CLAIMS

1. A mutein of human tear lipocalin having binding affinity to IL-4 receptor alpha, characterised in at least one substitution at a position corresponding to the amino acid p of mature human tear lipocalin selected from the group consisting of: Pro, Leu, Asn, Tyr, Lys or Glu at position 26; Lys at position 32; Trp, Asn, Val, His or Arg at position 34; Leu, Ala, His, Lys or Met at position 56; Leu at position 58; or Lys, Ser, Gln or Pro at position 63.

2. The mutein of human tear lipocalin according to claim 1, wherein the mutein comprises:

(i) a mutated amino acid residue at any one or more of the sequence positions 27, 28, 30, 31, 33, 53, 57, 61, 64, 66, 80, 83, 104-106 and 108 of the linear polypeptide sequence of the mature human tear lipocalin,

(ii) a mutated amino acid residue at any 2 or more of the sequence positions 26, 32, 34, 55, 56, 58 and 63 of the linear polypeptide sequence of the mature human tear lipocalin, wherein the amino acid sequence of the mutein comprises one of the following sets of amino acid combinations:

(1) Ser 26, Glu 34, Leu 55, Lys 58,
(2) Ser 26, Asn 34, Ala 55, Lys 58,
(3) Ser 26, Val 34,
(4) Pro 26, Ser 34,
(5) Pro 26, Ala 55,
(6) Leu 26, Trp 34, Ala 55,
(7) Leu 26, Trp 34, Ile 58,
(8) Asn 26, Asp 34,
(9) Asn 26, Ala 55,
(10) Tyr 26, His 34, Ala 55,
(11) Tyr 26, His 34, Ala 58,
(12) Lys 26, Arg 34, Ala 55,
(13) Lys 26, Arg 34, Asn 58,
(14) Glu 26, Gly 34, Ala 55,
(15) Glu 26, Gly 34, Leu 58.

3. The mutein of claim 1 or 2, further comprising at least one of the following: Ala 31, Tyr 33, Trp 61, Ser 80, Leu 104, Pro 106 and Gln 108.

4. The mutein of any claims 1-3, further comprising at least one of the following: Phe 53, Leu 53, Tyr 64, Met 64, Leu 66 and Asp 66.

5. The mutein of claims 1-4, further comprising at least one of the following: Arg 27; Cys 28, Arg 30, Ala 31, Tyr 33, Arg 57, Trp 61, Ser 80, Arg 83, Leu 104, Cys 105, Pro 106 and Gln 108.

6. The mutein of claims 1-5, further comprising one or both of the sets:
   (1) Phe 53, Tyr 64, Leu 66;
   (2) Leu 53, Met 64, Asp 66.

7. The mutein of claim 1 or 2, comprising one of the following sets:
   (1) Ser 26; Tyr 32; Leu 55; Gln 56; Lys 58;
   (2) Pro 26; Tyr 32; Ser 34; Ala 55; Gln 56; Lys 63;
   (3) Leu 26; Phe 32; Trp 34; Ala 55; Ile 58; Ser 63;
   (4) Ser 26; Tyr 32; Val 34; Ala 55; Ala 56; Ile 58; Ser 63;
   (5) Ser 26; Val 32; Asn 34; Ala 55; Gln 56; Lys 58; Lys 63;
   (6) Asn 26; Lys 32; Asp 34; Ala 55; His 56; Arg 58; Gln 63;
   (7) Tyr 26; Tyr 32; His 34; Ala 55; His 56; Ala 58; Lys 63;
   (8) Lys 26; Tyr 32; Arg 34; Ala 55; Lys 56; Asn 58; Pro 63; and
   (9) Glu 26; His 32; Gly 34; Ala 55; Met 56; Leu 58; Lys 63.

8. The mutein of claim 1 or 2 as set forth in any one of SEQ ID NOs: 3-11 or of a fragment or variant thereof.

9. A method of generating a mutein of human tear lipocalin, wherein the mutein binds to IL-4 receptor alpha, comprising:

(a) subjecting a nucleic acid molecule encoding a human tear lipocalin to mutagenesis at
(i) any one or more of the amino acid sequence positions 27, 28, 30, 31, 33, 53, 57, 61, 64, 66, 80, 83, 104-106 and 108 of the linear polypeptide sequence of mature human tear lipocalin,
(ii) any two or more of the amino acid sequence positions 26, 32, 34, 55, 56, 58 and 63 of the linear polypeptide sequence of the mature human tear lipocalin,
thereby obtaining one or more nucleic acids encoding a mutein of human tear lipocalin, wherein
if mutated at position 26, the mutation comprises Ser, Pro, Leu, Asn, Tyr, Lys or Glu;
if mutated at position 32, the mutation comprises Tyr, Val, Lys or His;
if mutated at position 34, the mutation comprises Glu, Ser, Trp, Asn, Val, Asp, His, Arg or Gly;
if mutated at position 55, the mutation comprises Leu or Ala;
if mutated at position 56, the mutation comprises Gln, Leu, Ala, His, Lys or Met;
if mutated at position 58, the mutation comprises Lys, Ser, Ile, Arg, Ala, Asn or Leu;
if mutated at position 63, the mutation comprises Glu, Lys, Ser, Gln or Pro;

(b) expressing the one or more mutein nucleic acid molecule(s) obtained in (a) in an expression system, thereby obtaining one or more mutein(s),

(c) enriching the one or more mutein(s) obtained in (b) and selecting muteins that binds to IL-4 receptor alpha with a dissociation constant of less than 10 nM by means of selection and/or isolation.

10. The method according to claim 9, wherein step (c) further comprises:
    (ci) providing IL-4 receptor alpha or an immunogenic fragment thereof,
    (cii) contacting the one or more muteins with the IL-4 receptor alpha or the immunogenic fragment thereof, thereby allowing the formation of a complex between the IL-4 receptor alpha or the immunogenic fragment thereof and a mutein having binding affinity.

11. The method of claim 9 or 10, wherein the selection in step (c) is carried out under competitive conditions.

12. The method according to any of claims 9 to 11, wherein the mutein comprises one of the following sets of amino acid:
    (1) Ser 26, Glu 34, Leu 55, Lys 58,
    (2) Ser 26, Asn 34, Ala 55, Lys 58,
    (3) Ser 26, Val 34,
    (4) Pro 26, Ser 34,
    (5) Pro 26, Ala 55,
    (6) Leu 26, Trp 34, Ala 55,
    (7) Leu 26, Trp 34, Ile 58,
    (8) Asn 26, Asp 34,
    (9) Asn 26, Ala 55,
    (10) Tyr 26, His 34, Ala 55,
    (11) Tyr 26, His 34, Ala 58,
    (12) Lys 26, Arg 34, Ala 55,
    (13) Lys 26, Arg 34, Asn 58,
    (14) Glu 26, Gly 34, Ala 55,
    (15) Glu 26, Gly 34, Leu 58.

13. A muteins obtainable or obtained according to any of claims 9-12.

14. A nucleic acid molecule comprising a nucleotide sequence encoding a mutein of any one of claims 1-8 and 13.

15. A host cell containing a nucleic acid molecule of claim 14.

16. A pharmaceutical or diagnostic composition comprising a mutein of any one of claims 1-8 and 13.

Claims

1. A mutein of human tear lipocalin having binding affinity to IL-4 receptor alpha, characterised in at least one substitution at a position corresponding to the amino acid position of mature human tear lipocalin selected from the group consisting of: Pro, Leu, Asn, Tyr, Lys or Glu at position 26; Lys at position 32; Trp, Asn, Val, His or Arg at position 34; Leu, Ala, His, Lys or Met at position 56; Leu at position 58; or Lys, Ser, Gln or Pro at position 63, wherein the mutein has a stronger binding affinity than S191.4-B24 (SEQ ID NO:2).

2. The mutein of human tear lipocalin according to claim 1, wherein the mutein comprises:

(i) a mutated amino acid residue at any one or more of the sequence positions 27, 28, 30, 31, 33, 53, 57, 61, 64, 66, 80, 83, 104 -106 and 108 of the linear polypeptide sequence of the mature human tear lipocalin,

(ii) a mutated amino acid residue at any 2 or more of the sequence positions 26, 32, 34, 55, 56, 58 and 63 of the linear polypeptide sequence of the mature human tear lipocalin, wherein the amino acid sequence of the mutein comprises one of the following sets of amino acid combinations:

(1) Ser 26, Glu 34, Leu 55, Lys 58,
(2) Ser 26, Asn 34, Ala 55, Lys 58,
(3) Ser 26, Val 34,
(4) Pro 26, Ser 34,
(5) Pro 26, Ala 55,
(6) Leu 26, Trp 34, Ala 55,
(7) Leu 26, Trp 34, Ile 58,
(8) Asn 26, Asp 34,
(9) Asn 26, Ala 55,
(10) Tyr 26, His 34, Ala 55,
(11) Tyr 26, His 34, Ala 58,
(12) Lys 26, Arg 34, Ala 55,
(13) Lys 26, Arg 34, Asn 58,
(14) Glu 26, Gly 34, Ala 55,
(15) Glu 26, Gly 34, Leu 58.

3. The mutein of claim 1 or 2, further comprising at least one of the following: Ala 31, Tyr 33, Trp 61, Ser 80, Leu 104, Pro 106 and Gln 108.

4. The mutein of any one of claims 1-3, further comprising at least one of the following: Phe 53, Leu 53, Tyr 64, Met 64, Leu 66 and Asp 66.

5. The mutein of any one of claims 1-4, further comprising at least one of the following: Arg 27, Cys 28, Arg 30, Ala 31, Tyr 33, Arg 57, Trp 61, Ser 80, Arg 83, Leu 104, Cys 105, Pro 106 and Gln 108.

6. The mutein of any one of claims 1-5, further comprising one of the following sets of amino acid substitutions:

(1) Phe 53, Tyr 64, Leu 66

(2) Leu 53, Met 64, Asp 66.

7. The mutein of any one of claims 1 or 2, comprising one of the following sets of amino acid substitutions:

Ser 26; Tyr 32; Leu 55; Gln 56; Lys 58;
Pro 26; Tyr 32; Ser 34; Ala 55; Gln 56; Lys 63;
Leu 26; Phe 32; Trp 34; Ala 55; Ile 58; Ser 63;
Ser 26; Tyr 32; Val 34; Ala 55; Ala 56; Ile 58; Ser 63;
Ser 26; Val 32; Asn 34; Ala 55; Gln 56; Lys 58; Lys 63;
Asn 26; Lys 32; Asp 34; Ala 55; His 56; Arg 58; Gln 63;
Tyr 26; Tyr 32; His 34; Ala 55; His 56; Ala 58; Lys 63;
Lys 26; Tyr 32; Arg 34; Ala 55; Lys 56; Asn 58; Pro 63; and
Glu 26; His 32; Gly 34; Ala 55; Met 56; Leu 58; Lys 63.

8. The mutein of any one of claims 1 or 2 as set forth in any one of SEQ ID NOs: 3-11 or a fragment thereof.

9. A method of generating a mutein of human tear lipocalin, wherein the mutein binds to IL 4 receptor alpha and wherein the mutein has a stronger binding affinity than S191.4-B24 (SEQ ID NO:2), comprising:

(a) subjecting a nucleic acid molecule encoding a human tear lipocalin to mutagenesis at

(i) any one or more of the amino acid sequence positions 27, 28, 30, 31, 33, 53, 57, 61, 64, 66, 80, 83, 104 -106 and 108 of the linear polypeptide sequence of mature human tear lipocalin,

(ii) any two or more of the amino acid sequence positions 26, 32, 34, 55, 56, 58 and 63 of the linear polypeptide sequence of the mature human tear lipocalin,

thereby obtaining one or more nucleic acids encoding a mutein of human tear lipocalin, wherein

if mutated at position 26, the mutation comprises Ser, Pro, Leu, Asn, Tyr, Lys or Glu;

if mutated at position 32, the mutation comprises Tyr, Val, Lys or His;

if mutated at position 34, the mutation comprises Glu, Ser, Trp, Asn, Val, Asp, His, Arg or Gly;

if mutated at position 55, the mutation comprises Leu or Ala;

if mutated at position 56, the mutation comprises Gln, Leu, Ala, His, Lys or Met;

if mutated at position 58, the mutation comprises Lys, Ser, Ile, Arg, Ala, Asn or Leu;

if mutated at position 63, the mutation comprises Glu, Lys, Ser, Gln or Pro,

(b) expressing the one or more mutein nucleic acid molecule(s) obtained in (a) in an expression system, thereby obtaining one or more mutein(s), and

(c) enriching the one or more mutein(s) obtained in (b) and selecting muteins that bind to IL 4 receptor alpha with a dissociation constant of less than 10 nM by means of selection and/or isolation.

10. The method according to claim 9, wherein step (c) further comprises:

(ci) providing IL 4 receptor alpha or an immunogenic fragment thereof,

(cii) contacting the one or more muteins with the IL 4 receptor alpha or the immunogenic fragment thereof, thereby allowing the formation of a complex between the IL 4 receptor alpha or the immunogenic fragment thereof and a mutein having binding affinity.

11. The method of claims 9 or 10, wherein the selection in step (c) is carried out under competitive conditions.

12. The method according to any one of claims 9 to 11, wherein the mutein comprises one of the following sets of amino acids:

(1) Ser 26, Glu 34, Leu 55, Lys 58,

(2) Ser 26, Asn 34, Ala 55, Lys 58,

(3) Ser 26, Val 34,

(4) Pro 26, Ser 34,

(5) Pro 26, Ala 55,

(6) Leu 26, Trp 34, Ala 55,

(7) Leu 26, Trp 34, Ile 58,

(8) Asn 26, Asp 34,

(9) Asn 26, Ala 55,

(10) Tyr 26, His 34, Ala 55,

(11) Tyr 26, His 34, Ala 58,

(12) Lys 26, Arg 34, Ala 55,

(13) Lys 26, Arg 34, Asn 58,

(14) Glu 26, Gly 34, Ala 55,

(15) Glu 26, Gly 34, Leu 58.

13. A mutein obtainable or obtained according to any one of claims 9 to 12.

14. A nucleic acid molecule comprising a nucleotide sequence encoding a mutein of any one of claims 1 to 8 and 13.

15. A host cell containing a nucleic acid molecule of claim 14.

16. A pharmaceutical composition comprising a mutein of human tear lipocalin as defined in any one of claims 1 to 8 and 13 and a pharmaceutically acceptable excipient.

17. A diagnostic composition comprising a mutein of human tear lipocalin as defined in any one of claims 1 to 8 and 13 and means for diagnosis.