immune cells. This results in an increase the therapeutic efficacy and a decrease in the risk of side effects.

[0006] The glycocalyx consists in particular of proteoglycans, which are glycoproteins comprising one or more unbranched glycosaminoglycan (GAG) chains. Among the latter, the family of heparan sulfate proteoglycans (HSPG) corresponds to proteins associated with sulfated GAGs: heparan sulfates (HS). HSPGs, which have a central role in numerous biological processes (cell proliferation, cell adhesion, inflammation, coagulation, cell penetration of pathogenic microorganisms, in particular viruses and parasites) are found at the surface of the majority of mammalian cells and in extracellular matrices (Dreyfuss et al., Annuals of the Brazilian Academy of Sciences, 2009, 81, 409-429). This ubiquitous expression therefore means that HSPGs and their HS domains are not considered to represent relevant immunomodulatory targets. Moreover, the capacity of HS ligands to induce immune cell activation has not been identified to date.

Summary

[0007] During the course of their work, the inventors first discovered that a ligand of a sulfated sugar of the GAG family, heparan sulfates (HS), is incapable of activating dendritic cells *in vitro*. They then observed that a molecular complex containing this ligand and a ligand of a surface molecule of antigen-presenting cells (APC) potentiates the immunomodulatory effect of the heparan sulfate (HS) ligand. Indeed, this molecular complex induces the APC even more strongly, as shown by the increased secretion of the cytokines IL-6 and IL-12, and enables the subsequent activation of T lymphocytes. The inventors have demonstrated that the association between the ligands may be produced in the form of a covalent or non-covalent complex, in particular in the form of a fusion protein. The inventors have moreover observed that the immunomodulatory properties of this complex make it possible to better control disease processes and in particular to slow down the progression of a tumor.

[0008] These results are surprising because, although described as being able to serve as receptor or co-receptor, it had never been demonstrated that HSPGs could potentiate activation mediated by a ligand of a receptor expressed specifically at the surface of the APCs. Finally, the molecular complex which targets ubiquitously-expressed HS could spread toward the extracellular matrix or toward cells of no interest for the disease. Thus, theoretically, it should not be capable of modulating the activity of APCs and of a large repertoire of T lymphocytes with sufficient efficacy for an immunotherapeutic effect to occur.

[0009] The inventors have also discovered that the results obtained with the APCs could be transposed to other cells of the innate immune response, NK and NKT cells. Thus, the inventors have demonstrated that a ligand of a surface molecule of NK or NKT cells, incapable of inducing these cells alone, becomes capable of increasing the number of activated cells when it is associated, in a molecular complex, with a ligand of a sulfated sugar of the GAG family.

[0010] Consequently, a subject of the present disclosure is a molecular complex for use as immunomodulatory medicament, preferably immunostimulatory medicament, said complex consisting of at least one ligand of a sulfated sugar of the glycosaminoglycan family (first ligand or L1) and at least one ligand of another surface molecule of APC or of NK or NKT cells (second ligand or L2), said ligands being bonded to one another, and said complex being free of a specific antigen of the disease to be treated. In one aspect, the present disclosure refers to use of a molecular complex in the manufacture of an immunostimulatory medicament for treating cancer or an infectious disease, wherein the molecular complex consists of at least one ligand of a sulfated sugar of the glycosaminoglycan family expressed at the surface of antigen presenting cells or NK or NKT cells (first ligand), and at least one ligand of a surface molecule of antigen-presenting cells or of NK or NKT cells other than a sulfated sugar of the glycosaminoglycan family (second ligand), wherein the ligands are bonded to one another, and the complex is free of a specific antigen of the disease to be treated.

[0011] According to preferred examples, the first ligand is a heparan sulfate-binding peptide selected from the group consisting of: a peptide derived from the HIV Tat protein, comprising at least the basic region Tat 49-57 (SEQ ID NO: 3) such as the peptides Tat 49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8) and Tat22-57C(22-37)S (SEQ ID NO:9); an R7 to R11 polyarginine peptide; and a peptide derived from the R domain of the diphtheria toxin, comprising the R domain of the diphtheria toxin (SEQ ID NO: 5) or at least the fragment DT453-467 (SEQ ID NO 7) of said domain which comprises the heparan sulfate binding region.

[0012] According to preferred examples, the second ligand targets a surface molecule of APCs selected from the group consisting of: C-type lectin receptors, membrane-bound immunoglobulins, receptors for the constant region of immunoglobulins, and immune checkpoint (ICP) molecules.

[0013] According to preferred examples, the second ligand targets a surface molecule of NK or NKT cells selected from the group consisting of: NKG2D, NKp30, NKp44, NKp46, NKp80, Ly49H receptors, KIR, NKG2A receptors, and ICP PD-1, CTLA-4, TIM-3, TIGIT, LAG-3.

[0014] The second ligand is preferably selected from the group consisting of: (i) antibodies directed against said surface molecules of the APCs or of NK or NKT cells and fragments thereof containing at least the paratope; (ii) immunoglobulins, preferably IgG, and fragments thereof comprising at least the Fc region; and (iii) proteins and protein fragments which bind to the Fc and/or Fab region of the antibodies, particularly

protein A of *S. aureus*, the BB fragment thereof (SEQ ID NO: 1) and the ZZ derivative thereof (SEQ ID NO: 2).

[0015] According to preferred examples, said complex is in the form of oligomers or a mixture of monomers and oligomers.

[0016] According to preferred examples, said complex consists of a fusion protein between the first and the second ligand. A type of complex which is more particularly preferred according to the disclosure consists of a fusion protein comprising a first ligand selected from: Tat49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8), Tat22-57C(22-37)S (SEQ ID NO 9), the R domain of the diphtheria toxin (SEQ ID NO: 5) or the fragment DT453-467 (SEQ ID NO 7) and a second ligand selected from: the BB fragment of the protein A (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2).

[0017] According to other preferred examples, the second ligand is an antibody or an antibody fragment and the first ligand forms a fusion protein with an immunoglobulin-binding element, preferably the protein A of *S. aureus*, the BB fragment thereof (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2). Preferably, said fusion protein comprises a first ligand selected from Tat49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8), Tat22-57C(22-37)S (SEQ ID NO 9), the R domain of the diphtheria toxin (SEQ ID NO: 5) or the fragment DT453-467 (SEQ ID NO 7) and an immunoglobulin-binding element selected from the BB fragment of the protein A (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2). In a type of complex which is more particularly preferred according to the disclosure, the immunoglobulin-binding element is the BB fragment of the protein A (SEQ ID NO: 1) and said fusion protein is complexed to the second ligand which consists of a whole immunoglobulin. In another type of complex which is more particularly preferred according to the disclosure, said fusion protein is complexed to the second ligand which is selected from an anti-RFcgamma I, II and/or III, anti-DEC-205, anti-DC-SIGN, anti-CD74, anti-CD275, anti-CD335, anti-CD336, anti-CD56, anti-CTLA-4, anti-PD-L1, anti-OX40 antibody, or a fragment of the preceding antibodies comprising at least the paratope.

[0018] According to preferred examples, the complex is for use as immunostimulatory medicament, preferably for activating antigen-presenting cells, particularly dendritic cells or monocytes, for activating NK or NKT cells, and/or for activating the secretion of the cytokines IL-6 and/or IL-12.

[0019] Another subject of the present disclosure is a composition for use as immunomodulatory medicament, preferably immunostimulatory medicament, comprising at least one molecular complex according to the disclosure, and at least one pharmaceutically acceptable carrier, a carrier substance and/or an adjuvant. The adjuvant is preferably a CpG oligodeoxynucleotide, polyinosinic:polycytidylic acid or a mixture of CpG oligodeoxynucleotide(s) and of polyinosinic:polycytidylic acid, and/or the carrier substance is a nanoparticle.

[0020] According to preferred examples, said composition comprises at least one other therapeutic agent, preferably at least one immune checkpoint inhibitor, preferably an anti-PD-1, an anti-PD-L1 or an anti-CTLA-4.

[0021] According to preferred examples, the composition is for use in the immunotherapy of cancer or infectious diseases.

Disclosure of the invention

[0022] A subject of the present invention is a molecular complex for use as immunomodulatory medicament, preferably immunostimulatory medicament, said complex consisting of at least one ligand of a sulfated sugar of the glycosaminoglycan family (first ligand) and at least one ligand of another surface molecule of APC or of NK or NKT cells (second ligand), said ligands being bonded to one another, and said complex being free of a specific antigen of the disease to be treated.

[0023] Such a complex is capable of inducing cells of the immune system favorably for triggering the immune response. Thus, when splenocytes are incubated with these complexes, the activation of dendritic cells is observed, which dendritic cells become capable of secreting IL-6 and IL-12 cytokines. Moreover, the induction of the DC can be obtained for second ligands which are incapable of triggering an effect of this kind when they are not associated, such as the isolated form of the APC ligand referred to as ZZ. The association of the HS ligand can also make it possible to potentiate the effect of a ligand which is capable, in the isolated state, of activating APCs such as a non-specific polyclonal human Ab. The association of the HS ligand with a ligand of NK or NKT cells can also make it possible to activate these cell types, whereas it is devoid of this capacity in isolated form. Finally, an immunomodulatory complex, preferably an immunostimulatory complex, according to the invention can induce a therapeutic effect, in particular an anti-tumor effect, which is superior to that obtained with an anti-ICP (anti-PD1) Ab, particularly when the composition comprises an adjuvant.

Definitions

Claims

1. Use of a molecular complex in the manufacture of an immunostimulatory medicament for treating cancer or an infectious disease, wherein the molecular complex consists of at least one ligand of a sulfated sugar of the glycosaminoglycan family expressed at the surface of antigen presenting cells or NK or NKT cells (first ligand), and at least one ligand of a surface molecule of antigen-presenting cells or of NK or NKT cells other than a sulfated sugar of the glycosaminoglycan family (second ligand), wherein the ligands are bonded to one another, and the complex is free of a specific antigen of the disease to be treated.

2. The use of claim 1, wherein the first ligand is a heparan sulfate-binding peptide selected from the group consisting of: a peptide derived from the HIV Tat protein, comprising at least the basic region Tat49-57 (SEQ ID NO: 3) such as the peptides Tat49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8) and Tat22-57$C_{(22\text{-}37)S}$ (SEQ ID NO:9); an R7 to R11 polyarginine peptide; and a peptide comprising the R domain of the diphtheria toxin (SEQ ID NO: 5) or at least the fragment DT453-467 (SEQ ID NO 7) of said domain, comprising the heparan sulfate binding region.

3. The use of claim 1 or claim 2, wherein the second ligand targets a surface molecule of antigen-presenting cells selected from the group consisting of: C-type lectin receptors, membrane-bound immunoglobulins, receptors for the constant region of immunoglobulins, and immune checkpoint molecules and ligands thereof.

4. The use of any one of claims 1 to 3, wherein the second ligand is selected from the group consisting of: (i) antibodies directed against said surface molecules of antigen-presenting cells or of NK or NKT cells and fragments thereof containing at least the paratope; (ii) immunoglobulins, preferably IgG, and fragments thereof comprising at least the Fc region; and (iii) proteins and protein fragments which bind to the Fc and/or Fab region of the antibodies, particularly protein A of *S. aureus*, the BB fragment thereof (SEQ ID NO: 1) and the ZZ derivative thereof (SEQ ID NO: 2).**5.** The use of any one of claims 1 to 4, wherein the molecular complex is in the form of oligomers or a mixture of monomers and oligomers.

6. The use of any one of claims 1 to 5, wherein the molecular complex consists of a fusion protein between the first and the second ligand.

7. The use of claim 6, wherein the molecular complex consists of a fusion protein comprising a first ligand selected from: Tat49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8), Tat22-57C(22-37)S (SEQ ID NO 9), the R domain of the diphtheria toxin (SEQ ID NO: 5) or the fragment DT453-467 (SEQ ID NO 7) and a second ligand selected from: the BB fragment of the protein A (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2).

8. The use of any one of claims 1 to 5, wherein the second ligand is an antibody or an antibody fragment and the first ligand forms a fusion protein with an immunoglobulin-binding element, preferably the protein A of *S. aureus*, the BB fragment thereof (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2).

9. The use of claim 8, wherein said fusion protein comprises a first ligand selected from Tat49-57 (SEQ ID NO: 3), Tat37-57 (SEQ ID NO: 8), Tat22-57C(22-37)S (SEQ ID NO 9), the R domain of the diphtheria toxin (SEQ ID NO: 5) or the fragment DT453-467 (SEQ ID NO 7) and an immunoglobulin-binding element selected from the BB fragment of the protein A (SEQ ID NO: 1) or the ZZ derivative thereof (SEQ ID NO: 2).

10. The use of claim 9, wherein the immunoglobulin-binding element is the BB fragment of the protein A (SEQ ID NO: 1) and said fusion protein is complexed to the second ligand which consists of a whole immunoglobulin.

11. The use of claim 8, wherein said fusion protein is complexed to the second ligand which is selected from an anti-RFcgamma I, II and/or III, anti-DEC-205, anti-DC-SIGN, anti-CD74, anti-CD275, anti-CD56, anti-CD335, anti-CD336, anti-CTLA-4, anti-PD-L1, anti-OX40 antibody, or a fragment of the preceding antibodies comprising at least the paratope.

12. The use of any one of claims 1 to 11, wherein said medicament activates antigen-presenting cells, particularly dendritic cells or monocytes, activates NK or NKT cells, and/or activates the secretion of the cytokines IL-6 and/or IL-12.

13. The use of any one of claims 1 to 12, wherein the immunostimulatory medicament comprises at least one pharmaceutically acceptable carrier, a carrier substance and/or an adjuvant.

14. The use of claim 13, wherein the adjuvant is a CpG oligodeoxynucleotide, polyinosinic-polycytidylic acid or a mixture of CpG oligodeoxynucleotide(s) and polyinosinic:polycytidylic acid, and/or the carrier substance is a nanoparticle.

15. The use of claim 13 or 14, wherein the immunostimulatory medicament further comprises at least one other therapeutic agent, preferably at least one immune checkpoint inhibitor, preferably an anti-PD-1, an anti-PDL-1 or an anti-CTLA4.