

INTERLEUKIN-2 MUTEINS FOR THE EXPANSION OF T-REGULATORY CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/146,136 filed April 10, 2015, which is incorporated in its entirety by reference herein.

BACKGROUND

IL-2 binds three transmembrane receptor subunits: IL-2R β and IL-2R γ which together activate intracellular signaling events upon IL-2 binding, and CD25 (IL-2R α) which serves to stabilize the interaction between IL-2 and IL-2R $\beta\gamma$. The signals delivered by IL-2R $\beta\gamma$ include those of the PI3-kinase, Ras-MAP-kinase, and STAT5 pathways.

T cells require expression of CD25 to respond to the low concentrations of IL-2 that typically exist in tissues. T cells that express CD25 include both FOXP3⁺ regulatory T cells (Treg cells), which are essential for suppressing autoimmune inflammation, and FOXP3⁻ T cells that have been activated to express CD25. FOXP3⁻ CD25⁺ T effector cells (Teff) may be either CD4⁺ or CD8⁺ cells, both of which may contribute to inflammation, autoimmunity, organ graft rejection, or graft-versus-host disease. IL-2-stimulated STAT5 signaling is crucial for normal T-reg cell growth and survival and for high FOXP3 expression.

In co-owned WO 2010/085495, we describe the use of IL-2 muteins to preferentially expand or stimulate Treg cells. When administered to a subject, the effect on Treg cells is useful for treating inflammatory and autoimmune diseases. Although the IL-2 muteins described therein are useful for expanding Treg over Teff cells *in vivo*, it was desirable to create IL-2 muteins that had optimal attributes for a human therapeutic.

SUMMARY

Described herein are IL-2 muteins, anti-IL-2 antibodies, and anti-IL-2 antibody/IL-2 complexes that are amenable to high-yield manufacturability and have pharmacological activity. In the effort to produce such molecules for use as human therapeutics, a number of unexpected and unpredictable observations occurred. The compositions and methods described herein resulted from that effort.

In one aspect, the present disclosure refers to a human interleukin-2 (IL-2) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1,

wherein said IL-2 mutein has at least one mutation selected from H16K, H16R, H16T and preferentially stimulates T regulatory cells relative to other T cells or NK cells, both in in vitro assays and in humanized mice (NSG mice reconstituted with CD34+ hematopoietic stem cells). In another aspect, the present disclosure refers to a Fc-fusion protein comprising an Fc and the human IL-2 mutein of the present invention. In yet another aspect, the present disclosure refers to an *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, or increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject, or increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of the present invention, or an Fc-fusion protein of the present invention. In still another aspect, the present invention refers to the use of an IL-2 mutein of claim 1 or the Fc-fusion protein of the present invention in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.

The IL-2 muteins described herein have a relatively low likelihood of creating an immune response against the IL-2 mutein and/or endogenous IL-2 and provide Treg preferential expansion and

activation. Moreover, in certain examples, 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 examples, 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.

In one example, the present disclosure provides a human interleukin-2 (IL-2) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein has at least one mutation selected from L12G, L12K, L12Q, L12S, Q13G, E15A, E15G, E15S, H16A, H16D, H16G, H16K, H16M, H16N, H16R, H16S, H16T, H16V, H16Y, L19A, L19D, L19E, L19G, L19N, L19R, L19S, L19T, L19V, D20A, D20E, D20F, D20G, D20T, D20W, M23R, R81A, R81G, R81S, R81T, D84A, D84E, D84G, D84I, D84M, D84Q, D84R, D84S, D84T, S87R, N88A, N88D, N88E, N88F, N88G, N88M, N88R, N88S, N88V, N88W, V91D, V91E, V91G, V91S, I92K, I92R, and E95G and preferentially stimulates T regulatory cells relative to other T cells or NK cells, both in in vitro assays and in humanized mice (NSG mice reconstituted with CD34+ hematopoietic stem cells). In one example, said mutein is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1. In another example, said mutein is at least 97% identical to the amino acid sequence set forth in SEQ ID NO:1. In another example, the amino acid sequence of said mutein differs from the amino acid sequence set forth in SEQ ID NO:1 only at C125A and at one position selected from L12G, L12K, L12Q, L12S, Q13G, E15A,

E15G, E15S, H16A, H16D, H16G, H16K, H16M, H16N, H16R, H16S, H16T, H16V, H16Y, L19A, L19D, L19E, L19G, L19N, L19R, L19S, L19T, L19V, D20A, D20E, D20F, D20G, D20T, D20W, M23R, R81A, R81G, R81S, R81T, D84A, D84E, D84G, D84I, D84M, D84Q, D84R, D84S, D84T, S87R, N88A, N88D, N88E, N88F, N88G, N88M, N88R, N88S, N88V, N88W, V91D, V91E, V91G, V91S, I92K, I92R, and E95G. In another example, the amino acid sequence of said mutein differs from the amino acid sequence set forth in SEQ ID NO:1 only at C125A and at one position selected from D20E, D20G, D20W, D84A, D84S, H16D, H16G, H16K, H16R, H16T, H16V, I92K, I92R, L12K, L19D, L19N, L19T, N88D, N88R, N88S, V91D, V91G, V91K, and V91S.

In another example, the present disclosure provides an Fc-fusion protein comprising an Fc and the human IL-2 mutein as described above. In one example, the Fc is a human IgG1 Fc. In another example, the human IgG1 Fc comprises one or more mutations altering effector function of said Fc. In another example, the human IgG1 comprises a substitution at N297. In another example, the substitution at N297 is N297G. In another example, the Fc-fusion protein comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc. In another example, the C-terminal lysine of said human IgG Fc is deleted. In another example, a linker connects the Fc and human IL-2 mutein portions of said protein. In another example, the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7). In another example, the linker is GGGGS (SEQ ID NO: 5). In another example, the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells. In another example, the IL-2 mutein comprises a T3 substitution. In another example, the IL-2 mutein comprises a T3N or T3A substitution. In another example, the IL-2 mutein comprises a T3N substitution. In another example, the IL-2 mutein further comprises an S5 mutation. In another example, the IL-2 mutein further comprises an S5T mutation. In another example, said Fc-fusion protein comprises an Fc dimer. In another example, said Fc-fusion protein comprises two IL-2 muteins. In another example, said Fc-fusion protein comprises a single IL-2 mutein.

In another example, the present disclosure provides an isolated nucleic acid encoding a human IL-2 mutein as described above.

In another example, the present disclosure provides an isolated nucleic acid encoding an Fc portion of an antibody and a human IL-2 mutein as described above. In one example, said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame. In another example, the Fc is a human IgG1 Fc. In another example, the human IgG1 Fc comprises one or

more mutations altering effector function of said Fc. In another example, the human IgG1 comprises a substitution at N297. In another example, the substitution at N297 is N297G. In another example, the nucleic acid encodes a substitution or deletion of the C-terminal lysine of said human IgG Fc. In another example, the C-terminal lysine of said human IgG Fc is deleted. In another example, the nucleic acid further encodes a linker connecting the Fc portion of an antibody and the human IL-2 mutein. In another example, the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7). In another example, the linker is GGGGS (SEQ ID NO: 5). In another example, the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of a protein comprising said IL-2 mutein when expressed in mammalian cells. In another example, the IL-2 mutein comprises a T3 substitution. In another example, the IL-2 mutein comprises a T3N or T3A substitution. In another example, the IL-2 mutein comprises a T3N substitution. In another example, the IL-2 mutein further comprises an S5 mutation. In another example, the IL-2 mutein further comprises an S5T mutation.

In another example, the present disclosure provides an expression vector comprising an isolated nucleic acid described above operably linked to a promoter.

In another example, the present disclosure provides a host cell comprising an isolated nucleic acid described above. In one example, the isolated nucleic acid is operably linked to a promoter. In another example, said host cell is a prokaryotic cell. In another example, the host cell is *E. coli*. In another example, said host cell is a eukaryotic cell. In another example, the host cell is a mammalian cell. In another example, the host cell is a Chinese hamster ovary (CHO) cell line.

In another example, the present disclosure provides a method of making a human IL-2 mutein, comprising culturing a host cell as described above under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.

In another example, the present disclosure provides a method of making a Fc-fusion protein, comprising culturing a host cell as described above under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein as described above. In one

example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein as described above. In one example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein as described above. In one example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein as described above. In one example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein as described above. In one example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases. In another example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

In another example, the present disclosure provides a method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein as described above. In one example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases. In another example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

In another example, the present disclosure provides a method of treating a subject with an inflammatory or autoimmune disease, said method comprising administering to said subject a therapeutically effective amount of an IL-2 mutein as described above or a therapeutically effective amount of an Fc-fusion protein as described above. In one example, administration causes reduction of at least one symptom of the disease. In another example, the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after the administration. In another example, the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject remains essentially the same after the administration. In another example, the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.

In another example, the present disclosure provides 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. In one example, said Fc region of a human IgG1 comprises at least 95% identity to the amino acid sequence set forth in SEQ ID NO:3. In another example, said Fc region of a human IgG1 comprises the amino acid sequence set forth in SEQ ID NO:3. In another example, said Fc region of a human IgG1 further comprises one or more mutations to stabilize the polypeptide. In another example, one or more amino acids set forth in SEQ ID NO:3 are substituted with cysteine. In another example, V259, A287, R292, V302, L306, V323, or I332 of the amino acid sequence set forth in SEQ ID NO:3 is substituted with cysteine. In another example, said Fc region comprises an A287C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another example, said Fc region comprises an V259C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another example, said Fc region comprises an R292C and V302C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another example, said Fc region comprises an V323C and I332C substitution within the amino acid sequence set forth in SEQ ID NO:3.

In another example, the present disclosure provides an antibody comprising an Fc region as described above.

In another example, the present disclosure provides an Fc-fusion protein comprising an Fc region as described above.

In another example, the present disclosure provides a polypeptide comprising a linker, wherein the linker is GGNGT (SEQ ID NO: 6) or YGNGT (SEQ ID NO: 7). In one example, the linker comprises N-glycosylation. In another example, the linker is inserted into or replaces a loop in the polypeptide structure.

In another example, the present disclosure provides a method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising:

- a) expressing a nucleic acid encoding a polypeptide as described above in a mammalian cell culture; and
- b) harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.

In another example, the present disclosure provides 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.

In another example, the present disclosure provides an Fc-fusion protein wherein the amino acid sequence of said Fc-fusion protein is at least 90% identical to the amino acid sequence of a human IL-2 mutein fusion protein illustrated in Figure 24. In one example, the amino acid sequence of said Fc-fusion protein is at least 95% identical to the amino acid sequence of a human IL-2 mutein fusion protein illustrated in Figure 24. In another example, the amino acid sequence of said Fc-fusion protein is at least 97% identical to the amino acid sequence of a human IL-2 mutein fusion protein illustrated in Figure 24. In another example, the amino acid sequence of said Fc-fusion protein is at least 99% identical to the amino acid sequence of a human IL-2 mutein fusion protein illustrated in Figure 24. In another example, the amino acid sequence of said Fc-fusion protein is identical to the amino acid sequence of a human IL-2 mutein fusion protein illustrated in Figure 24.

In another example, the present disclosure provides a nucleic acid encoding the Fc-fusion as described above.

In another example, the present disclosure provides a cell comprising the nucleic acid as described above.

In another example, the present disclosure provides a method of making an Fc-fusion protein comprising incubating the cell as described above under conditions allowing it to express said Fc-fusion protein.

In another example, the present disclosure provides a method of treating an inflammatory or auto-immune condition in a subject comprising administering an effective amount of the Fc-fusion protein as described above to said subject. In one example, said inflammatory or auto-immune condition is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.

In another example, the present disclosure provides a method of monitoring the response of a subject to treatment with the human interleukin-2 (IL-2) mutein as described above, the Fc-fusion protein as described above, or the Fc-fusion protein as described above, comprising detecting a change in said subject, said change being: an increase in body temperature, an increase in CRP in said subject's peripheral blood, a decrease in platelets in said subject's peripheral blood, a decrease in neutrophils in said subject's peripheral blood, or a decrease in albumin in said subject's peripheral blood, wherein said treatment is terminated, suspended, reduced in dosing frequency, or reduced in dosing amount after said change is detected. In one example, said change comprises: an increase in body temperature of at least 0.5° C, an increase in CRP in said subject's peripheral blood of at least 0.2 mg/mL, a decrease in platelets in said subject's peripheral blood of at least 0.8-fold, a decrease in neutrophils in said subject's peripheral blood of at least 0.8-fold, or a decrease in albumin in said subject's peripheral blood of at least 0.4-fold.

In another example, the present disclosure provides an isolated anti-human IL-2 antibody, wherein said antibody: comprises a heavy chain variable domain that is at least 90% identical to the heavy variable domain of a reference antibody, and a light chain variable domain that is at least 90% identical to the light chain variable domain of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain and light chain variable domain of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively; or comprises a heavy chain variable domain that comprises CDR1, CDR2, and CDR3 of the heavy chain variable domain of a

reference antibody, and a light chain variable domain that comprises CDR1, CDR2, and CDR3 of the light chain variable domain of said reference antibody, and wherein said heavy chain CDRs and said light chain CDRs are as illustrated in Figure 28 and Figure 26, respectively; or cross-competes for binding to wild-type human IL-2 cytokine with a reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9. In one example, said antibody comprises a heavy chain variable domain amino acid sequence that is at least 90% identical to the heavy chain variable domain amino acid sequence of a reference antibody, and a light chain variable domain amino acid sequence that is at least 90% identical to the light chain variable domain amino acid sequence of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain amino acid sequence and light chain variable domain amino acid sequence of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively. In another example, said antibody comprises a heavy chain variable domain amino acid sequence that is at least 95% identical to the heavy variable domain amino acid sequence of a reference antibody, and a light chain variable domain amino acid sequence that is at least 95% identical to the light chain variable domain amino acid sequence of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain amino acid sequence and light chain variable domain amino acid sequence of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively. In another example, said antibody comprises a heavy chain variable domain amino acid sequence that is at least 97% identical to the heavy variable domain amino acid sequence of a reference antibody, and a light chain variable domain amino acid sequence that is at least 97% identical to the light chain variable domain amino acid sequence of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain amino acid sequence and light chain variable domain amino acid sequence of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively. In another example, said antibody comprises a heavy chain variable domain amino acid sequence that

is at least 99% identical to the heavy variable domain amino acid sequence of a reference antibody, and a light chain variable domain amino acid sequence that is at least 99% identical to the light chain variable domain amino acid sequence of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain amino acid sequence and light chain variable domain amino acid sequence of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively. In another example, said antibody comprises a heavy chain variable domain amino acid sequence of a reference antibody, and a light chain variable domain amino acid sequence of said reference antibody, wherein said reference antibody is 9D6, 2C3, 14C9, 8B12, 16A4, 16E1, 13A1, 8F10, 12C4, 9B12, 3H5, 18A6, 10A6, 10H7, 15A10, 12D2, 9B10, 17D3, 15G11, 14D7, 18F3, 17D9, 21F8, 22B9, 221D10, 14A6, 11D6, 10A9, 16E3, 14G7, 5H3, 2B12, 26H7, 26C12, 2H11, or 18H9, and wherein the heavy chain variable domain amino acid sequence and light chain variable domain amino acid sequence of said reference antibody is as illustrated in Figure 28 and Figure 26, respectively. In another example, said isolated antibody is: a human antibody; a humanized antibody; a chimeric antibody; a monoclonal antibody; a polyclonal antibody; a recombinant antibody; an antigen-binding antibody fragment; a single chain antibody; a diabody; a triabody; a tetrabody; a Fab fragment; a F(ab')₂ fragment; a domain antibody; an IgD antibody; an IgE antibody; an IgM antibody; an IgG1 antibody; an IgG2 antibody; an IgG3 antibody; an IgG4 antibody; or an IgG4 antibody having at least one mutation in a hinge region that alleviates a tendency to form intra-H chain disulfide bond. In another example, said isolated antibody comprises a human IgG1 Fc. In another example, said human IgG1 Fc has one or more mutations altering effector function of said Fc. In another example, said human IgG1 Fc comprises a substitution at N297. In another example, said substitution at N297 is N297G. In another example, the antibody comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc. In another example, the C-terminal lysine of said human IgG Fc is deleted. In another example, said isolated antibody comprises a human IgG1 Fc. In another example, said human IgG1 Fc has one or more mutations altering effector function of said Fc. In another example, said human IgG1 Fc comprises a substitution at N297. In another example, said substitution at N297 is N297G. In another example, the antibody comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc. In another example, the C-terminal lysine of said human IgG Fc is deleted.

In another example, the present disclosure provides an isolated complex comprising an isolated anti-human IL-2 antibody as described above bound to a human IL-2 cytokine.

In another example, the present disclosure provides an isolated nucleic acid encoding the light chain, the heavy chain, or both the light chain and the heavy chain of the isolated anti-human IL-2 antibody as described above.

In another example, the present disclosure provides an expression vector comprising the isolated nucleic acid as described above operably linked to a promoter.

In another example, the present disclosure provides a host cell comprising the isolated nucleic acid as described above. In one example, the isolated nucleic acid is operably linked to a promoter. In another example, said host cell is a prokaryotic cell. In another example, the host cell is *E. coli*. In another example, said host cell is a eukaryotic cell. In another example, the host cell is a mammalian cell. In another example, the host cell is a Chinese hamster ovary (CHO) cell line.

In another example, the present disclosure provides a method of making an anti-human IL-2 antibody, comprising culturing a host cell as described above under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.

In another example, the present disclosure provides a method of treating an inflammatory or auto-immune condition in a subject comprising administering an effective amount of the anti-human IL-2 antibody or isolated complex comprising an isolated anti-human IL-2 antibody as described above to said subject. In one example, said inflammatory or auto-immune condition is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.

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.

What is claimed is:

1. A human interleukin-2 (IL-2) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein has at least one mutation selected from H16K, H16R, H16T and preferentially stimulates T regulatory cells relative to other T cells or NK cells, both in in vitro assays and in humanized mice (NSG mice reconstituted with CD34+ hematopoietic stem cells).
2. The human IL-2 mutein of claim 1 further comprising a mutation at C125A.
3. An Fc-fusion protein comprising an Fc and the human IL-2 mutein of claim 1.
4. The Fc-fusion protein of claim 1, wherein the Fc is a human IgG1 Fc or wherein the Fc is a human IgG1 Fc comprising one or more mutations altering effector function of said Fc.
5. The Fc-fusion protein of claim 4, wherein the human IgG1 comprises a substitution at N297.
6. The Fc-fusion protein of claim 5, wherein the substitution at N297 is N297G.
7. The Fc-fusion protein of claim 3, wherein the Fc is a human IgG1 Fc comprising a substitution or deletion of the C-terminal lysine of said human IgG1 Fc.
8. The Fc-fusion protein of claim 3, wherein a linker connects the Fc and human IL-2 mutein portions of said protein.
9. The Fc-fusion protein of claim 8, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).
10. The Fc-fusion protein of claim 3, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells.
11. The Fc-fusion protein of claim 10, wherein the IL-2 mutein comprises a T3 substitution or an S5 mutation.
12. The Fc-fusion protein of claim 11, wherein the IL-2 mutein comprises a T3N or T3A substitution.
13. The Fc-fusion protein of claim 11, wherein the IL-2 mutein further comprises an S5T mutation.
14. The Fc-fusion protein of claim 3, wherein said Fc-fusion protein comprises an Fc dimer.

15. The Fc-fusion protein of claim 14, wherein said Fc-fusion protein comprises two IL-2 muteins.
16. The Fc-fusion protein of claim 14, wherein said Fc-fusion protein comprises a single IL-2 mutein.
17. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, or increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject, or increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of claim 1 or 2, or an Fc-fusion protein of any one of claims 3 to 16.
18. The method of claim 17, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.
19. Use of an IL-2 mutein of claim 1 or the Fc-fusion protein of claim 3 in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.
20. The use of claim 19, wherein the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.