(19) DANMARK

(10) **DK/EP 3715372 T5**

Patent- og Varemærkestyrelsen

(12) Rettet oversættelse af europæisk patentskrift

(51) Int.Cl.: ***C 07 K 16/28 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2024-08-26**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2024-01-24**

(86) Europæisk ansøgning nr.: **20162800.5**

(86) Europæisk indleveringsdag: **2009-10-27**

(87) Den europæiske ansøgnings publiceringsdag: **2020-09-30**

(30) Prioritet: **2008-10-29 US 26030708**

(62) Stamansøgningsnr: **18158965.6**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR**

(73) Patenthaver: **Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591-6706, USA**

(72) Opfinder: **Papadopoulos, Nicholas J, 59 Heritage Lane, Lagrangeville, NY 12540, USA**
Fairhurst, Jeanette L, 23 Parkview Court, White Plains, NY 10603, USA
HUANG, Tammy T, 28 Gideon Reynolds Road, Cross River, NY 10518, USA
Martin, Joel H, 244 Church Road, Putnam Valley, NY 10579, USA

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **Humane antistoffer med høj affinitet for human IL-4 receptor**

(56) Fremdragne publikationer:
WO-A2-01/92340
WO-A2-2005/047331
WO-A2-2008/054606

DESCRIPTION

BACKGROUND

[0001] Interleukin-4 (IL-4, also known as B cell stimulating factor or BSF-1) was originally characterized by its ability to stimulate the proliferation of B cells in response to low concentrations of antibodies directed to surface immunoglobulin. IL-4 has been shown to possess a broad spectrum of biological activities, including growth stimulation of T cells, mast cells, granulocytes, megakaryocytes and erythrocytes. IL-4 induces the expression of class II major histocompatibility complex molecules in resting B cells, and enhances the secretion of IgE and IgG1 isotypes by stimulated B cells.

[0002] The biological activities of IL-4 are mediated by specific cell surface receptors for IL-4. Human IL-4 receptor alpha (hIL-4R) (SEQ ID NO:274) is described in, for example, U.S. Patent No. 5,599,905, 5,767,065, and 5,840,869. Antibodies to hIL-4R are described in U.S. Patent No. 5,717,072 and 7,186,809.

[0003] Methods to produce antibodies useful as human therapeutics include generating chimeric antibodies and humanized antibodies (see, for example, US 6,949,245). See, for example, WO 94/02602 and US 6,596,541, describing methods of generating nonhuman transgenic mice capable of producing human antibodies.

[0004] Methods for using antibodies to hIL-4R are described in U.S. Patent Nos. 5,714,146; 5,985,280; and 6,716,587.

[0005] The following documents are also mentioned:

- WO 2008/054606 A2 which refers to antibodies specific for human IL4R and medical uses thereof;
- WO 01/92340 A2 which refers to IL-4 antagonists and compositions thereof; and
- WO 2005/047331 A2 which refers to antibodies that bind the IL-4R.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention is defined by the appended claims.

[0007] The present invention provides a recombinant expression vector comprising:

a first nucleic acid molecule comprising a nucleic acid sequence encoding a heavy chain variable region (HCVR) of an antibody that binds human interleukin-4 receptor (IL-4R), wherein

the nucleotide sequence encoding the HCVR has at least 95% homology to SEQ ID NO:161, wherein the encoded HCVR comprises the amino acid sequence of SEQ ID NO:162; and

a second nucleic acid molecule comprising a nucleic acid sequence encoding the light chain variable region (LCVR) of an antibody that binds human IL-4R, wherein the nucleotide sequence encoding the LCVR has at least 95% homology to SEQ ID NO:163, wherein the encoded LCVR comprises the amino acid sequence of SEQ ID NO:164.

[0008] The present invention further provides a host cell comprising a recombinant expression vector of the present invention.

[0009] The present invention also provides a host cell comprising:

- an isolated nucleic acid molecule comprising a nucleotide sequence encoding an HCVR comprising the amino acid sequence of SEQ ID NO:162 operably linked to DNA encoding a human heavy chain constant region, wherein the encoded HCVR comprises the amino acid sequence of SEQ ID NO:162; and
- an isolated nucleic acid molecule comprising a nucleotide sequence encoding an LCVR comprising the amino acid sequence of SEQ ID NO:164 operably linked to DNA encoding a human light chain constant region, wherein the encoded LCVR comprises the amino acid sequence of SEQ ID NO:164,

wherein the cell expresses a fully human antibody that binds human interleukin-4 receptor (IL-4R).

[0010] The present invention also provides a method of producing an antibody or antigen-binding fragment thereof that specifically binds human IL-4R, the method comprising growing a host cell of the present invention under conditions in which the antibody or antigen-binding fragment is expressed, and recovering the fully human antibody.

[0011] In one aspect, the antibodies encoded by a vector or host cell of the invention are human antibodies, preferably recombinant human antibodies, that specifically bind human interleukin-4 receptor (hIL-4R). The human antibodies are characterized by binding to hIL-4R with high affinity and by the ability to neutralize hIL-4 activity. In specific embodiments, the human antibodies are capable of blocking hIL-13/hIL-13R1 complex binding to hIL-4R, and thus inhibit signaling by hIL-13. The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to effect functionality, e.g., to eliminate residual effector functions (Reddy et al. (2000) J. Immunol. 164:1925-1933).

[0012] In a general embodiment, the antibody or antigen-binding fragment thereof, encoded by a vector or host cell of the present invention specifically binds hIL-4R (SEQ ID NO:274) with a K_D of about 300 pM or less, as measured by surface plasmon resonance in a monomeric or

dimeric assay. In a more specific embodiment, the antibody or antigen-binding portion thereof exhibits a K_D of about 200 pM or less, about 150 or less, about 100 pM or less, or about 50 pM. In various embodiments, the antibody or antigen-binding fragment blocks hIL-4 activity with an ICso of about 100 pM or less, as measured by luciferase bioassay. In more specific embodiments, the antibody or antigen-binding fragment exhibits an IC_{50} of about 50 pM or less, about 30 pM or less, or about 25 pM or less, as measured by STAT6 luciferase bioassay. In various embodiments, the antibody or antigen-binding fragment blocks hIL-13 activity with an IC_{50} of about 100 pM or less, about 90 pM or less, about 50 pM or less, or about 20 pM or less, as measured by STAT6 luciferase bioassay.

[0013] Also disclosed but not part of the invention is an antibody that comprises a heavy chain variable region (HCVR) sequence selected from the group consisting of SEQ ID NO:2, 18, 22, 26, 42, 46, 50, 66, 70, 74, 90, 94, 98, 114, 118, 122, 138, 142, 146, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258 and 262, or a substantially similar sequence thereof.

[0014] Also disclosed but not part of the invention is an antibody that comprises a light chain variable region (LCVR) sequence selected from the group consisting of SEQ ID NO:10, 20, 24, 34, 44, 48, 58, 68, 72, 82, 92, 96, 106, 116, 120, 130, 140, 144, 154, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260 and 264, or a substantially similar sequence thereof.

[0015] Also disclosed but not part of the invention is an antibody or antibody fragment of that comprises HCVR and LCVR sequence pairs (HCVR/LCVR) selected from the group consisting of SEQ ID NO: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260 and 262/264. In a preferred instance, the antibody or antibody fragment comprises HCVR/LCVR sequence pairs SEQ ID NO:162/164, 210/212 or 18/20; exemplary antibodies having these HCVR/LCVR sequence pairs include the antibodies designated H4H083P (SEQ ID NOs:210/212), and H4H095P (SEQ ID NOs:18/20).
An antibody encoded by a vector according to the invention is H4H098P (SEQ ID Nos: 162/164).

[0016] Also disclosed but not part of the invention are nucleic acid molecules encoding an HCVR, wherein the nucleic acid molecule is a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 17, 21, 25, 41, 45, 49, 65, 69, 73, 89, 93, 97, 113, 117, 121, 137, 141, 145, 165, 169, 185, 189, 193, 209, 213, 217, 233, 237, 241, 257 and 261, or a substantially identical sequence having at least 95% homology thereof.

[0017] Also disclosed but not part of the invention are nucleic acid molecules encoding a LCVR, wherein the nucleic acid molecule is a sequence selected from the group consisting of SEQ ID NO: 9, 19, 23, 33, 43, 47, 57, 67, 71, 81, 91, 95, 105, 115, 119, 129, 139, 143, 153, 167, 177, 187, 191, 201, 211, 215, 225, 235, 239, 249, 259 and 263, or a substantially identical sequence having at least 95% homology thereof.

[0018] Also disclosed but not part of the invention is an antibody of the disclosure which comprises a HCVR and LCVR encoded by a nucleotide sequence pairs selected from the group consisting of SEQ ID NO: 1/9, 17/19, 21/22, 25/33, 41/43, 45/47, 49/57, 65/67, 69/71, 73/81, 89/91, 93/95, 97/105, 113/115, 117/119, 121/129, 137/139, 141/143, 145/153, 165/167, 169/177, 185/187, 189/191, 193/201, 209/211, 213/215, 217/225, 233/235, 237/239, 241/249, 257/259 and 261/263. In a preferred instance, the antibody or antibody fragment comprise HCVR/LCVR sequences encoded by nucleic acid sequences selected from SEQ ID NO: 209/211 and 17/19. In an even more preferred embodiment, the antibody or antibody fragment encoded by a vector or host cells of the present invention comprises HCVR/LCVR encoded by nucleic acid sequences SEQ ID NO:161/163.

[0019] Also disclosed but not part of the invention is an antibody or antigen-binding fragment comprising a HCDR3 and a LCDR3, wherein the HCDR3 domain is selected from the group consisting of SEQ ID NO:8, 32, 56, 80, 104, 128, 152, 176, 200, 224 and 248; and the LCDR3 domain selected from the group consisting of SEQ ID NO:16, 40, 64, 88, 112, 136, 160, 184, 208, 232 and 256. In a preferred instance, the HCDR3/LCDR3 sequences are SEQ ID NO:152/160, 8/16 or 200/208. In an even more preferred instance, the HCDR3 and LCDR3 sequences are SEQ ID NO:152 and 160.

[0020] In a further instance , the antibody or antibody fragment further comprises a HCDR1 sequence selected from the group consisting of SEQ ID NO:4, 28, 52, 76, 100, 124, 148, 172, 196, 220 and 244, or a substantially similar sequence thereof; a HCDR2 sequence selected from the group consisting of SEQ ID NO:6, 30, 54, 78, 102, 126, 150, 174, 198, 222 and 246, or a substantially similar sequence thereof; a HCDR3 sequence selected from the group consisting of SEQ ID NO:8, 32, 56, 80, 104, 128, 152, 176, 200, 224 and 248, or a substantially similar sequence thereof; a LCDR1 sequence selected from the group consisting of SEQ ID NO:12, 36, 60, 84, 108, 132, 156, 180, 204, 228 and 252, or a substantially similar sequence thereof; a LCDR2 sequence selected from the group consisting of SEQ ID NO:14, 38, 62, 86, 110, 134, 158, 182, 206, 230 and 252, or a substantially similar sequence thereof; and a LCDR3 sequence selected from the group consisting of SEQ ID NO:16, 40, 64, 88, 112, 136, 160, 184, 208, 232 and 256 or a substantially similar sequences thereof. In a preferred instance, the antibody or antigen-binding fragment comprise HCDR sequences SEQ ID NO:148, 150 and 152 and LCDR sequences SEQ ID NO:156, 158 and 160; HCDR sequences SEQ ID NO:4, 6 and 8 and LCDR sequences SEQ ID NO:12, 14 and 16; and HCDR sequences SEQ ID NO:196, 198 and 200 and LCDR sequences SEQ ID NO:204, 206 and 208.

[0021] Also disclosed but not part of the invention are anti-hIL-4R antibodies, or antigen-binding fragments thereof, having HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences selected from the group consisting of: SEQ ID NOs: 148/150/152/156/158/160; 4/6/8/12/14/16; and 196/198/200/204/206/208. Exemplary antibodies having these HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences include the antibodies designated H4H098P (SEQ ID NOs:148/150/152/156/158/160), H4H083P (SEQ ID NOs:196/198/200/204/206/208), and H4H095P (SEQ ID NOs:4/6/8/12/14/16). In one embodiment, a vector or host cells of the present invention encodes the antibody designated

H4H098P (SEQ ID NOs:148/150/152/156/158/160).

[0022] Also disclosed but not part of the invention is a human antibody or antibody fragment comprising a HCDR3 and LCDR3, wherein the HCDR3 is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:7, 31, 55, 79, 103, 127, 151, 175, 199, 223 and 247, or a substantially identical sequence having at least 95% homology thereof; and the LCDR3 is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:15, 39, 63, 87, 111, 135, 159, 183, 207, 231 and 255, or a substantially identical sequence having at least 95% homology thereof.

[0023] Also disclosed but not part of the invention is a human antibody or antibody fragment comprising a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:3, 27, 51, 75, 99, 123, 147, 171, 195, 219 and 243, or a substantially identical sequence having at least 95% homology thereof; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:5, 29, 53, 77, 101, 125, 149, 173, 197, 221 and 245, or a substantially identical sequence having at least 95% homology thereof; a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 31, 55, 79, 103, 127, 151, 175, 199, 223 and 247, or a substantially similar sequence having at least 95% homology thereof; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:11, 35, 59, 83, 107, 131, 155, 179, 203, 227 and 251, or a substantially similar sequence having at least 95% homology thereof; a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:13, 37, 61, 85, 109, 133, 157, 181, 205, 229 and 253, or a substantially similar sequence having at least 95% homology thereof; and LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 39, 63, 87, 111, 135, 159, 183, 207, 231 and 255, or a substantially similar sequence having at least 95% homology thereof. In a preferred instance, the antibody or antigen-binding fragment comprise HCDR and LCDR sequences encoded by nucleotide sequences SEQ ID NO:147, 149, 151, 155, 157 and 159; 195, 197, 199, 203, 205 and 207; and 3, 5, 7, 11, 13 and 15.

[0024] In a specific instance, the anti-hIL-4R antibody or antigen-binding fragment thereof encoded by a vector or host cell of the present invention comprises HCVR comprising the amino acid sequence shown in SEQ ID NO:162 and LCVR comprising the amino acid sequence shown in SEQ ID NO:164, and is characterized by a K_D of about 100 pM or less (monomeric substrate) or 70 pM or less (dimeric substrate); a K_D of about 160 pM or less (monomeric substrate) or 40 pM or less (dimeric substrate) at 25°C and 37°C, respectively; and an IC_{50} of about 10 pM or less (25 pM dimer substrate) or about 100 pM or less (200 pM monomer substrate), which is capable of blocking both hIL-4 and hIL-13 activity with an IC_{50} of about 30 pM or less (as measured by bioassay) and cross-reacts with monkey IL-4R.

[0025] Also disclosed but not part of the invention is the anti-hIL-4R antibody or antigen-binding fragment thereof comprising HCVR comprising the amino acid sequence shown in SEQ ID NO:18 and LCVR comprising the amino acid sequence shown in SEQ ID NO:20, and which

is characterized by a K_D of about 450 pM or less (monomeric or dimeric substrate); and an IC_{50} of about 40 pM or less (25 pM dimer substrate) or about 100 pM or less (200 pM monomer substrate), which is capable of blocking both hIL-4 and hIL-13 activity with an IC_{50} of about 100 pM or less (as measured by bioassay).

[0026] Also disclosed is the anti-hIL-4R antibody or antigen-binding fragment thereof comprising HCVR comprising the amino acid sequence shown in SEQ ID NO:210 and LCVR comprising the amino acid sequence shown in SEQ ID NO:212, and which is characterized by a K_D of about 50 pM or less (monomeric substrate) or 30 pM or less (dimeric substrate); a K_D of about 200 pM or less (monomeric substrate) or 40 pM or less (dimeric substrate) at 25°C and 37°C, respectively; and an IC_{50} of about 10 pM or less (25 pM dimer substrate) or about 90 pM or less (200 pM monomer substrate), which is capable of blocking both hIL-4 and hIL-13 activity with an IC_{50} of about 25 pM or less (as measured by bioassay) and does not cross-reacts with monkey IL-4R.

[0027] Also disclosed but not part of the invention is an antibody or antigen-binding fragment of an antibody that specifically binds hIL-4R, comprising three heavy chain and three light chain complementarity determining regions (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3), wherein the HCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:265), wherein X^1 = Gly; X^2 = Phe; X^3 = Thr; X^4 = Phe; X^5 = Asp or Arg; X^6 =Asp or Ser; X^7 = Tyr; and X^8 = Ala or Gly; the HCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:266), wherein X^1 = Ile or Leu, X^2 = Ser, X^3 = Gly, Tyr or Arg, X^4= Ser, Asp or Thr, X^5 = Gly or Ser, X^6 = Gly, Ser or Val, X^7 = Ser or Asn, and X^8 = Thr, Lys or Ile; the HCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} (SEQ ID NO:267) wherein X^1 = Ala, X^2 = Lys, X^3 = Asp, Glu or Trp, X^4 = Gly or Arg, X^5 = Leu, Thr or Arg, X^6 = Gly, Arg or Ser, X^7 = Ile or Gly, X^8 = Thr, Phe or Tyr, X^9 = Ile, Asp or Phe, X^{10} = Arg, Tyr or Asp, X^{11} = Pro, Tyr or absent, X^{12} = Arg or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Gly or absent, X^{16} = Leu or absent, X^{17} = Asp or absent, and X^{18} = Val or absent; the LCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} (SEQ ID NO:268) wherein X^1 = Gln, X^2 = Asp, Ser or Val, X^3 = Ile or Leu, X^4 = Ser, Leu or Asn, X^5 = Asn, Tyr or Ile, X^6 = Trp, Ser or Tyr; X^7 = Ile or absent; X^8 = Gly or absent; X^9 = Tyr or absent; X^{10} = Asn or absent; and X^{11} = Tyr or absent; the LCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:269) wherein X^1 = Leu, Ala or Val, X^2 = Ala or Gly, and X^3 = Ser; and the LCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 (SEQ ID NO:270) wherein X^1 = Gin or Met, X^2 = Gln, X^3 = Ala or Tyr, X^4 = Leu or Asn, X^5 = Gln or Ser, X^6 = Thr, Phe or His, X^7 = Pro, X^8 = Tyr, Ile or Trp, and X^9 = Thr.

[0028] In a more specific instance, the HCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:265), wherein X^1 = Gly; X^2 = Phe; X^3 = Thr ; X^4 = Phe; X^5 = Arg; X^6 =Asp or Ser; X^7 = Tyr; and X^8 = Ala or Gly; the HCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:266), wherein X^1 = Ile, X^2 = Ser, X^3 = Gly or Tyr, X^4= Ser or Thr, X^5 = Gly, X^6 = Gly or Ser, X^7 = Asn, and X^8 = Thr or Lys; the HCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} (SEQ ID NO:267) wherein X^1 = Ala, X^2 = Lys, X^3 = Asp or Glu, X^4 = Gly or Arg, X^5 = Leu or Arg, X^6 = Gly or Ser, X^7 = Ile or Gly, X^8 = Thr or Phe, X^9 = Ile or Asp, X^{10} = Arg or Tyr, X^{11} = Pro or absent, X^{12} = Arg or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Gly or absent, X^{16} = Leu or absent, X^{17} = Asp or absent, and X^{18} = Val or absent; the LCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} (SEQ ID NO:268) wherein X^1 = Gln, X^2 = Ser or Val, X^3 = Ile or Leu, X^4 = Leu or Asn, X^5 = Asn or Tyr, X^6 = Ser or Tyr; X^7 = Ile or absent; X^8 = Gly or absent; X^9 = Tyr or absent; X^{10} = Asn or absent; and X^{11} = Tyr or absent; the LCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:269) wherein X^1 = Leu or Ala, X^2 = Ala or Gly, and X^3 = Ser; and the LCDR3 comprises an amino acid sequence of the formula X^1 X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 (SEQ ID NO:270) wherein X^1 = Gln or Met, X^2 = Gln, X^3 = Ala or Tyr, X^4 = Leu or Asn, X^5 = Gln or Ser, X^6 = Thr or His, X^7 = Pro, X^8 = Tyr or Trp, and X^9 = Thr.

[0029] In another more specific instance, the HCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:265), wherein X^1 = Gly; X^2 = Phe; X^3 = Thr ; X^4 = Phe; X^5 = Asp or Arg; X^6 =Asp; X^7 = Tyr; and X^8 = Ala; the HCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:266), wherein X^1 = lie or Leu, X^2 = Ser, X^3 = Gly or Arg, X^4= Ser or Thr, X^5 = Gly or Ser, X^6 = Gly or Val, X^7 = Ser or Asn, and X^8 = Thr or Ile; the HCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17}-X^{18} (SEQ ID NO:267) wherein X^1 = Ala, X^2 = Lys, X^3 = Asp or Trp, X^4 = Gly or Arg, X^5 = Leu or Thr, X^6 = Arg or Ser, X^7 = lie or Gly, X^8 = Thr or Tyr, X^9 = lie or Phe, X^{10} = Arg or Asp, X^{11} = Pro, Tyr or absent, X^{12} = Arg or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Gly or absent, X^{16} = Leu or absent, X^{17} = Asp or absent, and X^{18} = Val or absent; the LCDR1 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6- X^7 - X^8 - X^9 - X^{10} - X^{11} (SEQ ID NO:268) wherein X^1 = Gln, X^2 = Asp or Ser, X^3 = lie or Leu, X^4 = Ser or Leu, X^5 = Tyr or Ile, X^6 = Trp or Ser; X^7 = lie or absent; X^8 = Gly or absent; X^9 = Tyr or absent; X^{10} = Asn or absent; and X^{11} = Tyr or absent; the LCDR2 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:269) wherein X^1 = Leu or Val, X^2 = Ala or Gly, and X^3 = Ser; and the LCDR3

comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 (SEQ ID NO:270) wherein X^1 = Gln or Met, X^2 = Gln, X^3 = Ala, X^4 = Leu or Asn, X^5 = Gln or Ser, X^6 = Thr or Phe, X^7 = Pro, X^8 = Tyr or Ile, and X^9 = Thr.

[0030] Also disclosed but not part of the invention is an antibody or antigen-binding fragment comprising HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences from a HCVR and LCVR pair, wherein the HCVR/LCVR sequences are selected from the group consisting of SEQ ID NO:162/164, 210/212 and 18/20. The heavy and light chain CDR sequences in the antibody or antigen-binding fragment thereof encoded by a vector or host cell of the present invention are those contained in HCVR SEQ ID NO:162 and LCVR SEQ ID NO:164. In another more specific instance, heavy and light chain CDR sequences are those contained in HCVR SEQ ID NO:18 and LCVR SEQ ID NO:20. In yet another specific instance, heavy and light chain CDR sequences are those contained in HCVR SEQ ID NO:210 and LCVR SEQ ID NO:212.

[0031] The invention encompasses the encoded anti-hIL-4R antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of a galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0032] The invention provides recombinant expression vectors as claimed, and host cells into which such vectors have been included, methods of making the antibodies or antigen-binding fragments obtained by culturing the host cells of the invention are also provided. A host cell of the present invention may be a prokaryotic or eukaryotic cell, preferably the host cell is an E. *coli* cell or a mammalian cell, such as a CHO cell.

[0033] Also disclosed is a composition comprising a recombinant human antibody that specifically binds hIL-4R and an acceptable carrier.

[0034] Also disclosed but not part of the invention are methods for inhibiting hIL-4 activity using an antibody, or antigen-binding portion thereof, of the disclosure. In specific instances, the antibodies of the disclosure also block hIL-13/hIL-13R1 complex binding to hIL-4R. In one instances, the method comprises contacting hIL-4R with the antibody of the disclosure, or antigen-binding portion thereof, such that hIL-4 or hIL-4/hIL-13 activity is inhibited. In another instance, the method comprises administering an antibody of the disclosure, or antigen-binding portion thereof, to a human subject suffering from a disorder that is ameliorated by inhibition of hIL-4 or hIL-4/hIL-13 activity. The disorder treated is any disease or condition that is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of hIL-4 or hIL-4/hIL-13 activity.

[0035] IL-4 related disorders which are treated though not part of the invention by the antibodies or antibody fragments of the disclosure include, for example, arthritis (including

septic arthritis), herpetiformis, chronic idiopathic urticaria, scleroderma, hypertrophic scarring, Whipple's Disease, benign prostate hyperplasia, lung disorders, such as mild, moderate or severe asthma, inflammatory disorders such as inflammatory bowel disease, allergic reactions, Kawasaki disease, sickle cell disease, Churg-Strauss syndrome, Grave's disease, pre-eclampsia, Sjogren's syndrome, autoimmune lymphoproliferative syndrome, autoimmune hemolytic anemia, Barrett's esophagus, autoimmune uveitis, tuberculosis, and nephrosis.

[0036] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0037] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0039] The term "human IL4R" (hIL-4R), as used herein, is intended to refer to a human cytokine receptor that specifically binds interleukin-4 (IL-4), IL-4Rα (SEQ ID NO:274). The term "human interleukin-13" (hIL-13) refers to a cytokine that specifically binds IL-13 receptor, and "hIL-13/hIL-13R1 complex" refers to the complex formed by hIL-13 binding to hIL-13R1 complex, which complex binds hIL-4 receptor to initiate biological activity.

[0040] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of

three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0041] The term "antigen-binding portion" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hIL-4R). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL1 and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two F(ab)' fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) Nature 241:544-546), which consists of a VH domain; and (vi) an CDR. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single contiguous chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883. Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed (see e.g., Holliger et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448).

[0042] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to hIL-4R results in inhibition of the biological activity of hIL-4 and/or hIL-13. This inhibition of the biological activity of hIL-4 and/or IL-13 can be assessed by measuring one or more indicators of hIL-4 and/or hIL-13 biological activity known to the art, such as hIL-4- and/or IL-13-induced cellular activation and hIL-4 binding to hIL-4R (see examples below).

[0043] A "CDR" or complementarity determining region is a region of hypervariability interspersed within regions that are more conserved, termed "framework regions" (FR). In different embodiments of the anti-hIL-4R antibody or fragment of the disclosure, the FRs may be identical to the human germline sequences, or may be naturally or artificially modified.

[0044] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB).

[0045] The term "epitope" is an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different

segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sufonyl groups on the antigen.

[0046] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below.

[0047] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalaninetyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0048] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide

sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the disclosure to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, e.g., Altschul et al. (1990) J. Mol. Biol. 215:403-410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389-402.

Preparation of Human Antibodies

[0049] Methods for generating human antibodies include those described in, for example, US 6,596,541, Green et al. (1994) Nature Genetics 7:13-21), US 5,545,807, US 6,787,637.

[0050] Rodents can be immunized by any method known in the art (see, for example, Harlow and Lane (1988) Antibodies: A Laboratory Manual 1988 Cold Spring Harbor Laboratory; Malik and Lillehoj (1994) Antibody Techniques, Academic Press, CA). Antibodies of the disclosure are preferably prepared with the use of VELOCIMMUNE™ technology (US 6,596,541). A transgenic mouse in which the endogenous immunoglobulin heavy and light chain variable regions are replaced with the corresponding human variable regions is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable regions of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0051] The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody. In a specific embodiment, the cell is a CHO cell.

[0052] Antibodies may be therapeutically useful in blocking a ligand-receptor interaction or inhibiting receptor component interaction, rather than by killing cells through fixation of complement (complement-dependent cytotoxicity) (CDC) and participation antibody-dependent cell-mediated cytotoxicity (ADCC). The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0053] Human immunoglobulins can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four-chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via interchain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification. The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. In fact, a single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30: 105) to levels typically observed using a human IgG1 hinge. The instant disclosure encompasses antibodies having one or more mutations in the hinge, CH2 or CH3 region that may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0054] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As described below, the antibodies are characterized and selected for desirable characteristics, including binding affinity to hIL-4R, ability to block hIL-4 binding to hIL-4R, and/or selectivity for the human protein. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the disclosure, for example wild-type or modified IgG4 or IgG1 (for example, SEQ ID NO:271, 272, 273). While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Epitope Mapping and Related Technologies

[0055] To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described in Harlow and Lane *supra* can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science: 9:487-496).

[0056] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US Patent Application Publication No. 2004/0101920). Each category may reflect a unique epitope either distinctly different from, or partially overlapping with, an epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones with desired characteristics. MAP may be

used to sort the hIL-4R antibodies of the disclosure into groups of antibodies binding different epitopes.

[0057] Agents useful for altering the structure of the immobilized antigen are enzymes, such as, for example, proteolytic enzymes and chemical agents. The antigen protein may be immobilized on either biosensor chip surfaces or polystyrene beads. The latter can be processed with, for example, an assay such as a multiplex LUMINEX™ detection assay (Luminex Corp., TX). Because of the capacity of LUMINEX™ to handle multiplex analysis with up to 100 different types of beads, LUMINEX™ provides almost unlimited antigen surfaces with various modifications, resulting in improved resolution in antibody epitope profiling over a biosensor assay.

Bispecifics

[0058] The antibodies of the present disclosure may be monospecific, bispecific, or multispecific. Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al. (1991) J. Immunol. 147:60-69. The human anti-IL-4R antibodies can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment, to produce a bispecific or a multispecific antibody with a second binding specificity.

Therapeutic Administration and Formulations

[0059] Also disclosed but not part of the invention are therapeutic compositions comprising the anti-IL-4R antibodies or antigen-binding fragments thereof of the present disclosure. The administration of therapeutic compositions in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0060] The dose may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present disclosure is used for treating various conditions and diseases associated with IL-4R, in an adult patient, it is advantageous to intravenously administer the antibody of the present disclosure normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted.

[0061] Various delivery systems are known and can be used to administer the pharmaceutical composition of the disclosure, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0062] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533; Treat et al. (1989) in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.

[0063] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one instance, a pump may be used (see Langer, supra; Sefton (1987) CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974). In yet another instance, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984). Other controlled release systems are discussed in the review by Langer (1990) Science 249:1527-1533.

[0064] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50

(polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0065] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to 100 mg and in about 10 to 250 mg for the other dosage forms.

[0066] Single and combination therapies. The antibodies and antibody fragments of the disclosure are useful for treating diseases and disorders which are improved, inhibited or ameliorated by reducing IL-4 activity. These disorders include those characterized by abnormal or excess expression of IL-4, or by an abnormal host response to IL-4 production. IL-4 related disorders which are treated by the antibodies or antibody fragments of the include, for example, arthritis (including septic arthritis), herpetiformis, chronic idiopathic urticaria, scleroderma, hypertrophic scarring, Whipple's Disease, benign prostate hyperplasia, pulmonary disorders such as asthma (mild, moderate or severe), inflammatory disorders such as inflammatory bowel disease, allergic reactions, Kawasaki disease, sickle cell disease, Churg-Strauss syndrome, Grave's disease, pre-eclampsia, Sjogren's syndrome, autoimmune lymphoproliferative syndrome, autoimmune hemolytic anemia, Barrett's esophagus, autoimmune uveitis, tuberculosis, atopic dermatatis, ulcerative colitis, fibrosis, and nephrosis (see U.S. 7,186,809).

[0067] The disclosure encompasses combination therapies in which the anti-IL-4R antibody or antibody fragment is administered in combination with a second therapeutic agent. Coadministration and combination therapy are not limited to simultaneous administration, but include treatment regimens in which an anti-IL-4R antibody or antibody fragment is administered at least once during a course of treatment that involves administering at least one other therapeutic agent to the patient. A second therapeutic agent may be another IL-4 antagonist, such as another antibody/antibody fragment, or a soluble cytokine receptor, an IgE antagonist, an anti-asthma medication (corticosteroids, non-steroidal agents, beta agonists, leukotriene antagonists, xanthines, fluticasone, salmeterol, albuterol) which may be delivered by inhalation or other appropriate means. In a specific instance, the anti-IL-4R antibody or antibody fragment of the disclosure may be administered with an IL-1 antagonist, such as rilonacept, or an IL-13 antagonist. The second agent may include one or more leukotriene receptor antagonists to treat disorders such as allergic inflammatory diseases, e.g., asthma and allergies. Examples of leukotriene receptor antagonists include but are not limited to montelukast, pranlukast, and zafirlukast. The second agent may include a cytokine inhibitor such as one or more of a TNF (etanercept, ENBREL™), IL-9, IL-5 or IL-17 antagonist.

[0068] The present disclosure though not part of the invention also includes the use of any anti-IL-4R antibody or antigen binding fragment described herein in the manufacture of a medicament for the treatment of a disease or disorder, wherein the disease or disorder is improved, ameliorated or inhibited by removal, inhibition or reduction of human interleukin-4 (hIL-4) activity. Examples of such diseases or disorders include, e.g., arthritis, Dermatitis herpetiformis, chronic idiopathic urticaria, scleroderma, hypertrophic scarring, Whipple's Disease, benign prostate hyperplasia, lung disorders, asthma, inflammatory disorders, allergic reactions, Kawasaki disease, sickle cell disease, Churg-Strauss syndrome, Grave's disease, pre-eclampsia, Sjogren's syndrome, autoimmune lymphoproliferative syndrome, autoimmune hemolytic anemia, Barrett's esophagus, autoimmune uveitis, tuberculosis, nephrosis, atopic dermatitis and asthma.

EXAMPLES

[0069] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to Human IL-4 Receptor.

[0070] VELOCIMMUNE™ mice (Regeneron Pharmaceuticals, Inc.; US 6,596,541) were immunized with human IL-4R (hIL-4R, SEQ ID NO:274) or a combination of hIL-4R and monkey (*Macaca fascicularis*) IL-4R (mfIL-4R, SEQ ID NO:275) protein or DNA. To obtain optimal immune response, animals were subsequently boosted every 3-4 weeks and bleeds obtained 10 days after each boost for assessment of progression of anti-antigen response.

[0071] When the mice attained maximum immune response, antibody-expressing B cells were harvested and fused with mouse myeloma cells to form hybridomas. Alternatively, antigen-specific antibodies were isolated directly from the B cells without fusion to myeloma cells, as described in U.S. Patent Publication 2007/0280945A1. Stable recombinant antibody-expressing CHO cell lines were established from the isolated proper recombinants. Functionally desirable monoclonal antibodies were selected by screening conditioned media of the hybridomas or transfected cells for specificity, antigen-binding affinity, and potency in blocking hIL-4 binding to hIL-4R (described below).

[0072] Several anti-hIL-4R antibodies were obtained by the foregoing methods including the

exemplary antibodies designated H4H083P, H4H094P and H4H095P, H4H098P and H4H099P. These exemplary anti-hIL-4R antibodies, and their biological properties, are described in greater detail in the following Examples.

Example 2. Antigen Binding Affinity Determination.

[0073] Binding affinity (K_D) of selected antibodies with respect to hIL-4R at either 25°C or 37°C was determined using a real-time biosensor surface plasmon resonance assay (BIACORE™ 2000). Briefly, antibody was captured on a goat anti-hFc polyclonal antibody surface created through direct coupling to a BIACORE™ chip to form a captured antibody surface. Various concentrations (ranging from 50 nM to 12.5 nM) of monomeric hIL-4R (R&D Systems) or dimeric hIL-4R-mFc were injected over the captured antibody surface at 10 µl/min for 2.5 min at either 25°C or 37°C. Binding of antigen to antibody and dissociation of the bound complex, were monitored in real time. Equilibrium dissociation constants (K_D) and dissociation rate constants were ascertained by performing kinetic analysis using BIA evaluation software. BIA evaluation software was also used to calculate the half-life of antigen/antibody complex dissociation ($T_{1/2}$). Results are shown in Table 1. NB: No antibody-antigen binding was observed under the experimental condition. Control: a fully human anti-IL-4R antibody (U.S. Patent No. 7,186, 809; SEQ ID NOs:10 and 12).

Table 1

Antibody	25°C				37°C			
	Monomeric		Dimeric		Monomeric		Dimeric	
	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)
Control	1100	18	94	186	3970	4	114	158
H4H083P	48	361	28	245	183	87	38.1	163
H4H094P	NB	-	NB	-	NB	-	NB	-
H4H095P	274	131	302	156	437	49	314	116
H4H098P	94.1	243	67.6	237	157	129	38.8	158
H4H099P	NB	-	NB	-	NB	-	NB	-

[0074] Binding affinity (K_D) of selected antibodies with respect to monkey (*Macaca fascicularis*) IL-4R (mfIL-4R) at either 25°C or 37°C was also determined using a real-time biosensor surface plasmon resonance assay described above with various concentrations (ranging from 100 nM to 25 nM) of monomeric mfIL-4R-myc-myc-his (mfIL-4R-mmh) or dimeric mfIL-4R-mFc. Only antibody H4H098P was able to bind both monomeric and dimeric mfIL-4R at 25°C with K_D of 552 nM and 9.08 nM, respectively. In addition, antibody H4H098P also binds to dimeric mfIL-4R at 37°C with a K_D of 24.3 nM. H4H083P had very weak binding to dimeric

mfIL-4R.

[0075] Antibody-antigen binding affinity was also assessed using an ELISA-based solution competition assay. Briefly, a 96-well MAXISORP™ plate was first coated with 5 µg/ml avidin overnight followed by BSA blocking for 1 hr. The avidin-coated plate was then incubated with 250 ng/ml biotin-hIL4 for 2 hr. The plate was used to measure either free hIL-4R-mFc (dimeric hIL-4R) or free hIL-4R-myc-myc-his (hIL4R-mmh, monomeric hIL4R) in the antibody titration sample solutions. To make the antibody titration sample, a constant amount either 25 pM of hIL-4R-mFc or 200 pM of hIL-4R-mmh was premixed with varied amounts of antibody, ranging from 0 to about 10 nM in serial dilutions, followed by 1 hr incubation at room temperature to allow antibody-antigen-binding to reach equilibrium. The equilibrated sample solutions were then transferred to the hIL-4 coated plates for measurement of either free hIL-4R-mFc or free hIL-4R-mmh. After 1 hr binding, the plate was washed and bound hIL-4R-mFc was detected using either an HRP-conjugated mouse anti-mFc polyclonal antibody or an HRP-conjugated goat anti-myc polyclonal antibodies. IC_{50} values were determined (Table 2).

Table 2

Antibody	**IC_{50} (pM)**	
	25 pM hIL-4R-mFc	**200 pM hIL-4R-mmh**
Control	8.2	87
H4H083P	9.6	80
H4H094P	>10,000	>10,000
H4H095P	40	90
H4H098P	8.8	74
H4H099P	>10,000	>10,000

[0076] The ELISA-based solution competition assay was also used to determine the cross reactivity of the antibodies to monkey IL-4R. Antibody H4H098P exhibits an IC_{50} for mfIL-4R-mFc of 300 pM and an IC_{50} for mfIL-4R-mmh of 20 nM.

Example 3. Neutralization of Biological Effect of hIL-4 and hIL-13 *In Vitro*

[0077] A bioassay was developed to determine the ability of purified anti-hIL-4R antibodies to neutralize hIL-4R-mediated cellular function *in vitro* using an engineered HK293 cell line that contains human STAT6 and a STAT6 luciferase reporter. Inhibition of hIL-4R-inducible luciferase activity was determined as follows: Cells were seeded onto 96-well plates at 1×10^4 cells/well in media and incubated overnight at 37°C, 5% CO_2. Antibody proteins ranging from 0 to 20 nM in serial dilutions were added to the cells along with either 10 pM hIL-4 or 40 pM of hIL-13. Cells were then incubated at 37°C, 5% CO_2 for 6 hrs. The extent of cellular response

was measured in a luciferase assay (Promega Biotech). Results are shown in Table 3. NB: Luciferase activity was not blocked under the experimental condition described above. In addition, H4H098P was able to block mfIL-4R-mediated cellular function in the presence of 360 fM mfIL-4 with an IC_{50} of 150 nM.

Table 3

Antibody	IC_{50} (pM)	
	10 pM hIL-4	**40 pM hIL-13**
Control	47	38
H4H083P	25	19
H4H094P	NB	NB
H4H095P	98	86
H4H098P	27	25
H4H099P	NB	11,000

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US5599905A [0002]
- US5767065A [0002]
- US5840869A [0002]
- US5717072A [0002]
- US7186809B [0002] [0066]
- US6949245B [0003]
- WO9402602A [0003]
- US6596541B [0003] [0049] [0050] [0070]
- US5714146A [0004]
- US5985280A [0004]

- US6716587B [0004]
- WO2008054606A2 [0005]
- WO0192340A2 [0005]
- WO2005047331A2 [0005]
- US5545807A [0049]
- US6787637B [0049]
- US20040101920 [0056]
- US20070280945A1 [0071]
- US7186809A [0073]

Non-patent literature cited in the description

- **REDDY et al.**J. Immunol., 2000, vol. 164, 1925-1933 [0011]
- **SHIELD et al.**JBC, 2002, vol. 277, 26733- [0031]
- **WARD et al.**Nature, 1989, vol. 241, 544-546 [0041]
- **BIRD et al.**Science, 1988, vol. 242, 423-426 [0041]
- **HUSTON et al.**Proc. Natl. Acad. Sci. USA, 1988, vol. 85, 5879-5883 [0041]
- **HOLLIGER et al.**Proc. Natl. Acad Sci. USA, 1993, vol. 90, 6444-6448 [0041]
- **PEARSON**Methods Mol. Biol., 1994, vol. 24, 307-331 [0047]
- **GONNET et al.**Science, 1992, vol. 256, 1443-1445 [0047]
- **ALTSCHUL et al.**J. Mol. Biol., 1990, vol. 215, 403-410 [0048]
- **ALTSCHUL et al.**Nucleic Acids Res., 1997, vol. 25, 3389-402 [0048]
- **GREEN et al.**Nature Genetics, 1994, vol. 7, 13-21 [0049]
- **HARLOWLANE**Antibodies: A Laboratory ManualCold Spring Harbor Laboratory19880000 [0050]
- **MALIKLILLEHOJ**Antibody TechniquesAcademic Press19940000 [0050]
- **ANGAL et al.**Molecular Immunology, 1993, vol. 30, 105- [0053]
- **REINEKE**Methods Mol Biol, 2004, vol. 248, 443-63 [0055]
- **TOMER**Protein Science, 2000, vol. 9, 487-496 [0055]
- **TUTT et al.**J. Immunol., 1991, vol. 147, 60-69 [0058]
- Remington's Pharmaceutical SciencesMack Publishing Company [0059]
- **POWELL et al.**Compendium of excipients for parenteral formulationsJ Pharm Sci Technol, 1998, vol. 52, 238-311 [0059]
- **WU et al.**J. Biol. Chem., 1987, vol. 262, 4429-4432 [0061]
- **LANGER**Science, 1990, vol. 249, 1527-1533 [0062] [0063]
- **TREAT et al.**Liposomes in the Therapy of Infectious Disease and Cancer19890000353-365 [0062]
- **SEFTON**CRC Crit. Ref. Biomed. Eng., 1987, vol. 14, 201- [0063]
- Medical Applications of Controlled ReleaseCRC Pres.19740000 [0063]
- **GOODSON**Medical Applications of Controlled Release, 1984, vol. 2, 115-138 [0063]

PATENTKRAV

1. Rekombinant ekspressionsvektor, der omfatter:

et første nukleinsyremolekyle omfattende en nukleinsyresekvens, der koder for et variabelt tungkædeområde (HCVR) af et antistof, der binder human interleukin-4 receptor (IL-4R), hvor nukleotidsekvensen, der koder for HCVR, har mindst 95 % homologi med SEQ ID NO: 161, hvor det HCVR, der kodes for, omfatter aminosyresekvensen ifølge SEQ ID NO:162; og

et andet nukleinsyremolekyle omfattende en nukleinsyresekvens, der koder for det variable letkædeområde (LCVR) af et antistof, der binder human IL-4R, hvor nukleotidsekvensen, der koder for LCVR, har mindst 95 % homologi med SEQ ID NO: 163, hvor det LCVR, der kodes for, omfatter aminosyresekvensen ifølge SEQ ID NO: 164.

2. Rekombinant ekspressionsvektor ifølge krav 1, hvor nukleinsyresekvensen, der koder for HCVR, omfatter SEQ ID NO: 161.

3. Rekombinant ekspressionsvektor ifølge krav 1, hvor nukleinsyresekvensen, der koder for LCVR, omfatter SEQ ID NO: 163.

4. Værtscelle, der omfatter den rekombinante ekspressionsvektor ifølge et hvilket som helst af krav 1 til 3.

5. Værtscelle, der omfatter:

- et isoleret nukleinsyremolekyle omfattende en nukleotidsekvens, der koder for et HCVR omfattende aminosyresekvensen ifølge SEQ ID NO: 162 operativt bundet til DNA, der koder for et humant, konstant tungkædeområde, hvor det HCVR, der kodes for, omfatter aminosyresekvensen ifølge SEQ ID NO:162; og

- et isoleret nukleinsyremolekyle omfattende en nukleotidsekvens, der koder for et LCVR omfattende aminosyresekvensen ifølge SEQ ID NO:164 operativt bundet til DNA, der koder for et humant, konstant letkædeområde, hvor det LCVR, der kodes for, omfatter aminosyresekvensen ifølge SEQ ID NO: 164,

hvor cellen udtrykker et fuldt humant antistof, der binder human interleukin-4-receptor (IL-4R).

6. Værtscelle ifølge krav 4 eller 5, hvor værtscellen er en pattedyrscelle.

7. Værtscelle ifølge krav 6, hvor værtscellen er er en kinesisk hamsterovarie- (CHO) celle.

8. Fremgangsmåde til fremstilling af et antistof eller antigenbindende fragment deraf, der specifikt binder human IL-4R, hvilken fremgangsmåde omfatter dyrkning af værtscellen ifølge et hvilket som helst af krav 4 til 7 under forhold, hvor antistoffet eller det antigenbindende fragment udtrykkes, og genvinding af det fulde, humane antistof.

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.