IL-2, yet maintain Treg preferential expansion and activation. In one aspect, the present invention refers to a polypeptide comprising a Fc region of a human IgG1 antibody wherein said Fc region comprises a N77G mutation wherein the numbering is as provided in SEQ ID NO: 3 or 4, and said Fc region of a human IgG1 comprises at least 90% identity to the amino acid sequence set forth in SEQ ID NO:3, and wherein said Fc region comprises an A67C and L86C, or an V39C and L86C, or an R72C and V82C, or an V103C and I112C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4. In another aspect, the present invention refers to an antibody comprising a Fc region of the present invention. In another aspect, the present invention refers to a Fc-fusion protein comprising the Fc region of the present invention. In another aspect, the present invention refers to a nucleic acid encoding a polypeptide of the present invention. In another aspect, the present invention refers to an expression vector comprising the nucleic acid of the present invention. In another aspect, the present invention refers to a host cell comprising the nucleic acid of the present invention. In another aspect, the present invention refers to a host cell comprising the expression vector of the present invention. In another aspect, the present invention refers to a method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising: a) expressing a nucleic acid encoding a polypeptide of the present invention in a mammalian cell culture; and b) harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.

Moreover, also described herein are in certain examples, that the IL-2 mutein is fused to a molecule, e.g. an antibody Fc, that increases the serum half-life when administered to a subject. IL-2 muteins have a short serum half-life (3 to 5 hrs for sub-cutaneous injection). Exemplary IL-2 mutein Fc fusions described herein have a half-life in humans of at least 1 day, at least 3 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days, or at least 25 days. This effect on the pharmacokinetics of the IL-2 muteins allows for decreased or less frequent dosing of the IL-2 mutein therapeutic.

Moreover, when creating a pharmaceutical large molecule, consideration must be made for the ability to produce the large molecule in large quantities, while minimizing aggregation and maximizing the stability of the molecule. The IL-2 mutein Fc-fusion molecules demonstrate such attributes.

Additionally, in certain embodiments, the IL-2 mutein Fc-fusion protein contains an IgG1 Fc region. When it is desirable to abolish the effector functions of IgG1 (e.g., ADCC activity), it was found that mutation of the asparagine at position 297 to glycine (N297G; EU numbering scheme) provided greatly improved purification efficiency and biophysical properties over other mutations that lead to an aglycosylation IgG1 Fc. In preferred examples, cysteines are engineered into the Fc to allow disulfide bonds, which increased stability of the aglycosylated Fc-containing molecule. The usefulness of the aglycosylated Fc goes beyond the IL-2 mutein Fc-fusion context. Thus, provided herein are Fc-containing molecules, Fc-fusions and antibodies, comprising a N297G substitution and optionally substitution of one or more additional residues to cysteine.

Another example includes glycosylated peptide linkers. Preferred linker peptides that are amenable to N-glycosylation include GGNGT (SEQ ID NO:6) or YGNGT (SEQ ID NO:7).

Brief Description of the Figures

Claims

What is claimed is:

1. A polypeptide comprising a Fc region of a human IgG1 antibody wherein said Fc region comprises a N77G mutation wherein the numbering is as provided in SEQ ID NO: 3 or 4, and said Fc region of a human IgG1 comprises at least 90% identity to the amino acid sequence set forth in SEQ ID NO:3, and wherein said Fc region comprises an A67C and L86C, or an V39C and L86C, or an R72C and V82C, or an V103C and I112C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

2. The polypeptide of claim 1, wherein said Fc region of a human IgG1 comprises at least 95% identity to the amino acid sequence set forth in SEQ ID NO:3.

3. An antibody comprising a Fc region of claim 1 or 2.

4. A Fc-fusion protein comprising the Fc region of claim 1 or 2.

5. A nucleic acid encoding a polypeptide of claim 1 or 2.

6. An expression vector comprising the nucleic acid of claim 5.

7. A host cell comprising the nucleic acid of claim 5.

8. A host cell comprising the expression vector of claim 6.

9. The host cell of claim 7 or claim 8, wherein the host cell is a mammalian host cell.

10. A method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising:
    a) expressing a nucleic acid encoding a polypeptide of claim 1 or 2 in a mammalian cell culture; and
    b) harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.