an apolipoprotein fused to a rerouting molecule; bottom right depicts an apolipoprotein fused to an immunomodulatory biomolecule and a rerouting molecule.

Fig. 3 shows SDS-PAGE gels demonstrating the expression and purification of different apolipoprotein fusion constructs (ApoA1-IL1B, ApoA1-IL38, ApoA1-IL2). The rectangles indicate the bands corresponding to the desired proteins. (P: pellet containing cell debris, SN: supernatant containing soluble protein fraction, FT: flow through of SN applied to Ni-NTA column, A: first wash using 10 mM imidazole, A50: second wash using 50 mM imidazole, E1: elution fraction 1, E2: elution fraction 2, E3: elution fraction 3, E4: elution fraction 4, FW: final wash using 500 mM imidazole.)

Fig. 4 shows results of dynamic light scattering measurements for four lipid nanoparticles, three of which each contain a different apolipoprotein (ApoA1-IL1B, ApoA1-IL38, ApoA1-IL4) and one containing apoA1 as a control nanoparticle. Mean number diameter (darker grey) and polydispersity index (PdI) (lighter grey) have been determined for these nanoparticles over the course of 11 days.

Fig. 5 shows an SDS-PAGE gel demonstrating the expression and purification of apoA1 in which serine to cysteine mutations have been introduced, at either position 147 or 279. The rectangle indicates the band corresponding to the mutated apoA1.

CLAIMS

1. An apolipoprotein lipid nanoparticle comprising

a fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and a rerouting molecule; and

phospholipids;

wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity.

2. The apolipoprotein lipid nanoparticle according to claim 1, wherein the fusion protein further comprises an immunomodulatory biomolecule;

wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response.

3. The apolipoprotein lipid nanoparticle according to any one of claims 1 or 2, wherein the apolipoprotein lipid nanoparticle further comprises sterols.

4. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 3, wherein the apolipoprotein lipid nanoparticle further comprises lipids, preferably triglycerides.

5. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 4, wherein said apolipoprotein lipid nanoparticle is a sphere, a ribbon or a disc.

6. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 5, wherein at least a part of said fusion protein is exposed to the environment surrounding said apolipoprotein lipid nanoparticle, preferably wherein said immunomodulatory biomolecule and/or said rerouting molecule is exposed to the environment surrounding said apolipoprotein lipid nanoparticle.

7. The apolipoprotein lipid nanoparticle according to any one of claims 2 to 6, wherein the immunomodulatory biomolecule is selected from the group consisting of

a cytokine, a chemokine, a hormone, a growth factor, a hematopoietic growth factor, and combinations thereof.

8. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 7, wherein the rerouting molecule is selected from an antibody or an antigen binding fragment thereof, a rerouting peptide or a rerouting protein, preferably wherein the rerouting peptide or rerouting protein is a ligand of a receptor present on the target.

9. The apolipoprotein lipid nanoparticle according to claim 8, wherein the antibody or antigen binding fragment thereof is selected from the group consisting of a Fab, a Fab_2, a scFv, a scFv-Fc, a dAb-Fc, a free light chain antibody, a half antibody, a bispecific Fab2, a Fab_3, a trispecific Fab3 a diabody, a bispecific diabody, a triabody, a trispecific triabody, a minibody, an IgG, an IgNAR, a monovalent IgG, a V_hH, and a variable new antigen receptor (VNAR).

10. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 9, wherein the rerouting molecule is capable of binding to a hematopoietic stem and progenitor cell (HSPC), such as a hematopoietic stem cell (HSC), a multipotent progenitor (MPP), or a common myeloid progenitor cell (CMP).

11. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 9, wherein the rerouting molecule is capable of binding to a myeloid cell selected from the group consisting of megakaryocyte, eosinophil, basophil, erythrocyte, monocyte such as dendritic cell or macrophage, and a neutrophil.

12. The apolipoprotein lipid nanoparticle according to claim 11, wherein the rerouting peptide is SIRPα.

13. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 9, wherein the rerouting molecule is capable of binding to a non-myeloid cell, such as a non-myeloid immune cell or an endothelial cell.

14. The apolipoprotein lipid nanoparticle according to claim 13, wherein the rerouting molecule is capable of binding to lymphocytes, preferably T cells.

15. The apolipoprotein lipid nanoparticle according to claim 13, wherein the rerouting peptide is PD1, CD40L or GP120.

16. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 15, wherein the apolipoprotein is an ApoA1, ApoA-1 Milano, ApoA4, ApoC3, ApoD, ApoE, ApoL1, ApoL3 or the apolipoprotein mimetic is a mimetic of an ApoA1, ApoA-1 Milano, ApoA4, ApoC3, ApoD, ApoE, ApoL1, ApoL3.

17. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 16, wherein the apolipoprotein lipid nanoparticle comprises a payload, preferably wherein the payload is selected from a nucleic acid or a nucleic acid analog, a therapeutic, a biologic or combinations thereof.

18. Method of manufacturing an apolipoprotein lipid nanoparticle as defined in any one of claims 1 to 17, the method comprising the steps of:

a1) expressing and isolating one or more apolipoprotein fusion proteins to obtain one or more isolated apolipoprotein fusion proteins,

wherein the one or more apolipoprotein fusion proteins are selected from the group consisting of:

an apolipoprotein or apolipoprotein mimetic fused to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic fused to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and/or

a2) chemically conjugating one or more apolipoproteins or apolipoprotein mimetics and isolating the one or more conjugated apolipoproteins to obtain one or more isolated conjugated apolipoproteins,

wherein the one or more conjugated apolipoproteins are selected from the group consisting of:

an apolipoprotein or apolipoprotein mimetic conjugated to a rerouting molecule;

an apolipoprotein or apolipoprotein mimetic conjugated to an immunomodulatory biomolecule and a rerouting molecule; and combinations thereof; and

b) combining the one or more isolated apolipoprotein fusion proteins obtained in step a1 and/or the one or more isolated conjugated apolipoproteins obtained in step a2 with phospholipids, and optionally sterols and/or lipids, to obtain an apolipoprotein lipid nanoparticle.

19. A pharmaceutical composition comprising the apolipoprotein lipid nanoparticle according to any one of claims 1 to 17, and a pharmaceutically acceptable carrier.

20. The apolipoprotein lipid nanoparticle according to any one of claims 1 to 17 or the pharmaceutical composition according to claim 19 for use as a medicament.

21. Use of the apolipoprotein lipid nanoparticle according to any one of claims 1 to 17 or the pharmaceutical composition according to claim 19 in the manufacture of a medicament for the treatment of an immune related disorder.

22. A fusion protein comprising an apolipoprotein or an apolipoprotein mimetic and a rerouting molecule, wherein the rerouting molecule is a molecule that allows that fusion protein to bind to a different target than to which the apolipoprotein or apolipoprotein mimetic would have bound and/or to bind to its intended target with a higher affinity.

23. The fusion protein according to claim 22, wherein the rerouting molecule is a rerouting molecule as defined in any one of claims 8 to 15.

24. The fusion protein according to claim 22 or 23, wherein the fusion protein further comprises an immunomodulatory biomolecule, wherein the immunomodulatory biomolecule is a protein that enhances or suppresses an immune response, preferably wherein the immunomodulatory biomolecule is an immunomodulatory biomolecule as defined in claim 7.