FcRn-BINDING POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 63/351,363, filed June 11, 2022, and US Provisional Application No. 63/437,776, filed January 9, 2023; each of which is incorporated by reference herein in its entirety for any purpose.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application incorporates by reference a Sequence Listing submitted with this application in electronic format entitled 01202-0057-00PCT_Sequence_Listing, created June 8, 2023, which is 54,032 bytes in size.

FIELD

[0003] The present invention relates to FcRn-binding polypeptides, including FcRn- and albumin-binding polypeptides, and methods of using the polypeptides, for example, to treat immunological diseases or disorders.

BACKGROUND

[0004] Plasma proteins are eliminated from circulation by two primary mechanisms: renal filtration of molecules below 60 kDa, and micropinocytosis by endothelial cells. Proteins below the renal threshold are rapidly cleared from circulation resulting in a half-life of 1 day or less, while proteins larger than the renal threshold are primarily cleared through micropinocytosis with half-lives around 3-5 days. Albumin and immunoglobulin G (IgG) are proteins with long plasma half-lives of around 15-30 days due to their large size (66 and 150 kDa respectively) and their ability to recycle from endothelial micropinocytosis through pH dependent binding to the neonatal Fc receptor (FcRn).

[0005] Long plasma half-life is useful for therapeutic agents to accurately and precisely control plasma drug concentrations, optimize efficacy while limiting toxicity, and reduce the frequency and amount of drug needed.

[0006] Therefore, there exists a need for FcRn-binding polypeptides that have improved plasma half-life.

SUMMARY

Example 1. A polypeptide comprising at least one VHH domain that binds FcRn, wherein at least one VHH domain that binds FcRn comprises a CDR1 sequence selected

from SEQ ID NOs: 80-81, a CDR2 sequence selected from SEQ ID NOs: 83-84, and a CDR3 sequence of SEQ ID NO: 85.

Example 2. The polypeptide of example 1, wherein each VHH domain that binds FcRn comprises, independently, a CDR1 sequence selected from SEQ ID NOs: 80-81, a CDR2 sequence selected from SEQ ID NOs: 83-84, and a CDR3 sequence of SEQ ID NO: 85.

Example 3. The polypeptide of example 1 or example 2, wherein at least one VHH domain that binds FcRn comprises CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 80, 83, and 85 SEQ ID NOs: 81, 83, and 85; and SEQ ID NOs: 81, 84, and 85.

Example 4. The polypeptide of example 3, wherein each VHH domain that binds FcRn comprises, independently, CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 80, 83, and 85 SEQ ID NOs: 81, 83, and 85; and SEQ ID NOs: 81, 84, and 85.

Example 5. The polypeptide of any one of examples 1-4, wherein at least one VHH domain that binds FcRn is humanized.

Example 6. The polypeptide of example 5, wherein each VHH domain that binds FcRn is humanized.

Example 7. The polypeptide of any one of examples 1-6, wherein at least one VHH domain that binds FcRn comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 86-93.

Example 8. The polypeptide of example 7, wherein each VHH domain that binds FcRn comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 86-93.

Example 9. The polypeptide of any one of examples 1-7, wherein at least one VHH domain that binds FcRn comprises a sequence selected from SEQ ID NOs: 86-93.

Example 10. The polypeptide of any one of examples 1-9, wherein each VHH domain that binds FcRn comprises a sequence selected from SEQ ID NOs: 86-93.

Example 11. The polypeptide of any one of examples 1-10, wherein at least one VHH domain that binds FcRn binds human FcRn with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 12. The polypeptide of any one of examples 1-11, wherein each VHH domain that binds FcRn binds human FcRn with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 13. The polypeptide of any one of examples 1-12, wherein at least one VHH domain that binds FcRn binds human FcRn at pH 6 and at pH 7.4.

Example 14. The polypeptide of any one of examples 1-13, wherein each VHH domain that binds FcRn binds human FcRn at pH 6 and at pH 7.4.

Example 15. The polypeptide of any one of examples 1-14, wherein at least one VHH domain that binds FcRn blocks binding of human IgG to human FcRn.

Example 16. The polypeptide of any one of examples 1-15, wherein each VHH domain that binds FcRn blocks binding of human IgG to human FcRn.

Example 17. The polypeptide of any one of examples 1-16, wherein the polypeptide comprises at least one VHH domain that binds albumin.

Example 18. The polypeptide of example 17, wherein at least one VHH domain that binds albumin comprises a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.

Example 19. That polypeptide of example 17, wherein each VHH domain that binds albumin comprises, independently, a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.

Example 20. The polypeptide of example 17 or example 18, wherein at least one VHH domain that binds albumin comprises CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.

Example 21. The polypeptide of example 17 or example 18, wherein each VHH domain that binds albumin comprises, independently, CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.

Example 22. The polypeptide of any one of examples 17-21, wherein at least one VHH domain that binds albumin is humanized.

Example 23. The polypeptide of example 22, wherein each VHH domain that binds albumin is humanized.

Example 24. The polypeptide of any one of examples 17-23, wherein at least one VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 97-100.

Example 25. The polypeptide of any one of examples 17-23, wherein each VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 97-100.

Example 26. The polypeptide of any one of examples 17-23, wherein at least one VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 97-100.

Example 27. The polypeptide of any one of examples 17-23, wherein each VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 97-100.

Example 28. The polypeptide of any one of examples 17-26, wherein the polypeptide comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 69-77.

Example 29. The polypeptide of any one of examples 17-26, wherein the polypeptide comprises a sequence selected from SEQ ID NOs: 69-77.

Example 30. The polypeptide of any one of examples 17-29, wherein at least one VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.

Example 31. The polypeptide of any one of examples 17-29, wherein each VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.

Example 32. The polypeptide of any one of examples 17-29, wherein at least one VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.

Example 33. The polypeptide of any one of examples 17-29, wherein each VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.

Example 34. The polypeptide of any one of examples 17-33, wherein at least one VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 35. The polypeptide of any one of examples 17-34, wherein at least one VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse,

and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 36. The polypeptide of any one of examples 17-35, wherein each VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 37. The polypeptide of any one of examples 17-36, wherein each VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse, and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Example 38. The polypeptide of any one of examples 17-37, wherein the each VHH domain that binds albumin does not bind albumin domain 3.

Example 39. The polypeptide of any one of examples 17-38, wherein the each VHH domain that binds albumin does not interfere with binding of albumin to FcRn.

Example 40. The polypeptide of any one of the preceding examples, wherein the polypeptide comprises at least two, at least three, or at least four VHH domains that bind FcRn and at least one VHH domain that binds albumin.

Example 41. The polypeptide of example 40, wherein the polypeptide comprises two, three, or four VHH domains that bind FcRn and one VHH domain that binds albumin.

Example 42. The polypeptide of any one of examples 1-41, wherein the polypeptide comprises at least one binding domain that binds a protein other than albumin or FcRn.

Example 43. The polypeptide of example 42, wherein at least one binding domain that binds a protein other than albumin or FcRn is a VHH.

Example 44. The polypeptide of example 43, wherein each binding domain that binds a protein other than albumin or FcRn is a VHH.

Example 45. The polypeptide of example 42 or example 43, wherein at least one binding domain that binds a protein other than albumin or FcRn comprises a heavy chain variable region and a light chain variable region.

Example 46. The polypeptide of example 45, wherein each binding domain that binds a protein other than albumin or FcRn comprises a heavy chain variable region and a light chain variable region.

Example 47. The polypeptide of any one of examples 42-46, wherein at least one binding domain that binds a protein other than albumin or FcRn is a binding domain of a therapeutic antibody.

Example 48. The polypeptide of example 47, wherein each binding domain that binds a protein other than albumin is a binding domain of a therapeutic antibody.

Example 49. The polypeptide of example 47 or example 48, wherein the therapeutic antibody is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

Example 50. The polypeptide of any one of examples 1-41, wherein the polypeptide comprises an amino acid sequence of a therapeutic protein.

Example 51. The polypeptide of example 50, wherein the therapeutic protein is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

Example 52. The polypeptide of any one of the preceding examples, wherein the half-life of the polypeptide is greater than the half-life of the same polypeptide lacking a VHH domain that binds FcRn or albumin.

Example 53. A pharmaceutical composition comprising the polypeptide of any one of examples 1-52 and a pharmaceutically acceptable carrier.

Example 54. An isolated nucleic acid that encodes the the polypeptide of any one of examples 1-52.

Example 55. A vector comprising the nucleic acid of example 54.

Example 56. A host cell comprising the nucleic acid of example 54 or the vector of example 55.

Example 57. A host cell that expresses the polypeptide of any one of examples 1-52.

Example 58. A method of producing the polypeptide of any one of examples 1-52, comprising incubating the host cell of example 56 or example 57 under conditions suitable for expression of the polypeptide.

Example 59. The method of example 58, further comprising isolating the antibody or polypeptide.

Example 60. A method comprising administering to a subject the polypeptide of any one of examples 1-52 or the pharmaceutical composition of example 53.

Example 61. A method of treating a disease or disorder comprising administering to a subject with the disease or disorder a pharmaceutically effective amount of the polypeptide of any one of examples 1-52, or the pharmaceutical composition of example 53.

Example 62. The method of example 61, wherein the disease or disorder is selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.
Example 63. The method of example 61 or example 62, wherein the disease or disorder is an autoantibody-mediated disease or disorder.
Example 64. The method of any one of examples 61-63, wherein the disease or disorder is pemphigus vulgaris, lupus nephritis, myasthenia gravis, Guillain-Barré syndrome, antibody-mediated rejection, antiphospholipid antibody syndrome, chronic inflammatory demyelinating polyneuropathy, immune complex-mediated vasculitis, glomerulitis, a channelopathy, neuromyelitis optica, autoimmune encephalitis, autoimmune Grave's disease, idiopathic thrombocytopenia purpura, autoimmune haemolytic anaemia, immune neutropenia, dilated cardiomyopathy, or serum sickness.
Example 65. The method of any one of examples 60-64, wherein the polypeptide is administered subcutaneously.
[0006a] In one aspect, the disclosure provides a polypeptide comprising at least one VHH domain that binds FcRn, wherein at least one VHH domain that binds FcRn comprises a CDR1 sequence selected from SEQ ID NOs: 80-81, a CDR2 sequence selected from SEQ ID NOs: 83-84, and a CDR3 sequence of SEQ ID NO: 85.
[0006b] In one aspect, the disclosure provides a pharmaceutical composition comprising the polypeptide as disclosed herein and a pharmaceutically acceptable carrier.
[0006c] In one aspect, the disclosure provides an isolated nucleic acid that encodes the polypeptide as disclosed herein.
[0006d] In one aspect, the disclosure provides a vector comprising the nucleic acid as disclosed herein.
[0006e] In one aspect, the disclosure provides a host cell comprising the nucleic acid as disclosed herein or the vector as disclosed herein.
[0006f] In one aspect, the disclosure provides a host cell that expresses the polypeptide as disclosed herein.
[0006g] In one aspect, the disclosure provides a method of producing the polypeptide as disclosed herein, comprising incubating the host cell as disclosed herein under conditions suitable for expression of the polypeptide, and isolating the antibody or polypeptide.
[0006h] In one aspect, the disclosure provides the polypeptide as disclosed herein, or the pharmaceutical composition as disclosed herein for use as a medicament for administration to a subject, optionally wherein the polypeptide is to be administered subcutaneously.
[0006i] In one aspect, the disclosure provides use of the polypeptide as disclosed herein or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease or disorder.

BRIEF DESCRIPTION OF THE FIGURES

[0007] FIG. 1A-1I. FIG. 1A shows shows an alignment of certain 4A01 humanized variants (SEQ ID NOs: 24-43). FIG. 1B-1C show human albumin (HSA) binding ELISA of certain 4A01 humanized variants. FIG. 1D-1E show cynomolgus monkey albumin (CSA) binding ELISA of certain 4A01 humanized variants. FIG. 1F-1G show murine albumin (MSA) binding ELISA of certain 4A01 humanized variants. FIG. 1H-1I show rat albumin (RSA) binding ELISA of certain 4A01 humanized variants.
[0008] FIG. 2A-2D show human albumin (HSA; FIG. 2A), cynomolgus monkey albumin (CSA; FIG. 2B), murine albumin (MSA; FIG. 2C), and rat albumin (RSA; FIG. 2D) binding ELISA of humanized hz4A01v51.
[0009] FIG. 3A-3B. FIG. 3A is a schematic showing the experimental design for biolayer interferometry assessing binding to albumin domain 3. FIG. 3B shows a biolayer interferometry trace showing 4A01-NNT-hFc (SEQ ID NOs: 23 and 68) does not bind to albumin domain 3, while 1C04 does.
[0010] FIG. 4A-4B. FIG. 4A is a schematic showing the experimental design for biolayer interferometry assessing B2M-FcRn binding to albumin. FIG. 4B is a biolayer interferometry trace showing 4A01-NNT-hFc (SEQ ID NOs: 23 and 68) and humanized hz4A01v51-NNT-hFc (SEQ ID NO: 102) do not compete with albumin for B2m-FcRn binding, while 1C04 does.
[0011] FIG. 5 shows certain exemplary formats tested in binding and functional assays. Formats (i) and (iii) are bivalent, bispecific polypeptides targeting FcRn (black) and albumin