mediated conditions, like inflammatory disorders, that can be given less frequently, at lower doses, in combination with existing drugs, and/or in a manner that facilitates self-administration. Antibody single domain-based therapeutics provided herein address these and other needs.

[0012] The foregoing discussion is presented solely to provide a better understanding of the nature of the problems confronting the art and should not be construed in any way as an admission as to prior art nor should the citation of any reference herein be construed as an admission that such reference constitutes "prior art" to the instant application.

SUMMARY ~~OF THE INVENTION~~

[0012a] In one aspect, there is provided a polypeptide comprising an antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, said domain comprising a light chain variable domain (VL), wherein said variable domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:1-18, or a TNF-alpha-binding fragment or derivative thereof.

[0013] One ~~aspect~~embodiment of the ~~invention~~disclosure relates to polypeptides comprising an antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, termed an anti-TNF-alpha polypeptide. In some embodiments, the domain comprises a light chain variable domain (VL), where the variable domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 (VL18); SEQ ID NO:2 (VL11); SEQ ID NOs:3-18, and a TNF-alpha-binding fragment or derivative thereof. In some preferred embodiments, the variable domain of the polypeptide antagonizes binding of human TNF-alpha to a TNF-alpha receptor, and/or further cross-reacts with at least one other mammalian TNF-alpha. In some preferred embodiments, the mammal is not a primate, *e.g.*, the mammal may be a rat or a mouse. In some more preferred embodiments, the variable domain cross-reacts with TNF-alpha of at least two other mammals, including a rodent and a non-rodent species.

[0014] In some embodiments, the anti-TNF-alpha polypeptide in accordance with the ~~invention~~disclosure further comprises a second antibody single domain, or antigen-binding fragment thereof, that also binds TNF-alpha, such that the single domains together form a dimer.

The second domain also may comprise a light chain variable domain. For example, in some particular embodiments, the dimer comprises sequences corresponding to SEQ ID NO:1 (VL18) and SEQ ID NO:2 (VL11), or a TNF-alpha-binding fragment or derivative thereof for one or both sequences. In some particular embodiments, the dimer comprises a sequence corresponding to SEQ ID NO:32 (VL18-3L-VL11), or a TNF-alpha-binding fragment or derivative thereof.

[0015] Another ~~aspect~~embodiment of the ~~invention~~disclosure relates to an anti-TNF-alpha polypeptide further comprising an albumin-binding domain linked thereto. The albumin-binding domain may be

linked to the polypeptide as a fusion, *e.g.*, as a fusion via a linker. In some particular embodiments, the linker is a peptide linker comprising an amino acid sequence corresponding to SEQ ID NO:29 (3L). In some preferred embodiments, the albumin-binding domain enhances the half-life of the polypeptide, *e.g.*, by five fold. In some more specific embodiments, the albumin-binding domain comprises a sequence corresponding to SEQ ID NO:30 (PEP), or an albumin-binding fragment or derivative thereof. For example, in some particular embodiments, the polypeptide comprises a sequence corresponding to SEQ ID NO: 33 (VL18-3L-VL11-PEP) or a TNF-alpha-binding fragment or derivative thereof.

[0016] In still another ~~aspect~~embodiment of the ~~invention~~disclosure, the anti-TNF-alpha polypeptides, and/or fusions or conjugates thereof with albumin-binding domains, are de-immunized. De-immunization reduces immunogenicity in the host and may be achieved by eliminating at least one T_H epitope in the variable domain and/or in the albumin-binding domain. In some particular embodiments, the variable domain comprises a sequence corresponding to SEQ ID NO:1 (VL18), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, (A51V-L54R/A51V-L54E), K63S, E79K, (C80S), T91A, and L111K, the substitutions referring to amino acid positions in SEQ ID NO:1. In some particular embodiments, the variable domain comprises a sequence corresponding to SEQ ID NO:2 (VL11), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, R31S, (A51V-L54R /A51V-L54E), K63S, E79K, (C80S), T91A, A100S, and E106K, the substitutions referring to amino acid positions in SEQ ID NO:2. In some particular embodiments, the variable domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:19-23 (five de-immunized VL18 variants), SEQ ID NOs: 24-28 (five de-immunized VL11 variants), and a TNF-alpha-binding fragment or derivative thereof. In some more specific embodiments, the polypeptide may comprise at least one sequence selected from the group consisting of SEQ ID NOs: 35-39 (VL18-3L-VL11 variants), or a TNF-alpha-binding fragment or derivative thereof. In some more specific embodiments, the polypeptide comprises at least one sequence selected from the group consisting of SEQ ID NOs: 34 and 40-44 (VL18-3L-VL11-PEP variants), or a TNF-alpha-binding fragment or derivative thereof.

[0017] In some particular embodiments, the albumin-binding domain is de-immunized, *e.g.*, by at least one amino acid substitution selected from the group consisting of E12D, T29H-K35D, and A45D, the substitutions referring to amino acid positions in SEQ ID NO:30. In some particular embodiments, the albumin-binding domain comprises an amino acid sequence corresponding to SEQ ID NO:31.

[0017a] In another aspect, there is provided the use of an effective amount of the polypeptide according to any one of the above claims for the preparation of a medicament for treating and/or delaying a TNF-alpha-related condition in a subject suffering therefrom and/or pre-disposed thereto, preferably wherein said TNF-alpha-related condition is an inflammatory disorder, and preferably wherein said inflammatory disorder is rheumatoid arthritis.

[0018] Yet another ~~aspect~~embodiment of the ~~invention~~disclosure relates to the use of an effective amount of one or more of the polypeptides according to the ~~invention~~disclosure for the preparation of a medicament for treating and/or delaying a TNF-alpha-related condition in a subject suffering therefrom and/or pre-disposed thereto. In some embodiments, the TNF-alpha-related condition is an inflammatory disorder, such as rheumatoid arthritis. In some preferred embodiments, the medicament is administered no more than twice a month or, more preferably, no more than once a month.

[0018a] In another aspect, there is provided a pharmaceutical composition comprising an effective amount of the polypeptide as defined above and a pharmaceutically acceptable carrier.

[0018b] In another aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding the polypeptide as defined above; or a vector comprising the nucleic acid; or a host cell comprising the vector.

[0019] Still yet another ~~aspect~~embodiment of the ~~invention~~disclosure relates to a pharmaceutical composition comprising an effective amount of an anti-TNF-alpha polypeptide in accordance with the ~~invention~~disclosure, and/or or a nucleic acid comprising a nucleotide sequence encoding same, and a pharmaceutically acceptable carrier.

[0020] Another ~~aspect~~embodiment relates to a nucleic acid comprising a nucleotide sequence encoding an anti-TNF-alpha polypeptide in accordance with the ~~invention~~disclosure, as well as vectors and/or host cells and/or pharmaceutical compositions comprising same.

[0020a] In another aspect, there is provided a method of making the polypeptide as defined above: (i) providing a host cell comprising a vector encoding said polypeptide; (ii) culturing said cell under conditions allowing expression of said polypeptide; and (iii) recovering said polypeptide from said culture.

[0021] Still yet another ~~aspect~~embodiment of the ~~invention~~disclosure relates to a method of making an anti-TNF-alpha polypeptide of the ~~invention~~invention comprising: (i) providing a host cell comprising a vector encoding the polypeptide; (ii) culturing the cell under conditions allowing expression of the polypeptide; and (iii) recovering the polypeptide from the culture.

BRIEF DESCRIPTION OF THE FIGURES

[0022] FIG. 1 illustrates a sequence comparison of VL single domains showing high affinity binding to human TNF-alpha.

[0023] FIGs. 2A-D illustrate results of (A-C) Western Blot expression analysis for VL monomers and dimers; and (D) binding affinities of VL monomers and dimers for human and rat TNF-alpha.

[0024] FIGs. 3A-F illustrate results of binding assays and pharmacokinetics of a VL-VL dimer of the invention. FIGs. 3A-C illustrate results of affinity determinations of the dimer to human TNF-alpha and albumin-binding fusion thereof by BIAcore as compared with Remicade™; FIGs. 3D-E illustrate relative binding and specificity of the dimer to TNF molecules of various species by ELISA; FIG. 3F illustrates pharmacokinetics of VL-VL constructs (VL18-3L-VL11 and VL18-3L-VL11-PEP) tested by administration to Wistar female rats to determine the serum half-life thereof *in vivo*, data were normalized considering maximal concentration as that assayed 5 minutes after administration (% of 5 min value).

CLAIMS

1. A polypeptide comprising an antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, said domain comprising a light chain variable domain (VL),

wherein said variable domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:1-18, or a TNF-alpha-binding fragment or derivative thereof.

2. The polypeptide according to claim 1 wherein said variable domain comprises SEQ ID NO:1 (VL18) or SEQ ID NO:2 (VL11).

3 The polypeptide according to claim 1 or 2 wherein said variable domain antagonizes binding of human TNF-alpha to a TNF-alpha receptor.

4. The polypeptide according to claim 3 wherein said variable domain further cross-reacts with at least one other mammalian TNF-alpha, wherein said mammal is not a primate.

5. The polypeptide according to claim 4 wherein said mammal is a rat or a mouse.

6. The polypeptide according to claim 4 wherein said variable domain cross-reacts with TNF-alpha of at least two other mammals, said at least two other mammals being a rodent and a non-rodent species.

7. The polypeptide according to any one of the above claims wherein said variable domain is de-immunized by eliminating at least one T_H epitope of said variable domain.

8. The polypeptide according to claim 7 wherein said variable domain comprises a sequence corresponding to SEQ ID NO:1 (VL18), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, (A51V-L54R/A51V-L54E), K63S, E79K, (C80S), T91A, and L111K, said substitutions referring to amino acid positions in SEQ ID NO:1.

9. The polypeptide according to claim 7 wherein said variable domain comprises a sequence corresponding to SEQ ID NO:2 (VL11), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, R31S, (A51V-L54R /A51V-L54E), K63S, E79K, (C80S), T91A, A100S, and E106K, said substitutions referring to amino acid positions in SEQ ID NO:2.

10. The polypeptide according to claim 7 wherein said variable domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:19-23 (five VL18 variants), SEQ ID NOs: 24-28 (five VL11 variants), and a TNF-alpha-binding fragment or derivative thereof.

24. The polypeptide according to any one of claims 21-23 wherein said dimer is de-immunized by eliminating at least one T_H epitope of in at least one of said variable domains.

25. The polypeptide according to claim 24 wherein said polypeptide comprises at least one sequence selected from the group consisting of SEQ ID NOs: 35-39 (VL18-3L-VL11 variants), or a TNF-alpha-binding fragment or derivative thereof

26. The polypeptide according to any one of claims 21-25 wherein said dimer further comprises an albumin-binding domain linked to at least one of said variable domains.

27. The polypeptide according to claim 26 wherein said said albumin-binding domain comprises a sequence corresponding to SEQ ID NO:30 (PEP), or an albumin-binding fragment or derivative thereof.

28. The polypeptide according to claim 27 wherein said polypeptide comprises a sequence corresponding to SEQ ID NO: 33 (VL18-3L-VL11-PEP) or a TNF-alpha-binding fragment or derivative thereof.

29. The polypeptide according to any one of claims 26-28 wherein said polypeptide is de-immunized by eliminating at least one T_H epitope in at least one of said variable domains and/or in said albumin-binding domain.

30. The polypeptide according to claim 29 wherein said polypeptide comprises at least one sequence selected from the group consisting of SEQ ID NOs: 34 and 40-44 (VL18-3L-VL11-PEP variants), or a TNF-alpha-binding fragment or derivative thereof.

31. Use of an effective amount of the polypeptide according to any one of the above claims for the preparation of a medicament for treating and/or delaying a TNF-alpha-related condition in a subject suffering therefrom and/or pre-disposed thereto.

32. The use of claim 31 wherein said TNF-alpha-related condition is an inflammatory disorder.

33. The use of claim 32 wherein said inflammatory disorder is rheumatoid arthritis.

34. The use of claim 31 wherein said medicament is administered no more than twice a month.

35. The use of claim 34 wherein said medicament is administered no more than once a month.

36. A pharmaceutical composition comprising an effective amount of the polypeptide according to any one of claims 1-30 and a pharmaceutically acceptable carrier.

37. A nucleic acid comprising a nucleotide sequence encoding the polypeptide according to any one of claims 1-30.

38. A vector comprising the nucleic acid according to claim 37.

39. A host cell comprising the vector according to claim 38.

11. The polypeptide according to any one of the above claims, further comprising an albumin-binding domain linked thereto.

12. The polypeptide according to claim 11 wherein said albumin-binding domain is linked to said polypeptide as a fusion.

13. The polypeptide according to claim 12 wherein said albumin-binding domain is linked to said polypeptide as a fusion via a linker.

14. The polypeptide according to claim 13 wherein said linker is a peptide linker comprising an amino acid sequence corresponding to SEQ ID NO:29 (3L).

15. The polypeptide according to any one of claims 11-14 wherein said albumin-binding domain enhances the half-life of said polypeptide by five fold.

16. The polypeptide according to any one of claims 11-15 wherein said albumin-binding domain corresponds to an albumin-binding domain isolated from *S. zooepidemicus* Z5.

17. The polypeptide according to claim 16 wherein said albumin-binding domain comprises a sequence corresponding to SEQ ID NO:30 (PEP), or an albumin-binding fragment or derivative thereof.

18. The polypeptide according to any one of claims 11-17 wherein said polypeptide is de-immunized by eliminating at least one T_H epitope in said variable domain and/or in said albumin-binding domain.

19. The polypeptide according to claim 17 wherein said albumin-binding domain is de-immunized by at least one amino acid substitution selected from the group consisting of E12D, T29H-K35D, and A45D, said substitutions referring to amino acid positions in SEQ ID NO:30.

20. The polypeptide according to claim 17 wherein said albumin-binding domain comprises an amino acid sequence corresponding to SEQ ID NO:31.

21. The polypeptide according to any one of the above claims, further comprising a second antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, wherein said second domain comprises a second light chain variable domain and wherein said single domains together form a dimer.

22. The polypeptide according to claim 21 wherein said dimer comprises sequences corresponding to SEQ ID NO:1 (VL18) and SEQ ID NO:2 (VL11), or a TNF-alpha-binding fragment or derivative thereof for one or both sequences.

23. The polypeptide according to claim 22 wherein said dimer comprises a sequence corresponding to SEQ ID NO:32 (VL18-3L-VL11), or a TNF-alpha-binding fragment or derivative thereof.

16. A method of making the polypeptide according to any one of claims 1-30 comprising:

(i) providing a host cell comprising a vector encoding said polypeptide;

(ii) culturing said cell under conditions allowing expression of said polypeptide; and

(iii) recovering said polypeptide from said culture.

Claims

1. A polypeptide comprising an antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, said domain comprising a light chain variable domain (VL),

wherein said variable domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:1-18, or a TNF-alpha-binding fragment or derivative thereof.

2. The polypeptide according to claim 1 wherein said variable domain comprises SEQ ID NO:1 (VL18) or SEQ ID NO:2 (VL11) and/or wherein said variable domain antagonizes binding of human TNF-alpha to a TNF-alpha receptor; and/or wherein said variable domain further cross-reacts with at least one other mammalian TNF-alpha, wherein said mammal is not a primate.

3. The polypeptide according to claim 1 or 2 wherein said mammal is a rat or a mouse and/or wherein said variable domain cross-reacts with TNF-alpha of at least two other mammals, said at least two other mammals being a rodent and a non-rodent species.

4. The polypeptide according to any one of the above claims wherein said variable domain

(i) is de-immunized by eliminating at least one T_H epitope of said variable domain;

(ii) comprises a sequence corresponding to SEQ ID NO:1 (VL18), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, (A51V-L54R/A51V-L54E), K63S, E79K, (C80S), T91A, and L111K, said substitutions referring to amino acid positions in SEQ ID NO:1;

(iii) comprises a sequence corresponding to SEQ ID NO:2 (VL11), which is de-immunized by at least one amino acid substitution selected from the group consisting of T7Q, V15P, R31S, (A51V-L54R /A51V-L54E), K63S, E79K, (C80S), T91A, A100S, and E106K, said substitutions referring to amino acid positions in SEQ ID NO:2; or

(iv) comprises at least one sequence selected from the group consisting of SEQ ID NOs:19-23 (five VL18 variants), SEQ ID NOs: 24-28 (five VL11 variants), and a TNF-alpha-binding fragment or derivative thereof.

5. The polypeptide according to any one of the above claims, further comprising an albumin-binding domain linked thereto,

preferably wherein said albumin-binding domain is linked to said polypeptide as a fusion;

preferably wherein said fusion is via a linker; and

preferably wherein said linker is a peptide linker comprising an amino acid sequence corresponding to SEQ ID NO:29 (3L).

6. The polypeptide according to claim 5 wherein said albumin-binding domain enhances the half-life of said polypeptide by five fold, and/or

wherein said albumin-binding domain corresponds to an albumin-binding domain isolated from *S. zooepidemicus* Z5,

preferably comprising a sequence corresponding to SEQ ID NO:30 (PEP), or an albumin-binding fragment or derivative thereof,

preferably wherein said albumin-binding domain is de-immunized by at least one amino acid substitution selected from the group consisting of E12D, T29H-K35D, and A45D, said substitutions referring to amino acid positions in SEQ ID NO:30,

preferably wherein said albumin-binding domain comprises an amino acid sequence corresponding to SEQ ID NO:31; and/or

wherein said polypeptide is de-immunized by eliminating at least one T_H epitope in said variable domain and/or in said albumin-binding domain.

7. The polypeptide according to any one of the above claims, further comprising a second antibody single domain, or antigen-binding fragment thereof, that binds TNF-alpha, wherein said second domain comprises a second light chain variable domain and wherein said single domains together form a dimer,

preferably wherein wherein said dimer comprises sequences corresponding to SEQ ID NO:1 (VL18) and SEQ ID NO:2 (VL11), or a TNF-alpha-binding fragment or derivative thereof for one or both sequences,

preferably wherein said dimer comprises a sequence corresponding to SEQ ID NO:32 (VL18-3L-VL11), or a TNF-alpha-binding fragment or derivative thereof.

8. The polypeptide according to any one of claims 4-7 wherein said dimer is de-immunized by eliminating at least one T_H epitope of in at least one of said variable domains,

preferably wherein said polypeptide comprises at least one sequence selected from the group consisting of SEQ ID NOs: 35-39 (VL18-3L-VL11 variants), or a TNF-alpha-binding fragment or derivative thereof.

9. The polypeptide according to claim 7 or 8 wherein said dimer further comprises an albumin-binding domain linked to at least one of said variable domains,

preferably wherein said said albumin-binding domain comprises a sequence corresponding to SEQ ID NO:30 (PEP), or an albumin-binding fragment or derivative thereof,

preferably wherein said polypeptide comprises a sequence corresponding to SEQ ID NO: 33 (VL18-3L-VL11-PEP) or a TNF-alpha-binding fragment or derivative thereof.

10. The polypeptide according to any one of claims 5-9 wherein said polypeptide is de-immunized by eliminating at least one T_H epitope in at least one of said variable domains and/or in said albumin-binding domain,

preferably wherein said polypeptide comprises at least one sequence selected from the group consisting of SEQ ID NOs: 34 and 40-44 (VL18-3L-VL11-PEP variants), or a TNF-alpha-binding fragment or derivative thereof.

11. Use of an effective amount of the polypeptide according to any one of the above claims for the preparation of a medicament for treating and/or delaying a TNF-alpha-related condition in a subject suffering therefrom and/or pre-disposed thereto,

preferably wherein said TNF-alpha-related condition is an inflammatory disorder, and

preferably wherein said inflammatory disorder is rheumatoid arthritis.

12. The use of claim 11 wherein said medicament is administered no more than twice a month,

preferably wherein said medicament is administered no more than once a month.

13. A pharmaceutical composition comprising an effective amount of the polypeptide according to any one of claims 1-12 and a pharmaceutically acceptable carrier.

14. A nucleic acid comprising a nucleotide sequence encoding the polypeptide according to any one of claims 1-12; or

a vector comprising the nucleic acid; or

a host cell comprising the vector.

15. A method of making the polypeptide according to any one of claims 1-12 comprising:

(i) providing a host cell comprising a vector encoding said polypeptide;

(ii) culturing said cell under conditions allowing expression of said polypeptide; and

(iii) recovering said polypeptide from said culture.