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 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 V49C and L86C, or an R72C and V82C, or an V103C and I111C 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 ~~embodiments~~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 ~~embodiments~~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 ~~aspect of the invention~~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

FIG. 1 In a short term stimulation assay, homodimerization by fusion to the C-terminus of IgG-Fc does not alter the activity of IL-2 muteins with reduced potency and with high affinity for CD25.

FIG. 2A and FIG. 2B IL-2 muteins with the indicated mutations and fused to the C-terminus of one side of an Fc-heterodimer were tested for their ability to stimulate STAT5 phosphorylation in T cells. These muteins also contained three mutations conferring high affinity for CD25 (V69A, N71R, Q74P). Their activity was compared to three forms of IL-2 without Fc fusion (open symbols): WT IL-2, HaWT (high affinity for CD25) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P), and HaD (high affinity for CD25 and reduced signaling activity) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D). Phospho-STAT5 responses are shown for gated FOXP3+CD4+ and FOXP3-CD4+ T cells.

FIG. 3 Proliferation of T cell subsets in response to titrations of IL-2 muteins fused to Fc-heterodimer. Activity of fusion proteins was compared to three forms of IL-2 without Fc fusion (open symbols): WT IL-2, HaWT (high affinity for CD25) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P), and HaD (high affinity for CD25 and reduced signaling activity) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D)

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 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 V49C and L86C, or an R72C and V82C, or an V103C and I111C 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.

Claims

What is claimed is:

1. A polypeptide comprising an Fc region of a human IgG1 antibody wherein said Fc region comprises an N297G mutation 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.

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. The polypeptide of claim 1, wherein said Fc region of a human IgG1 comprises the amino acid sequence set forth in SEQ ID NO:4.

4. The polypeptide of claim 1 or claim 3, wherein said Fc region of a human IgG1 further comprises one or more mutations to stabilize the polypeptide.

5. The polypeptide of claim 4, wherein one or more amino acids set forth in SEQ ID NO:3 or SEQ ID NO:4 are substituted with cysteine.

6. The polypeptide of claim 5, wherein V259, A287, R292, V302, L306, V323, or I332 of the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 is substituted with cysteine.

7. The polypeptide of claim 6, wherein said Fc region comprises an A287C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

8. The polypeptide of claim 6, wherein said Fc region comprises an V259C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

9. The polypeptide of claim 6, wherein said Fc region comprises an R292C and V302C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

10. The polypeptide of claim 6, wherein said Fc region comprises an V323C and I332C substitution within the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4.

11. An antibody comprising an Fc region of any of claims 1-10.

12. An Fc-fusion protein comprising the Fc region of any of claims 1-10.

13. A nucleic acid encoding a polypeptide of any of claims 1-10.

14. An expression vector comprising the nucleic acid of claim 13.

15. A host cell comprising the nucleic acid of claim 13.

16. A host cell comprising the expression vector of claim 14.

17. The host cell of claim 15 or claim 16, wherein the host cell is a mammalian host cell.

18. A polypeptide comprising a linker, wherein the linker is GGNGT (SEQ ID NO:6) or YGNGT (SEQ ID NO:7).

19. The polypeptide of claim 18, wherein the linker comprises N-glycosylation.

20. The polypeptide of claim 18, wherein the linker is inserted into or replaces a loop in the polypeptide structure.

21. A method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising:

a) expressing a nucleic acid encoding a polypeptide of any of claims 1-10 in a mammalian cell culture; and

b) harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.

22. A method of making an IgG1 Fc-containing molecule aglycosylated when expressed in mammalian cells, said method comprising the step of mutating a codon for N297 in the Fc region to a glycine codon.