and glycan structure for immunosuppression. (Anthony, *et al.*, 2008, Science 320: 373-376 and WO 2007/117505).

However, only a minor population of IgG in IVIG have glycans terminating in α2,6 sialic acids (sFc) and the anti-inflammatory activity. As a result, for the suppression of autoantibody triggered inflammation in a variety of clinical settings, one has to administer IVIG at high doses (1-2g/kg), to enrich sialylated IgGs, or otherwise to increase the sialylation of IgGs (US Application Nos. 20080206246, and 20090004179, and Nimmerjahn *et al. Annu Rev Immunol* 26, 513-533 (2008)).

The present invention addresses and meets the above-mentioned needs by identifying sialylation–free anti-inflammatory polypeptides.

SUMMARY

This disclosure relates to agents, such as polypeptides and antibodies, and methods for treating inflammatory disorders, e.g., autoimmune diseases. In one aspect, the present invention refers to an isolated polypeptide comprising a modified sequence that is free of sialylation and at least 90% identical to the sequence of an hIgG1 Fc region, which is SEQ ID NO: 1 and has a substitution at a position corresponding to F241 of SEQ ID NO: 1, according to the Kabat numbering system, wherein the polypeptide has an anti-inflammatory activity, wherein the substitution is an A or a conservative substitution thereof. In another example, the present invention refers to a method for making a polypeptide as disclosed herein having an anti-inflammatory activity, the method comprising providing a first nucleotide sequence encoding a parent polypeptide which is at least 90% identical to an hIgG1 Fc region with the sequence of SEG ID NO: 1; and modifying the first nucleotide sequence to obtain a second nucleotide sequence encoding a modified polypeptide so that the modified polypeptide is free of sialylation and has an A or a conservative substitution thereof at a position corresponding to F241 of SEQ ID NO: 1 according to the Kabat numbering system. In yet another aspect, the present invention refers to a method of producing a polypeptide as disclosed herein, comprising culturing the host cell comprising a nucleotide sequence encoding the polypeptide in a medium under conditions suitable for expression of the, and purifying the same polypeptide from the cultured cell or the medium of the cell. In one example, disclosed herein is an isolated polypeptide comprising a modified sequence that is at least 75% identical to an IgG Fc region and has a conservative substitution at a position corresponding to F241 of SEQ ID No: 1, wherein the modified sequence is free of sialylation and the polypeptide has an anti-inflammatory activity that is higher than that of a parent polypeptide. In one example, there is disclosed an isolated polypeptide comprising a modified sequence that is at least 75% identical to an IgG Fc region and has a conservative substitution at a position corresponding to F241 of SEQ ID No: 1, wherein the modified sequence is free of sialylation and the polypeptide has an anti-inflammatory activity that is higher than that of a parent polypeptide. In one example, the present invention provides an isolated polypeptide comprising a modified sequence that is at least 75% identical to an IgG Fc region, wherein the modified sequence is free of sialylation and the polypeptide has an anti-inflammatory activity that is higher than that of a parent polypeptide. Accordingly, one example of this disclosure features an isolated polypeptide comprising a modified sequence that is at least 75% (e.g., any number between 75% and 100%, inclusive, e.g., 70 %, 80%, 85%, 90%, 95%, 99%, and 100%) identical to an IgG Fc region. The modified sequence is free of sialylation and the polypeptide has an anti-inflammatory activity that is higher than that of a parent polypeptide. The parent polypeptide can comprise the IgG Fc region, such as the sequence of SEQ ID NO: 1 listed below. In some examples, the polypeptide has ability to bind to DC-SIGN, and to bind to hFcγRIIA or RIIB. In one example, the isolated polypeptide has an ability to bind to hFcγRIIA or RIIB at a K_D of $2x10^{-5}$ M or lower (i.e., K_A of 5.0×10^4 M^{-1} or higher). Preferably, the modified sequence has a FA241 mutation. The modified sequence can be at least 75% (e.g., any number between 75% and 100%, inclusive, e.g., 70 %, 80%, 85%, 90%, 95%, 99%, and 100%) identical to SEQ ID NO: 2. In some examples, the modified sequence comprises or consists essentially of SEQ ID NO: 2. In another example, disclosed herein is a method for making a polypeptide having an anti-inflammatory activity, the method comprising: providing a parent polypeptide having the sequence of an IgG Fc region or a first nucleic acid sequence encoding the parent polypeptide; and modifying the parent polypeptide to obtain a modified polypeptide so that the modified polypeptide is free of sialylation and mimics the structure of a sialylated

form of the IgG Fc region, wherein the modifying step comprises introducing a conservative substitution at a position corresponding to F241 of SEQ ID No: 1. In another example, there is disclosed a method for making a polypeptide having an anti-inflammatory activity, the method comprising: providing a parent polypeptide having the sequence of an IgG Fc region or a first nucleic acid sequence encoding the parent polypeptide; and modifying the parent polypeptide to obtain a modified polypeptide so that the modified polypeptide is free of sialylation and mimics the structure of a sialylated form of the IgG Fc region, wherein the modifying step comprises introducing a conservative substitution at a position corresponding to F241 of SEQ ID No: 1. In another example, the present invention provides a method for making a polypeptide having an anti-inflammatory activity, the method comprising: providing a parent polypeptide having the sequence of an IgG Fc region or a first nucleic acid sequence encoding the parent polypeptide; and modifying the parent polypeptide to obtain a modified polypeptide so that the modified polypeptide is free of sialylation and mimics the structural of a sialylated form of the IgG Fc region. In another example, the disclosure provides a method for making a polypeptide having an anti-inflammatory activity. The method includes, among others, steps of providing a parent polypeptide having the sequence of an IgG Fc region or a first nucleic acid sequence encoding the parent polypeptide; and modifying the parent polypeptide to obtain a modified polypeptide so that the modified polypeptide is free of sialylation and

mimics the structural of a sialylated form of the IgG Fc region. The modifying step can be conducted by modifying the first nucleic acid sequence to obtain a second nucleic acid encoding the modified polypeptide. The disclosure also provides a polypeptide made by the just-described method.

In another aspect, the present invention provides an isolated nucleic acid comprising a sequence encoding the polypeptide described above.

In another aspect, the present invention provides an expression vector comprising a nucleic acid.

In another aspect, the present invention provides a host cell comprising a nucleic acid.

In another example, the invention features an isolated nucleic acid comprising a sequence encoding the polypeptide described above; an expression vector comprising the nucleic acid; and a host cell comprising the nucleic acid. In another example, there is disclosed a method of producing a polypeptide, comprising culturing the host cell in a medium under conditions permitting expression of a polypeptide encoded by the nucleic acid, and purifying the polypeptide from the cultured cell or the medium of the cell.

The disclosure also features a method of producing a polypeptide. The method includes culturing the host cell in a medium under conditions permitting expression of a polypeptide encoded by the nucleic acid, and purifying the polypeptide from the cultured cell or the medium of the cell. In another aspect, the present invention provides a pharmaceutical formulation comprising (i) the polypeptide or a nucleic acid and (ii) a pharmaceutically acceptable carrier. In another example, the disclosure features a pharmaceutical formulation comprising (i) the polypeptide or nucleic acid described above, and (ii) a pharmaceutically acceptable carrier. In another aspect, the present invention provides use of the polypeptide of the present invention, or a nucleic acid encoding the polypeptide in the manufacture of a medicament for treating an inflammatory disease. In another example, the disclosure provides a method of treating an inflammatory disease. The method includes administering to a subject in need thereof a therapeutically effective amount of the above-described polypeptide or nucleic acid encoding the polypeptide. Also provided is use of the polypeptide or nucleic acid in the manufacture of a medicament for treating an inflammatory disease. In another aspect, the present invention provides use of a polypeptide or a nucleic acid in the manufacture of a medicament for treating an inflammatory disease.

The disclosure also features an isolated polypeptide, nucleic acid, expression vector, host cell, composition, or method for treating an inflammatory disease substantially as shown and described herein.

The details of one or more examples of the disclosure are set forth in the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims.

DESCRIPTION OF DRAWINGS

FIGs. 1a-c are diagrams and photographs showing that α2,6-linked sialic acid conferred DC-SIGN binding activity to recombinant human IgG1 Fc.

FIGs. 2a-b are diagrams and photographs showing that disrupting Fc-glycan interactions conferred DC-SIGN binding activity to recombinant human IgG1 Fc.

FIG. 3 is as set of diagrams showing that the FA241 mutation in hIgG1 Fc recapitulates anti-inflammatory activity of α2,6 sFc.

CLAIMS

1. An isolated polypeptide comprising a modified sequence that is free of sialylation and at least 90% identical to the sequence of an hIgG1 Fc region, which is SEQ ID NO: 1 and has a substitution at a position corresponding to F241 of SEQ ID NO: 1, according to the Kabat numbering system, wherein the polypeptide has an anti-inflammatory activity, wherein the substitution is an A or a conservative substitution thereof.

2. The isolated polypeptide of claim 1, characterized in that the modified sequence is at least 95% identical to the sequence of SEQ ID NO: 1.

3. The isolated polypeptide of claim 1 or 2, characterized in that the modified sequence is at least 99% identical to the sequence of SEQ ID NO: 1.

4. The isolated polypeptide of any one of claims 1-3, having an ability to bind to DC-SIGN.

5. The isolated polypeptide of any one of claims 1-4, having an ability to bind to hFcγRIIA or RIIB

6. The isolated polypeptide of any one of claims 1-5, having an ability to bind to hFcγRIIA or RIIB at a K_D of $2x10^{-5}$ M or lower (i.e., K_A of 5.0×10^4 M^{-1} or higher).

7. The isolated polypeptide of any one of claims 1-6, characterized in that the substitution is a FA241 mutation.

8. The isolated polypeptide of any one of claims 1-7, characterized in that the modified sequence is at least 75% identical to SEQ ID NO: 2.

9. The isolated polypeptide of claim 8, characterized in that the modified sequence is at least 80% identical to SEQ ID NO: 2.

10. The isolated polypeptide of claim 9, characterized in that the modified sequence is at least 90% identical to SEQ ID NO: 2.

11. The isolated polypeptide of claim 10, characterized in that the modified sequence is at least 95% identical to SEQ ID NO: 2.

12. The isolated polypeptide of claim 11, characterized in that the modified sequence is at least 99% identical to SEQ ID NO: 2.

13. The isolated polypeptide of claim 12, characterized in that the modified sequence comprises SEQ ID NO: 2.

14. The isolated polypeptide of claim 9, characterized in that the modified sequence consists essentially of SEQ ID NO: 2.

15. A method for making a polypeptide of any one of claims 1-14 having an anti-inflammatory activity, the method comprising:

providing a first nucleotide sequence encoding a parent polypeptide which is at least 90% identical to an hIgG1 Fc region with the sequence of SEG ID NO: 1; and

modifying the first nucleotide sequence to obtain a second nucleotide sequence encoding a modified polypeptide so that the modified polypeptide is free of sialylation and has an A or a conservative substitution thereof at a position corresponding to F241 of SEQ ID NO: 1 according to the Kabat numbering system.

16. The method of claim 15, characterized in that the modified sequence is at least 95% identical to the sequence of SEQ ID NO: 1.

17. A polypeptide made by the method of claim 15 or 16.

18. An isolated nucleic acid comprising a sequence encoding the polypeptide of any of claims 1-14 and 17.

19. An expression vector comprising a nucleic acid of claim 18.

20. A host cell comprising a nucleic acid of claim 18.

21. A method of producing a polypeptide of any one of claims 1-14, comprising culturing the host cell comprising a nucleotide sequence encoding the polypeptide in a medium under conditions suitable for expression of the, and purifying the same polypeptide from the cultured cell or the medium of the cell.

22. A pharmaceutical formulation comprising (i) the polypeptide of any of claims 1-14 and 17 or a nucleic acid of claim 18, and (ii) a pharmaceutically acceptable carrier.

23. Use of a polypeptide of any of claims 1-14 and 17 or a nucleic acid of claim 18 in the manufacture of a medicament for treating an inflammatory disease.