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 invention relates to agents, such as polypeptides and antibodies, and methods for treating inflammatory disorders, e.g., autoimmune diseases.

In one aspect, there is provided use of (i) a first isolated polypeptide comprising an IgG Fc region that is sialylated; or (ii) a second isolated polypeptide comprising a modified sequence that is at least 75% identical to the IgG Fc region or a nucleic acid encoding the second polypeptide, wherein the modified sequence has a FA241 mutation, in the manufacture of a medicament for treating a T cell-mediated autoimmune disease or increasing a level of regulatory T (T_{reg}) cells.

In one aspect, the present 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., 75 %, 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., 75%, 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, 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 example, the disclosure 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. 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 example, the disclosure features a pharmaceutical formulation comprising (i) the polypeptide or nucleic acid described above, and (ii) a pharmaceutically acceptable carrier.

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. 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.

In another example, the disclosure further provides a method of increasing a level of regulatory T (T_{reg}) cells in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first isolated polypeptide comprising an IgG Fc region that is sialylated; or (ii) a second isolated polypeptide comprising a modified sequence that is at least 75% (e.g., any number between 75% and 100%, inclusive, e.g., 75 %, 80%, 85%, 90%, 95%, 99%, and 100%) identical to the IgG Fc region or a nucleic acid encoding the second polypeptide, wherein the modified sequence has a FA241 mutation. In one example, the subject has an inflammatory disease, such as an autoimmune disease, including a T cell-mediated autoimmune disease. Examples of the T cell-mediated autoimmune disease include multiple sclerosis and type I diabetes. Accordingly, the disclosure also provides a method treating a T cell-mediated autoimmune disease in a subject in need thereof. The method comprises administering to the subject an effective amount of the above-mentioned first isolated polypeptide or second isolated polypeptide or nucleic acid.

In preferred examples, the IgG region in the first isolated polypeptide can be sialylated at a level higher than that of IgG in the subject. The modified sequence can be sialylated at different levels or non-sialylated. In some examples, it is (a) substantially free of sialylation or (b) sialylated at a level lower than that of IgG of the subject. The IgG Fc region can comprise the sequence of SEQ ID NO: 1. The modified sequence can be at least 75% (e.g., any number between 75% and 100%, inclusive, e.g., 75 %, 80%, 85%, 90%, 95%, 99%, and 100%) identical to SEQ ID NO: 2. The first or second isolated polypeptide has an ability to bind to DC-SIGN, hFcγRIIA, or hFcγRIIB. Preferably, the isolated polypeptide has an ability to bind to hFcγRIIA or hFcγRIIB at a K_D of $2x10^{-5}$ M or lower (i.e., K_A of 5.0×10^4 M^{-1} or higher).

The disclosure further provides uses of the above-described first isolated polypeptide or second isolated polypeptide in the manufacture of a medicament for treating a T cell-mediated autoimmune disease or for increasing a level of regulatory T (T_{reg}) cells in a subject in need thereof.

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

DESCRIPTION OF DRAWINGS

FIG. 1A, 1B, 1C and 1D show that F241A is capable of suppressing serum-induced arthritis. (Fig. 1A) Schematic depiction of the interaction between mannose residues of the non-sialylated core glycan (Asialo-Fc) with the phenylalanine residue at position 241 of the Cγ2 domain. Upon sialylation (Sial-Fc) this interaction no longer occurs, resulting in a higher conformational flexibility of the Fc portion to switch between the so-called open and closed conformation. (Fig. 1b) hDC-SIGN$^+$ bone marrow-derived macrophages were pulsed for 1 hour with sialylated (sFc) or non-sialylated (Fc) wild-type Fc or F241A-Fc. Whole cell extracts were used to analyze IL-33 production by western blotting. Actin served as loading control. (Fig. 1c) BMMΦ from either SIGN-R1$^{-/-}$ or hDC-SIGN$^+$ mice were pulsed with non-sialylated wild-type Fc (asialo-Fc), sialylated wild-type Fc (sFc) or F241A. After extensive washes the cells were adoptively transferred into K/BxN challenged C57BL/6 mice. (Fig. 1d) hDC-SIGN$^+$ BMMΦ were pulsed with either asialylated (asialo Fc) or sialylated (α2,6sFc) variants of wild-type Fc or F241A.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of (i) a first isolated polypeptide comprising an IgG Fc region that is sialylated; or (ii) a second isolated polypeptide comprising a modified sequence that is at least 75% identical to the IgG Fc region or a nucleic acid encoding the second polypeptide, wherein the modified sequence has a FA241 mutation, in the manufacture of a medicament for treating a T cell-mediated autoimmune disease or increasing a level of regulatory T (T_{reg}) cells.

2. The use of claim 1, wherein the IgG region in the first isolated polypeptide is sialylated at a level higher than that of IgG in the subject.

3. The use of any one of claims 1-2, wherein the subject has an inflammatory disease.

4. The use of claim 3, wherein the inflammatory disease is an autoimmune disease.

5. The use of claim 4, wherein the autoimmune disease is a T cell-mediated autoimmune disease.

6. The use of claim 5, wherein the T cell-mediated autoimmune disease is selected from the group consisting of multiple sclerosis and type I diabetes.

7. The use of any one of claims 1-6, wherein the IgG Fc region comprises the sequence of SEQ ID NO: 1.

8. The use of any one of claims 1-7, wherein the first or second isolated polypeptide has an ability to bind to DC-SIGN, hFcγRIIA, or hFcγRIIB.

9. The use of claim 8, wherein the isolated polypeptide has an ability to bind to hFcγRIIA or hFcγRIIB at a K_D of $2x10^{-5}$ M or lower (i.e., K_A of 5.0×10^4 M^{-1} or higher).

10. The use of any one of claims 1-9, wherein the modified sequence is (a) substantially free of sialylation or (b) sialylated at a level lower than that of IgG of the subject.

11. The use of any one of claims 1-10, wherein the modified sequence is at least 75% identical to SEQ ID NO: 2.