

quantitatively different effect, stimulating STAT5 phosphorylation primarily in less than 1% of the cells and primarily in CD25^{high} cells.

[0016] FIGURE 5 demonstrates the selectivity of IL-2 and IL-2 Selective Agonist proteins on 7 different immune cell types in human PBMC. Both IL2/N88R/C125S/D109C and IL2/N88R/C125S/D109C-PEG are highly selectivity for Tregs compared to *wt* IL-2, and shows greater selectivity in multiple cell types than IL-2.

SUMMARY

[0017] The disclosure described herein is a novel IL-2 protein with selective agonist activity for Regulatory T cells and with an additional amino acid substitution that enable chemical conjugation with Polyethylene Glycol (PEG) that increase circulating half-life compared to the IL-2 selective agonist alone. Substituted amino acid positions were selected based on their exposure to solvent within the IL-2-IL2R $\alpha\beta\gamma$ receptor complex and the predicted ability of the polymer-conjugated variants to retain the ability to bind to and activate the IL2R $\alpha\beta\gamma$ receptor complex. A series of recombinant IL-2 variants were constructed in which cysteine amino acid residues were introduced into selected amino acid positions and their variant proteins screened for activity. IL-2 variants that were successfully expressed and purified were refolded, conjugated to maleimido-PEG polymers, and then tested for selective agonist activity on Regulatory T cells. A preferred IL-2 selective agonist variant, IL2/N88R/C125S/D109C, has thus been identified. This novel protein and its PEG conjugate will selectively and efficiently boost the levels and the activity of Regulatory T cells in patients, suppress autoimmune pathology, and will thus be a safe and effective therapeutic for the treatment of autoimmune diseases.

[0018] The disclosure also provides IL-2 variant proteins having at least 95% to 98% and to 100% sequence identity to Seq. ID No. 1, containing the substitution D109C and having the ability to activate T cells. The proteins of this disclosure may be linked to a polyethylene glycol moiety linked to the cysteine at position 109. The polyethylene glycol moiety will optionally have a molecular weight of between 5 and 40 kDa. The IL-2 variant protein of this disclosure may contain at least one of the substitutions selected from the group consisting of: N88R, N88I, N88G, D20H, Q126L, and Q126F. Further the IL-2 variant proteins of this disclosure may optionally comprise the substitution C125S. More preferable is the IL-2 variant protein in which the IL-2 protein sequence contains the substitution N88R and the

substitution C125S. The proteins of this disclosure are optionally provided in a pharmaceutical compositions comprising the proteins of this disclosure and a pharmaceutically acceptable carrier.

[0019] The disclosure further provides for methods of increasing the circulating half life of a human IL-2 variant protein of this disclosure where the method comprises linking of a polyethylene glycol moiety to the cysteine residue at position 109 wherein the polyethylene glycol moiety is a of a length sufficient to increase the circulating half live of the protein compared to the same protein without the polyethylene glycol moiety. The methods of this disclosure further provide for the administration of the protein variants at a therapeutically effective dose sufficient to stimulate human regulatory T cell levels.

[0019a] In one aspect, there is provided an IL-2 variant protein containing the substitution D109C in the amino acid sequence of SEQ ID NO: 1, wherein the IL-2 variant protein contains at least one additional substitution selected from the group consisting of: C125S, N88R, N88I, N88G, D20H, Q126L, and Q126F. In another aspect, there is provided a pharmaceutical composition comprising the IL-2 variant protein disclosed herein and a pharmaceutically acceptable carrier,

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0020] This invention is a novel IL-2 variant protein that comprises an amino acid substitution that enables specific and efficient chemical conjugation of a PEG polymer to the IL-2 variant protein while maintaining high affinity for the IL-2 receptor, high bioactivity, and an extended circulating half life compared to IL-2 itself. This variant protein was discovered through a process in which candidate amino acid positions in the IL-2 sequence that were exposed to solvent in the published IL-2- IL2R $\alpha\beta\gamma$ receptor complex crystal structure were identified, and variant proteins were constructed in which cysteine residues were substituted at the selected positions. These IL-2 variants were expressed and purified, the variant proteins produced in sufficiently high levels were conjugated with PEG-malelimide, and IL2/N88R/D109C-PEG was then tested for bioactivity on Treg cells and shown to be active. The molecule defined by this invention will enable the safe and effective treatment of autoimmune diseases by the novel mechanism of stimulating the production of a small subpopulation of T cells that suppress autoimmune and inflammatory pathology. This paradigm-breaking therapeutic can potentially treat a number of different autoimmune diseases.

Definitions

WHAT IS CLAIMED IS:

1. An IL-2 variant protein containing the substitution D109C in the amino acid sequence of SEQ ID NO: 1, wherein the IL-2 variant protein contains at least one additional substitution selected from the group consisting of: C125S, N88R, N88I, N88G, D20H, Q126L, and Q126F.
2. The IL-2 variant protein of claim 1 having a polyethylene glycol moiety linked to the cysteine at position 109, wherein the polyethylene glycol moiety has a molecular weight of between 5 and 40 kDa.
3. The IL-2 variant protein of claim 2, wherein the IL-2 variant protein contains the substitution N88R and the substitution C125S.
4. A pharmaceutical composition comprising the IL-2 variant protein of claim 1 and a pharmaceutically acceptable carrier.
5. The IL-2 variant protein of claim 1, wherein the IL-2 variant protein contains the substitution N88R and the substitution C125S.