HIGH AFFINITY HUMAN ANTIBODIES TO HUMAN IL-4 RECEPTOR

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:1) 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.

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[0004] 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 (Abgenix) and US 6,596,541 (Regeneron Pharmaceuticals) (both of which publications are herein specifically incorporated by reference) describing methods of generating nonhuman transgenic mice capable of producing human antibodies.

[0005] Methods for using antibodies to hIL-4R are described in U.S. Patent Nos. 5,714,146; 5,985,280; and 6,716,587.

BRIEF SUMMARY OF THE INVENTION

[0006] In a first aspect, the invention provides 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).

[0007] In one embodiment, the invention provides an antibody or antigen-binding fragment thereof, that specifically binds hIL-4R (SEQ ID NO:1) with a K_D of about 200 pM or less, as

measured by surface plasmon resonance. In a more specific embodiment, the antibody or antigen-binding portion thereof exhibits a K_D of less than about 150 pM, or less than about 50 pM, or less than about 20 pM. In various embodiments, the antibody or antigen-binding fragment blocks hIL-4 activity with an IC_{50} of about 200 pM or less, as measured by STAT6 luciferase bioassay. In more specific embodiments, the antibody or antigen-binding fragment exhibits an IC_{50} of about 150 pM or less, or about 100 pM or less, or even about 50 pM or less as measured by 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, as measured by STAT6 luciferase bioassay. In more specific embodiments, the antibody or antigen-binding fragment exhibits an IC_{50} of about 75 pM or less, or about 50 pM or less, or even about 20 pM or less.

[0008] In a second aspect, the antibody of the invention comprises a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, 483, 487, 491, 495, 499, 503, 507, 511, 515, 519, 523, 527, 531, 535, 539, 543, 547, 551, 555, 559, 563, 567, 571, 575, 579 and 581, or a substantially similar sequence thereof.

[0009] In a third aspect, the antibody of the invention comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO:11, 27, 43, 59, 75, 91, 107, 123, 130, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 389, 393, 397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, 461, 465, 469, 473, 477, 481, 485, 489, 493, 497, 501, 505, 509, 513, 517, 521, 625, 529, 533, 537, 541, 545, 549, 553, 557, 561, 565, 569, 573, and 577, or a substantially similar sequence thereof.

[0010] In one embodiment, the antibody or antibody fragment of the invention comprises a HCVR and a LCVR (HCVR/LCVR) selected from the group consisting of SEQ ID NO: 3/11, 19/27, 35/43, 51/59, 67/75, 83/91, 99/107, 115/123, 131/139, 147/155, 163/171, 179/187, 195/203, 211/219, 227/235, 243/251, 259/267, 275/283, 291/299, 307/315, 323/331, 339/347, 355/363, 371/379, 387/389, 391/393, 395/397, 399/401, 403/405, 407/409, 411/413, 415/417, 419/421, 423/425, 427/429, 431/433, 435/437, 439/441, 443/445, 447/449, 451/453, 455/457, 459/461, 463/465, 467/469, 471/473, 475/477, 479/481, 483/485, 487/489, 491/493, 495/497, 499/501, 503/505, 507/509, 511/513, 515/517, 519/521, 523/525, 527/529, 531/533, 535/537, 539/541, 543/545, 547/549, 551.553, 555/557, 559/561, 563/565, 567/569, 571/573, 575/577, 579/59 and 581/59. In a preferred embodiment, the antibody or antibody fragment comprise HCVR/LCVR selected from SEQ ID NO: 51/59, 259/267, 275/283, 291/299, 579/59 or 581/59.

[0011] In a fourth aspect, the invention provides nucleic acid molecules encoding an HCVR, wherein the nucleic acid molecule is a nucleotide sequence selected from the group consisting of SEQ ID NO:2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 390, 394, 398, 402, 406, 410, 414, 418, 422, 426, 430, 434, 438, 442, 446, 450, 454, 458, 462, 466, 470, 474, 478, 482, 486, 490, 494, 498, 502, 506, 510,

514, 518, 522, 526, 530, 534, 538, 542, 546, 550, 554, 558, 562, 566, 570, 574, 578 and 580, or a substantially identical sequence having at least 95% homology thereof.

[0012] In a fifth aspect, the invention provides nucleic acid molecules encoding a LCVR, wherein the nucleic acid molecule is a sequence selected from the group consisting of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 129, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 388, 392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 444, 448, 452, 456, 460, 464, 468, 472, 476, 480, 484, 488, 492, 496, 500, 504, 508, 512, 516, 520, 524, 528, 532, 536, 540, 544, 548, 552, 556, 560, 564, 568, 572, and 576, or a substantially identical sequence having at least 95% homology thereof.

[0013] In one embodiment, the antibody of the invention comprises a HCVR and LCVR encoded by a nucleotide sequence pairs selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, 370/378, 386/388, 390/392, 394/396, 398/400, 402/404, 406/408, 410/412, 414/416, 418/420, 422/424, 426/428, 430/432, 434/436, 438/440, 442/444, 446/448, 450/452, 454/456, 458/460, 462/464, 466/468, 470/472, 474/476, 478/480, 482/484, 486/488, 490/492, 494/496, 498/500, 502/504, 506/508, 510/512, 514/516, 518/520, 522/524, 526/528, 530/532, 534/536, 538/540, 542/544, 546/548, 550/552, 554/556, 558/560, 562/564, 566/568, 570/572, 574/576, 578/59 and 580/59. In a preferred embodiment, the antibody or antibody fragment comprise HCVR/LCVR encoded by nucleotide sequences selected from SEQ ID NO: 50/58, 258/266, 274/282, 290/298, 578/58 or 580/58.

[0014] In a sixth aspect, the invention features an antibody or antigen-binding fragment of an antibody that specifically binds hIL-4R, comprising three heavy chain and three light chain complementary determining regions (CDR), wherein

the heavy chain CDR1 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:582), wherein X^1 = Gly; X^2 = Tyr or Phe; X^3 = Thr or I; X^4 = Phe; X^5 = Asn or Arg; X^6 =Ser; X^7 = Tyr; and X^8 = Gly;

the heavy chain CDR2 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:583), wherein X^1 = Ile, X^2 = Ser or Arg, X^3 = Thr or Tyr, X^4= Tyr or Asp, X^5 = Asn or Gly, X^6 = Gly or Ser, X^7 = Lys or Asn, and X^8 = Thr;

the heavy chain CDR3 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19} - X^{20} - X^{21}$ (SEQ ID NO:584) wherein X^1 = Ala or Val, X^2 = Arg or Lys, X^3 = Asp or Glu, X^4 = Gly or Glu, X^5 = Ala or Arg, X^6 = Arg or Ser, X^7 = Ile or Gly, X^8 = Val or Ser, X^9 = Val or Trp, X^{10} = Ala or Phe, X^{11} = Gly or Asp, X^{12} = Thr or Pro, X^{13} = Thr or absent, X^{14} = Pro or absent, X^{15} = Tyr or absent, X^{16} = Tyr or absent, X^{17} = Tyr or absent, X^{18} = Gly or absent, X^{19} = Met or absent, X^{20} = Asp or absent, and X^{21} = Val or absent;

the light chain CDR1 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 -$

$X^5 - X^6$ (SEQ ID NO:585) wherein X^1 = Gln, X^2 = Asp or Ala, X^3 = Ile, X^4 = Ser or Asn, X^5 = Asn or Ile, and X^6 = Trp or Phe;

the light chain CDR2 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3$ (SEQ ID NO:586) wherein X^1 = Ala or Val, X^2 = Ala or Thr, and X^3 = Ser; and

the light chain CDR3 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:587) wherein X^1 = Gln, X^2 = Gln, X^3 = Ala or Tyr, X^4 = Asn, X^5 = Ser, X^6 = Phe or His, X^7 = Pro, X^8 = Ile or Trp, and X^9 = Thr.

[0015] In a seventh aspect, the invention provides an antibody or antigen-binding fragment comprising a heavy chain CDR3 and a light chain CDR3, wherein the heavy chain CDR3 domain selected from the group consisting of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361 and 377; and the light chain CDR3 domain selected from the group consisting of SEQ ID NO:17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369 and 385. In a preferred embodiment, the heavy chain and light chain CDR3s are selected from the sequence pairs SEQ ID NO:57 and 65; 265 and 273; 281 and 289; and 297 and 305.

[0016] In a further embodiment, the invention features a human antibody or antibody fragment comprising a heavy chain CDR1 domain selected from the group consisting of SEQ ID NO:5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341, 357 and 373, or a substantially similar sequence thereof; a heavy chain CDR2 domain selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, 311, 327, 343, 359 and 375, or a substantially similar sequence thereof; a heavy chain CDR3 domain selected from the group consisting of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361 and 377, or a substantially similar sequence thereof; a light chain CDR1 domain selected from the group consisting of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365 and 381, or a substantially similar sequence thereof; a light chain CDR2 domain selected from the group consisting of SEQ ID NO:15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367 and 383, or a substantially similar sequence thereof; and a light chain CDR3 domain selected from the group consisting of SEQ ID NO:17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369 and 385, or a substantially similar sequences thereof. In a preferred embodiment, the antibody or antigen-binding fragment comprise heavy chain CDRs SEQ ID NO:53, 55, 57 and light chain CDRs SEQ ID NO:61, 63, 65; heavy chain CDRs SEQ ID NO: 261, 263, 265 and light chain CDRs SEQ ID NO:269, 271, 273; heavy chain CDRs SEQ ID NO: 277, 279, 281 and light chain CDRs SEQ ID NO:285, 287, 289; and heavy chain CDRs SEQ ID NO: 293, 295, 297 and light chain CDRs SEQ ID NO:301, 303, 305.

[0017] In an eighth aspect, the invention provides an antibody or antigen-binding fragment

comprising three CDRs from a HCVR and three CDRs from a LCVR, wherein the HCVR/LCVR are selected from the group consisting of 51/59, 579/59, 581/59, 259/267, 275/283, and 291/299.

[0018] In one embodiment, the invention features a human antibody or antibody fragment comprising a heavy chain and a light chain CDR3, wherein the heavy chain CDR3 is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360 and 376; and a light chain CDR3 is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368 and 384.

[0019] In a further embodiment, the invention features a human antibody or antibody fragment comprising a heavy chain CDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356 and 372, or a substantially identical sequence having at least 95% homology thereof; a heavy chain CDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358 and 374, or a substantially identical sequence having at least 95% homology thereof; a heavy chain CDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360 and 376, or a substantially similar sequence having at least 95% homology thereof; a light chain CDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, and 380, or a substantially similar sequence having at least 95% homology thereof; a light chain CDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366 and 382, or a substantially similar sequence having at least 95% homology thereof; and a light chain CDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368 and 384, or a substantially similar sequence having at least 95% homology thereof. In a preferred embodiment, the antibody or antigen-binding fragment comprise heavy chain CDRs encoded by nucleotide sequences SEQ ID NO:52, 54, 56 and light chain CDRs encoded by nucleotide sequences SEQ ID NO:60, 62, 64; heavy chain CDRs encoded by nucleotide sequences SEQ ID NO: 260, 262, 264 and light chain CDRs encoded by nucleotide sequences SEQ ID NO:268, 270, 272; heavy chain CDRs encoded by nucleotide sequences SEQ ID NO: 276, 278, 280 and light chain CDRs encoded by nucleotide sequences SEQ ID NO:284, 286, 288; and heavy chain CDRs encoded by nucleotide sequences SEQ ID NO: 292, 294, 296 and light chain CDRs

encoded by nucleotide sequences SEQ ID NO:300, 302, 304.

[0020] The invention encompasses 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).

[0021] In a ninth aspect, the invention provides recombinant expression vectors carrying the nucleic acid molecules of the invention, and host cells into which such vectors have been included, as are methods of making the antibodies or antigen-binding fragments of the invention obtained by culturing the host cells of the invention. The host cell 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.

[0022] In a tenth aspect, the invention features a composition comprising a recombinant human antibody that specifically binds hIL-4R and an acceptable carrier.

[0023] In an eleventh aspect, the invention features methods for inhibiting hIL-4 activity using an antibody, or antigen-binding portion thereof, of the invention. In specific embodiments, the antibodies of the invention also block hIL-13/hIL-13R1 complex binding to hIL-4R. In one embodiment, the method comprises contacting hIL-4R with the antibody of the invention, or antigen-binding portion thereof, such that hIL-4 or hIL-4/hIL-13 activity is inhibited. In another embodiment, the method comprises administering an antibody of the invention, 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.

[0024] Further encompassed by the invention is the use of an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 in the manufacture of a medicament for use to attenuate or inhibit an IL-4-mediated disease or disorder in a human.

[0025] IL-4-mediated or related disorders which are treated by the antibodies or antibody fragments of the invention 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.

[0026] Other objects and advantages will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF FIGURES

[0027] Fig. 1(A-C). Antibody binding profile assessment generated with OCTET™-based sequential binding assays. Fig. 1(A) is a bar graph showing results obtained when the first antibody exposed to bound antigen is the control antibody. Fig. 1(B) the first antibody exposed to bound antigen is VAB16F3-1. Fig. 1(C) the first antibody exposed to bound antigen is VAK5H4-4. Second antibody: 1= control; 2=VX4E7-9; 3= VX3F7-6; 4=VAB16G-1;5= VAB16F3-1; 6=VAB15C8-17; 7= VAB11G8-1; 8= VAB10C1-5; 9= VAB10G8-19; 10=VAB8G10-1; 11=VAB7B9-3; 12=VAB6C10-14; 13= VAB5C5-11; 14=VAB3B4-10; 15=VAB4D5-3; 16= VAB1H1-2; 17= VAK5H4-4; 18= VAK7G8-5; 19=VAK8G11-13; 20= VAK9C6-11; 21= VAK10G6-7; 22=VAK11D4-1; 23=VAK12B11-9; and 24=VAK10G12-5. Open bars show binding level of the first antibody, filled bars show additional binding by a second antibody.

DETAILED DESCRIPTION

[0028] 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.

[0029] 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

[0030] 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:1). 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.

[0031] 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 inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as 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 (abbreviated herein as 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 complementary 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.

[0032] 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 isolated complementary determining region (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).

[0033] 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).

[0034] A "CDR" or complementary 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 invention, the FRs may be identical to the human germline sequences, or may be naturally or artificially modified.

[0035] 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).

[0036] 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.

[0037] 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.

[0038] 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, phenylalanine-tyrosine, 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 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0039] 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 invention 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

[0040] Methods for generating human antibodies include, for example, VelocImmune™ (Regeneron Pharmaceuticals), XenoMouse™ technology (Green et al. (1994) Nature Genetics 7:13-21; Abgenix), the "minilocus" approach, and phage display (and see, for example, US 5,545,807, US 6,787,637). The VelocImmune™ technology (US 6, 596,541) encompasses a method of generating a high specificity fully human antibody to a select antigen.

[0041] Rodents can be immunized by any method known in the art (see, for example, Harlow and Lane (1988) *supra*; Malik and Lillehoj (1994) Antibody techniques, Academic Press, CA). In a preferred embodiment, hIL-4R antigen is administered directly to mice that comprise DNA loci encoding both a human Ig heavy chain variable region and a Kappa light chain variable region (VelocImmune™, Regeneron Pharmaceuticals, Inc.; US 6,596,541), with an adjuvant to stimulate the immune response, for example, complete and incomplete Freund's adjuvant, MPL+TDM adjuvant system (Sigma), or RIBI (muramyl dipeptides) (see O'Hagan (2000) Vaccine Adjuvant, Human Press, NJ). An adjuvant can prevent rapid dispersal of polypeptide by sequestering the antigen in a local depot, and may contain factors that can stimulate host immune response. VelocImmune™ technology involves generating a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. 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.

[0042] 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.

[0043] 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 invention 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.

[0044] Antibodies of the invention are preferably prepared with the use of VelocImmune™ technology. 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.

[0045] In one embodiment, the transgenic mouse comprises up to 18 functional human variable heavy chain genes and 12 functional human variable kappa light chain genes. In another embodiment, the transgenic mouse comprises up to 39 human variable heavy chain genes and 30 human variable kappa light chain genes. In yet another embodiment, the transgenic mouse comprises up to 80 human variable heavy chain genes and 40 human variable kappa light chain genes.

[0046] In general, the antibodies of the instant invention possess very high affinities, typically

possessing K_Ds of from about 10^{-9} through about 10^{-12} M, when measured by binding to antigen either immobilized on a solid phase or measured in solution.

[0047] 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 invention, for example wild-type or modified IgG4 or IgG1 (for example, SEQ ID NO:588, 589, 590). 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

[0048] To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described in *Antibodies: A Laboratory Manual* 1988 Cold Spring Harbor Laboratory, Harlow and Lane, eds. 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).

[0049] 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 invention into groups of antibodies binding different epitopes.

[0050] 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.

Therapeutic Administration and Formulations

[0051] Administration of therapeutic entities in accordance with the invention can be achieved 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: The Science and Practice of Pharmacy (2003, 20th ed, Lippincott Williams & Wilkins). 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. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

[0052] The therapeutic molecules of the invention may be administered to a patient in a manner appropriate to the indication, for example, parenterally, topically, or by inhalation. If injected, the antagonist can be administered, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes, by bolus injection, or continuous infusion. Localized administration at a site of disease or injury is contemplated, as are transdermal delivery and sustained release from implants. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebulizer, inhalation of the antagonist in aerosol form, and the like. Other alternatives include eyedrops; oral preparations including pills, syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, and ointments.

[0053] Specific dosages and the frequency of administration may vary according to such factors as the route of administration, the nature and severity of the disease to be treated, whether the condition is acute or chronic, and the size and general condition of the patient. Appropriate dosages can be determined by procedures known in the pertinent art, e.g. in clinical trials that may involve dose escalation studies. The therapeutic molecules of the invention may be once, or repeatedly. In particular embodiments, the antibody or antibody fragment is administered over a period of at least a month or more, e.g., for one, two, or three months or even indefinitely. For treating chronic conditions, long-term treatment is generally most effective. However, for treating acute conditions, administration for shorter periods, e.g. from one to six weeks, may be sufficient. In general, the therapeutic is administered until the patient manifests a medically relevant degree of improvement over baseline for the chosen indicator or indicators. The level of IL-4 may be monitored during and/or after treatment with the therapeutic molecule of the invention. Methods for measuring IL-4 serum levels are known in the art, for example by

ELISA, etc.

Therapeutic Use and Combination Therapies

[0054] The antibodies and antibody fragments of the invention 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).

[0055] The invention encompasses combination therapies in which the anti-IL-4R antibody or antibody fragment is administered in combination with a second therapeutic agent. Co-administration 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 embodiment, the anti-IL-4R antibody or antibody fragment of the invention 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.

EXAMPLES

[0056] 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.

[0057] Mice comprising DNA loci encoding both a human Ig heavy chain variable region and a kappa light chain variable region (VelocImmune™, Regeneron Pharmaceuticals, Inc.; US Patent No. 6,596,541) were immunized with human IL-4R (hIL-4R, SEQ ID NO:1). The hIL-4R was administered by direct injection of purified antigen in an adjuvant or indirectly by providing the DNA sequence of hIL-4R in a DNA plasmid that contains the hIL-4R gene and expresses hIL-4R using the host's cellular protein expression machinery to produce antigen polypeptide *in vivo*. To obtain optimal immune response, animals were subsequently boosted every 3-4 weeks and bleeds obtained 10 after each boost for assessment of progression of anti-antigen response.

[0058] When the mice attained maximum immune response, antibody-expressing B cells were harvested and fused with mouse myeloma cells to form hybridomas. 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).

[0059] Selected antibodies that displayed desirable antigen binding affinity, potency, and/or ability to block hIL-4 binding to hIL-4R included (HCVR/LCVR): VX 4E7-9 (SEQ ID NO:3/11; 387/389; 391/393); VX 3F7-6 (SEQ ID NO:19/27; 395/397; 399/401); VAB 16G1-1 (SEQ ID NO:35/43; 403/405; 407/409); VAB 16F3-1 (SEQ ID NO:51/59, 411/413, 415/417, 579/59 and 581/59); VAB 15C8-17 (SEQ ID NO:67/75, 419/421, 423/425); VAB 13A1-6 (SEQ ID NO:83/91, 427/429, 431/433); VAB 11G8-1 (SEQ ID NO:99/107, 435/437, 439/441); VAB 10G8-19 (SEQ ID NO:115/123, 443/445, 447/449); VAB 10C1-5 (SEQ ID NO:131/139, 451/453, 455/457); VAB 8G10-1 (SEQ ID NO:147/155, 459/461, 463/465); VAB 7B9-3 (SEQ ID NO:163/171, 467/469, 471/473); VAB 6C10-14 (SEQ ID NO:179/187, 475/477, 479/481); VAB 5C5-11 (SEQ ID NO:195/203, 483/485, 487/489); VAB 3B4-10 (SEQ ID NO:211/219, 491/493, 495/497); VAB 4D5-3 (SEQ ID NO:227/235, 499/501, 503/505); VAB 1H1-2 (SEQ ID NO:243/251, 507/509, 511/513); VAK5H4-4 (SEQ ID NO:259/267, 515/517, 519/521); VAK7G8-5 (SEQ ID NO:275/283, 523/525, 527/529); VAK8G11-13 (SEQ ID NO:291/299, 531/533, 535/537); VAK9C6-11 (SEQ ID NO:307/315, 539/541, 543/545); VAK10G6-7 (SEQ ID NO:323/331, 547/549, 551/553); VAK11D4-1 (SEQ ID NO:339/347, 555/557, 559/561); VAK12B11-9 (SEQ ID NO:355/363, 563/565, 567/569); and VAK10G12-5 (SEQ ID NO:371/379, 571/573, 575/577).

Example 2. Antigen Binding Affinity Determination.

[0060] Binding affinity (K_D) of selected antibodies with respect to hIL-4R was determined using a real-time biosensor surface plasmon resonance assay (BIAcore™ 2000). Briefly, antibody

was captured on a goat anti-mouse (GAM) IgG polyclonal antibody surface created through direct chemical coupling of the GAM IgG to a BIAcore™ chip to form a captured antibody surface. Various concentrations (ranging from 12.5 nM to 0.625 nM) of monomeric hIL-4R (R&D Systems) or dimeric hIL-4R-hFc were injected over the captured antibody surface. 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 I. Control: a fully human anti-IL-4R antibody (U.S. Patent No. 7,186, 809; SEQ ID NOs:10 and 12). The results of the kinetic analyses revealed that the selected antibodies included high affinity antibodies that were capable of binding to dimeric receptor with K_Ds under 1 nM. The binding affinity of anti-hIL-4R antibodies to either mouse or monkey (*Macaca fascicularis*) IL-4R was also tested. The selected antibodies (1) did not cross-react with mouse IL-4R; and (2) either failed to bind monkey IL-4R or bound monkey IL-4R with very low affinity.

Table 1

Antibody	K_D dimeric (pM)	$T_{1/2}$ dimeric (hr)	K_D Monomeric (nM)	$T_{1/2}$ monomeric (min)
VX 4E7-9	111	19.1	1.43	48
VX 3F7-6	654	2.7	3.91	16
VAB 8G10-1	5,510	4.1	7.71	28
VAB 7B9-3	52	6.1	1.01	13
VAB 6C10-14	286	6.2	1.77	73
VAB 5C5-11	27	7.7	0.13	47
VAB 4D5-3	32	20.4	2.02	35
VAB 3B4-10	53	20.4	0.59	102
VAB 1H1-2	139	8.2	34.90	8
VAB 16G1-1	38	7.3	0.93	15
VAB 16F3-1	3	41	0.02	254
VAB 15C8-17	45	3.6	0.82	15
VAB 11G8-1	108	8.7	1.63	25
VAB 10G8-19	52	2	11.40	2
VAB 10C1-5	51	6.7	4.47	6
VAK8G11-13	2	63	0.03	840
VAK5H4-4	4	30	0.02	960
VAK10G6-7	11	24	0.23	144
VAK9C6-11	68	7	2.05	28

VAK7G8-5	9	25	0.06	480
VAK11D4-1	6	21	0.05	480
VAK12B11-9	36	12	0.41	120
VAK10G12-5	7	46	0.09	360
Control	71	2.1	1.23	16

[0061] Antibody-antigen binding affinity was also assessed using an ELISA-based solution competition assay. Briefly, antibodies (purified proteins at 1 or 3.3 ng/ml) were premixed with serial dilutions of antigen protein (monomeric or dimeric) ranging from 0 to 10 μg/ml. Solutions of the antibody and antigen mixture were then incubated for two to four hours at room temperature to reach binding equilibrium. Free antibody in the mixtures was then measured using a quantitative sandwich ELISA. Briefly, 96-well Maxisorp™ plates (VWR, West Chester, PA) were coated with 2 μg/ml hIL-4R-hFc protein in PBS overnight at 4° C followed by blocking nonspecific binding with BSA. The antibody-antigen mixture solutions were then transferred to the coated Maxisorb™ plates followed by a one-hour incubation. The plates were then washed with washing buffer and plate-bound antibodies were detected with an HRP-conjugated goat anti-mouse IgG polyclonal antibody reagent (Jackson ImmunoResearch) or an HRP-conjugated goat anti-human IgG polyclonal antibody reagent (Jackson ImmunoResearch) for control antibody and developed using colorimetric substrates such as BD OptEIA™ (BD Biosciences Pharmingen, San Diego, CA). After the reaction was stopped with 1 M phosphoric or sulfuric acid, absorbances at 450 nm were recorded and the data were analyzed using GraphPad™ Prism software. The dependency of the signals on the concentrations of antigen in solution was analyzed with a four parameter fit analysis and reported as IC_{50}, the antigen concentration required to achieve 50% reduction of the signal from the antibody samples without the presence of antigen in solution. The IC_{50}s were determined as described above and are summarized in Table 2.. In a preferred embodiment, the antibody or antibody fragment of the invention exhibits an IC_{50} for dimeric hIL-4R of about 20 pM or less, and an IC_{50} for monomeric hIL-4R of about 150 pM or less, or about 100 pM or less, as measured by ELISA solution competition assay.

Table 2

Antibody	IC_{50} Dimeric hIL4R (pM)	IC_{50} Monomeric hIL4R (pM)
VX 4E7-9	94	175
VX 3F7-6	184	2,900
VAB 8G10-1	85	517
VAB 7B9-3	7	2,980
VAB 6C10-14	203	489

VAB 5C5-11	6	5,220
VAB 4D5-3	35	285
VAB 3B4-10	88	336
VAB 1H1-2	70	779
VAB 16G1-1	9	595
VAB 16F3-1	5	7
VAB 15C8-17	9	418
VAB 11G8-1	76	473
VAB 10G8-19	4	89
VAB 10C1-5	12	12,900
VAK 5H4-4	18	130
VAK 7G8-5	18	92
VAK 8G11-13	15	103
VAK 9C6-11	46	1,400
VAK 10G6-7	25	165
VAK 11D4-1	23	109
VAK 12B11-9	64	325
VAK 10G12-5	41	169
Control	14	3,500

Example 3. Inhibition of hIL-4 and hIL-4R Interaction

[0062] The ability of the selected antibodies to block hIL-4 binding to hIL-4R were screened by BIAcore™ hIL-4 blocking assay and potency was measured by a quantitative hIL-4 blocking immunoassay as described below. Results are summarized in Table 3.

[0063] The ability of the antibodies to block hIL-4 binding to the hIL-4R receptor was determined using surface plasmon resonance. Purified hIL-4R-hFc molecules were captured by goat anti-human IgG polyclonal antibody immobilized on CM-5 to a density of 260 RU, to prepare a receptor-coated surface. Human IL-4 (0.25 ml at 50 nM) was then injected over the receptor-coated surface and the amount of bound hIL-4 was recorded (first injection of hIL-4). The bound hIL-4 was then removed with a pulse of 3 M $MgCl_2$ followed by conditioning buffer. Purified anti-hIL4R antibodies were then injected over the receptor surface followed by a second injection of hIL-4 at the same concentration (second injection of hIL-4). The percent reduction in hIL-4 binding resulting from preformed antibody and receptor complex is reported in Table 3.

[0064] To further evaluate ability to block hIL-4 binding to hIL-4R, a quantitative immunoassay was performed. Briefly, solutions of 25 pM hIL-4R-Fc were premixed with antibody protein ranging from ~ 50 nM to 0 nM in serial dilutions, followed by a one hour incubation at room temperature. Concentration of free hIL-4R-Fc (no antibody bound) was determined using a hIL-

4R-specific sandwich ELISA. Detection plates for hIL-4R were prepared by binding biotinylated hIL-4 (0.5 µg/ml) on streptavidin-coated 96-well plates. Pre-bound antibody-antigen samples were transferred to the hIL-4-coated detection plate. After a one-hour incubation at room temperature, the detection plate was washed and plate-bound hIL-4R-hFc was detected using HRP conjugated goat anti-hFc polyclonal antibodies and developed using colorimetric substrates such as BD OptEIA™ (BD Biosciences Pharmingen, San Diego, CA). After the reaction was stopped with 1 M phosphoric or sulfuric acid, absorbances at 450 nm were recorded and the data were analyzed using GraphPad™ Prism software. IC_{50}s were determined as the amount of antibody required to reduce 50% of IL-4R-hFc detectable to plate bound hIL-4. In a preferred embodiment, the antibody or antibody fragment of the invention exhibits an IC_{50} for blocking 25 pM hIL-4R of less than about 50 pM, or less than about 40 pM, or less than about 30 pM, or less than about 20 pM, as measured by ELISA.

Table 3

Antibody	% Inhibition (BIAcore™)	IC_{50} (ELISA pM)
VX 4E7-9	79	118
VX 3F7-6	86	274
VAB 8G10-1	74	244
VAB 7B9-3	96	59
VAB 6C10-14	79	441
VAB 5C5-11	96	24
VAB 4D5-3	82	240
VAB 3B4-10	72	322
VAB 1H1-2	78	146
VAB 16G1-1	92	18
VAB 16F3-1	97	19
VAB 15C8-17	97	29
VAB 11G8-1	77	240
VAB 10G8-19	85	18
VAB 10C1-5	93	34
VAK 5H4-4	96	33
VAK 7G8-5	95	27
VAK 8G11-13	95	26
VAK 9C6-11	96	67
VAK 10G6-7	95	37
VAK 11D4-1	95	35
VAK 12B11-9	96	99

VAK 10G12-5	94	59

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

[0065] The IL-4-mediated signal transduction pathway has been documented extensively in the literature (for example, see review of Hebenstreit et al. 2006 Cytokine Growth Factor Rev.17(3):173-88, 2006). IL-4 can stimulate two receptor complexes, type I and type II. Type I receptor complexes are formed by the binding of IL-4 to IL-4R and the subsequent heterodimerization with the common gamma chain. Alternatively, IL4/IL4R complex can heterodimerize with the IL-13 receptor 1 to form type II receptor complexes. Both type I and type II complexes signal mainly through STAT6. Therefore, the ability of the selected antibodies to block signaling through STAT6 was assessed as described below.

[0066] A cell line with high sensitivity to hIL-4 and hIL-13 was established. HEK293 cells were stably transfected with human STAT6 and a STAT6 luciferase reporter plasmid, and was maintained in growth media (DMEM, 10% FBS, L-glutamine, penicillin, streptomycin). A robust IL-4 receptor-mediated response was achieved when 10 pM hIL-4 was added to the growth medium of the STAT6-transfected HEK293 cells. For bioassay of the hIL-4 response, cells were washed once in assay media (Optimem I (Gibco) plus 0.1% FBS) and plated at 1 x 10^4 cells/well (96 well plate) in 80 µl of assay media. Purified antibodies were serially diluted into assay media (final concentrations ranging from 20 nM to 0) and 10 µl of each of the test antibody was added to the cells along with 10 µl of hIL-4 (10 pM constant final concentration). 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 4.

[0067] Inhibition of IL-4-dependent biological activity *in vitro* was also confirmed using either a human erythroblast cell line, TF1, or a modified, Il-13Rα overexpressing TF1 cell line (TF1/A12). In this bioassay, 20,000 cells were seeded in each well of a 96-well plate in RPMI 1640 medium containing 10% FBS, 2mM L-glutamine, and penicillin and streptomycin. Twenty-five µl of purified antibodies ranging from 0 to 50 nM (final concentration) were added together with 25 µl of hIL-4 recombinant protein to a final concentration of either 50 pM for cell line TF1 or 20 pM for cell line TF1/A12. Cells were then allowed to grow for 3 days at 37°C and final cell counts were measured using a CCK8 kit (Dojindo, Japan). Ability of antibody to block TF-1 cell growth is reported in Table 4 as the concentration of antibody required to achieve 50% reduction of cell proliferation.

Table 4

Antibody	STAT6 Bioassay IC_{50} (nM)	TF1 Bioassay IC_{50} (nM)	TF1/A12 Bioassay IC_{50} (nM)
VX 4E7-9	7.90		10
VX 3F7-6	>10		>50
VAB 8G10-1	>10		>50

VAB 7B9-3	3.40		8.20
VAB 6C10-14	>10		23
VAB 5C5-11	0.14	0.21	0.37
VAB 4D5-3	3.80		21
VAB 3B4-10	>10		>50
VAB 1H1-2	>10		>50
VAB 16G1-1	0.14	0.12	0.70
VAB 16F3-1	0.032	0.046	0.067
VAB 15C8-17	0.32	0.44	4.70
VAB 11G8-1	>10		>50
VAB 10G8-19	1.80		12.8
VAB 10C1-5	1.90		22.8
VAK 5H4-4	0.132	0.150	0.182
VAK 7G8-5	0.06	0.13	
VAK 8G11-13	0.08	0.16	
VAK 9C6-11	0.48	0.31	
VAK 10G6-7	0.11	0.23	
VAK 11D4-1	0.20	0.20	
VAK 12B11-9	0.49	0.57	
VAK 10G12-5	0.36	0.58	

Example 5. Neutralization of Biological Effect of hIL-13 *In Vitro*

[0068] Since IL-4R has been shown to be a modulator for IL-13 activity through its binding to IL-13/IL-13R complex, the selected antibodies were tested for their ability to block IL-13 activity in a modification of the HEK293 STAT6 luciferase assay described above, with the modification being replacement of 10 pM IL-4 with 40 pM hIL-13. The antibodies were also assessed for potency in blocking hIL-13 activity in the TF-1 cell line assay described above, with hIL-13 at 150 pM in the presence of 0-50 nM antibodies. Results are shown in Table 5.

Table 5

Antibody	STAT6 Bioassay IC_{50} (nM)	TF1 Bioassay IC_{50} (nM)
VX 4E7-9	6.30	
VX 3F7-6	>10	
VAB 8G10-1	>10	
VAB 7B9-3	1.10	
VAB 6C10-14	>10	
VAB 5C5-11	0.06	0.82

VAB 4D5-3	3.30	
VAB 3B4-10	8.30	
VAB 1H1-2	>10	
VAB 16G1-1	0.09	0.44
VAB 16F3-1	0.018	0.079
VAB 15C8-17	0.20	2.60
VAB 11G8-1	8.80	
VAB 10G8-19	0.97	
VAB 10C1-5	1.00	
VAK 5H4-4	0.084	0.240
VAK 7G8-5	0.04	0.36
VAK 8G11-13	0.06	0.42
VAK 9C6-11	0.30	1.00
VAK 10G6-7	0.07	0.46
VAK 11D4-1	0.14	0.44
VAK 12B11-9	0.37	1.60
VAK 10G12-5	0.26	1.90

Example 6. Antibody Binding Profile Assessment

[0069] An antibody binding profile can be established by determining the effect that an antibody (bound to its antigen) can have on the ability of a panel of different antibodies to subsequent bind the same antigen. For example, the antigen can be immobilized on a support to form an antigen-coated surface, the antigen-coated surface can be saturated with the antibody, and then the antibody-saturated antigen-coated surface can be exposed to a panel of other antibodies. The extent of binding of the panel of other antibodies to the antibody-saturated antigen surface provides an antibody binding profile. OCTET™-based sequential binding assays were employed to generate an antibody binding profile. Briefly, a group of 24 strepavidin High Binding FA Biosensors (ForteBio, Inc., Menlo Park, CA) were first incubated with antigen, biotin-hIL-4R-hFc, at 2 μg/ml for 10 min at 30°C to achieve saturation and the amount of bound antigen was measured as a change in thickness (nm) of the biological layer due to bound protein, which is directly measured by the wavelength shift. Biotin-hIL-4R-hFc-bound biosensors were then incubated with the first antibody (control antibody) at 50 μg/ml for 15 min at 30°C to achieve saturation and the amount of bound control antibody was measured as a change in thickness (nm) of the biological layer. Each control antibody-bound sensor was then incubated with one of a panel of 24 different anti-hIL-4R antibodies (second antibody) at 50 μg/ml for 15 min at 30°C, and the amount of second antibody bound was measured as a change in thickness of the biological layer. The same assay was repeated using either

VAB16F3-1 or VAK5H4-4 anti-hIL-4R antibody as the first antibody. Results are shown in Fig. 1A-C.

[0070] Selected anti-hIL-4R antibodies were also assessed by Western blot (data not shown). Briefly, hIL-4R monomer (200 ng per lane) and mfIL-4R His-tagged monomer (200 ng per lane) were electrophoresed on SDS-PAGE gels using both reducing and non-reducing sample buffer. Four separate gels were each transferred to a PVDF membrane and each membrane was exposed to one of four primary antibodies: anti-His mAb (Qiagen), VAB 16F3-1, VAK 5H4-4, or control anti-hIL-4R antibody, with either HRP-conjugated goat anti-mIgG or anti-hIgG (Pierce) as secondary antibody. All three anti-hIL-4R antibodies recognized the non-reduced form of hIL-4R. Only VAB 16F3-1 and VAK 5H4-4 detected the reduced form of hIL-4R. None of the anti-hIL-4R antibodies detected monkey IL-4R.

Claims:

1. An antibody or antigen-binding fragment thereof, that specifically binds hIL-4R (SEQ ID NO:1) with a K_D of about 200 pM or less, preferably about 150 pM or less, as measured by surface plasmon resonance.

2. An antibody or antigen-binding fragment according to claim 1, comprising

a heavy chain variable region (HCVR) selected from SEQ ID NO:3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, 483, 487, 491, 495, 499, 503, 507, 511, 515, 519, 523, 527, 531, 535, 539, 543, 547, 551, 555, 559, 563, 567, 571, 575, 579 and 581; and

a light chain variable region (LCVR) selected from SEQ ID NO:11, 27, 43, 59, 75, 91, 107, 123, 130, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 389, 393, 397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, 461, 465, 469, 473, 477, 481, 485, 489, 493, 497, 501, 505, 509, 513, 517, 521, 625, 529, 533, 537, 541, 545, 549, 553, 557, 561, 565, 569, 573, and 577.

3. An antibody or antigen-binding according to claim 1 or 2, comprising HCVR/LCVR pairs selected from SEQ ID NO: 3/11, 19/27, 35/43, 51/59, 67/75, 83/91, 99/107, 115/123, 131/139, 147/155, 163/171, 179/187, 195/203, 211/219, 227/235, 243/251, 259/267, 275/283, 291/299, 307/315, 323/331, 339/347, 355/363, 371/379, 387/389, 391/393, 395/397, 399/401, 403/405, 407/409, 411/413, 415/417, 419/421, 423/425, 427/429, 431/433, 435/437, 439/441, 443/445, 447/449, 451/453, 455/457, 459/461, 463/465, 467/469, 471/473, 475/477, 479/481, 483/485, 487/489, 491/493, 495/497, 499/501, 503/505, 507/509, 511/513, 515/517, 519/521, 523/525, 527/529, 531/533, 535/537, 539/541, 543/545, 547/549, 551.553, 555/557, 559/561, 563/565, 567/569, 571/573, 575/577, 579/59 and 581/59.

4. An antibody or antigen-binding fragment according to claim 3, preferably comprising HCVR/LCVR selected from SEQ ID NO:51/59, 259/267, 275/283, 291/299, 579/59 or 581/59.

5. An antibody or antigen-binding fragment according to claim 1, comprising

a heavy chain CDR1 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:582), wherein X^1 = Gly, X^2 = Tyr or Phe, X^3 = Thr or I, X^4 = Phe, X^5 = Asn or Arg, X^6 =Ser, X^7 = Tyr, and X^8 = Gly;

a heavy chain CDR2 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:583), wherein X^1 = Ile, X^2 = Ser or Arg, X^3 = Thr or Tyr, X^4= Tyr or Asp, X^5 = Asn or Gly, X^6 = Gly or Ser, X^7 = Lys or Asn, and X^8 = Thr;

a heavy chain CDR3 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19} - X^{20} - X^{21}$ (SEQ ID NO:584) wherein X^1 = Ala or Val, X^2 = Arg or Lys, X^3 = Asp or Glu, X^4 = Gly or Glu, X^5 = Ala or Arg, X^6 = Arg or Ser, X^7 = Ile or Gly, X^8 = Val or Ser, X^9 = Val or Trp, X^{10} = Ala or Phe, X^{11} = Gly or Asp, X^{12} = Thr or Pro, X^{13} = Thr or absent, X^{14} = Pro or absent, X^{15} = Tyr or absent, X^{16} = Tyr or absent, X^{17} = Tyr or absent, X^{18} = Gly or absent, X^{19} = Met or absent, X^{20} = Asp or absent, and X^{21} = Val or absent;

a light chain CDR1 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6$ (SEQ ID NO:585) wherein X^1 = Gln, X^2 = Asp or Ala, X^3 = Ile, X^4 = Ser or Asn, X^5 = Asn or Ile, and X^6 = Trp or Phe;

a light chain CDR2 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3$ (SEQ IDNO:586) wherein X^1 = Ala or Val, X^2 = Ala or Thr, and X^3 = Ser; and

a light chain CDR3 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:587) wherein X^1 = Gln, X^2 = Gln, X^3 = Ala or Tyr, X^4 = Asn, X^5 = Ser, X^6 = Phe or His, X^7 = Pro, X^8 = Ile or Trp, and X^9 = Thr.

6. An antibody or antigen-binding fragment according to claim 1, comprising a heavy chain CDR3 and a light chain CDR3,

wherein the heavy chain CDR3 domain selected from the group consisting of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361 and 377; and

the light chain CDR3 domain selected from the group consisting of SEQ ID NO:17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369 and 385.

7. An antibody or antigen-binding fragment according to claim 6, further comprising

a heavy chain CDR1 domain selected from the group consisting of SEQ ID NO:5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341, 357 and 373;

a heavy chain CDR2 domain selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, 311, 327, 343, 359 and 375;

a light chain CDR1 domain selected from the group consisting of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365 and 381; and

a light chain CDR2 domain selected from the group consisting of SEQ ID NO:15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367 and 383.

8. An antibody or antigen-binding fragment according to claim 1, comprising CDRs from a HCVR and a LCVR sequence, wherein the HCVR/LCVR sequences are selected from the group consisting of 51/59, 579/59, 581/59, 259/267, 275/283, and 291/299.

9. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment according to any one of the preceding claims.

10. A vector comprising the nucleic acid sequence according to claim 9.

11. A host-vector system for the production of an antibody or antigen-binding fragment of an antibody which specifically binds IL-4 receptor, comprising a vector according to claim 10, in a suitable host cell.

12. A host-vector system according to claim 11, wherein the host cell is a prokaryotic or eukaryotic cell selected from one of *E. coli* or a CHO cell.

13. A method of producing an anti-IL-4R antibody or antigen-binding fragment thereof, comprising growing cells of a host-vector system according to claim 11 or 12 under conditions permissing production of the antibody or fragment thereof and recovering the antibody or fragment so expressed.

14. Use of an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 in the manufacture of a medicament for use to attenuate or inhibit an IL-4-mediated disease or disorder in a human.

15. A method of treating 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, by administration of an antibody or antigen-binding fragment according to any one of claims 1 to 9.

16. A method according to claim 15, wherein the disease or disorder is selected from arthritis, herpetiformis, chronic idiopathic urticaria, scleroderma, hypertrophic scarring, Whipple's Disease, benign prostate hyperplasia, lung disorders, 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, and nephrosis.