HIGH AFFINITY HUMAN ANTIBODIES TO HUMAN PROTEASE-ACTIVATED RECEPTOR-2

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for protease-activated receptor-2 (PAR-2).

BACKGROUND

[0002] Protease-activated receptors ("PARs") are a family of seven-transmembrane G-protein-coupled receptors. Among seven-transmembrane G-protein-coupled receptors PARs have a unique mode of activation; that is, PARs are activated by proteolytic cleavage at the amino terminus to generate a new N-terminal domain which serves as a "tethered ligand." The tethered ligand interacts with the extracellular loop-2 of the receptor thereby resulting in receptor activation. Currently, there are four known members of the PAR family, designated PAR-1, PAR-2, PAR-3 and PAR-4.

[0003] PAR-2 has also been referred to as "C140." (US 5,874,400). Both human and murine PAR-2 share the protease cleavage domain SKGRSLIG (residues 6-13 of SEQ ID NO:852, and residues 8-15 of SEQ ID NO:856). This sequence is cleaved between the R and S residues by a variety of proteases such as trypsin, as well as by mast cell tryptase, tissue factor/factor VIIa complex and factor Xa, neutrophil proteinase 3 (PR-3), human leukocyte elastase, and proteases originating from pathogenic organisms.

[0004] PAR-2 activity has been implicated in or associated with several diseases and conditions including inflammatory diseases, pain, gastrointestinal conditions, neurological diseases, and cardiovascular disorders (see, e.g., Linder et al., 2000, J. Immunol. 165:6504-6510; Vergnolle et al., 2001, Nature Medicine 7:821-826; Cenac et al., 2007, J. Clin. Investigation 117:636-647; Vergnolle, 2004, British J. Pharmacol. 141:1264-1274; Knight et al., 2001, J. Allergy Clin. Immunol. 108:797-803; Schmidlin et al., 2002, J. Immunol. 169:5315-5321). Antibodies that bind to PAR-2 have the potential to antagonize the activity of PAR-2 *in vivo*. Anti-PAR-2 antibodies are therefore potentially useful for treating and/or ameliorating a variety of disease conditions.

[0005] Antibodies that bind to PAR-2, and certain therapeutic uses thereof, are mentioned in US 5,874,400, US 2007/0237759, WO 2009/005726, and US 2010/0119506. Nonetheless, there remains a need in the art for novel PAR-2 modulating agents, including anti-PAR-2 antibodies, that can be used to treat PAR-2-mediated diseases and conditions.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides human antibodies that bind to human PAR-2. The antibodies of the invention are useful, *inter alia*, for inhibiting PAR-2-mediated signaling and for treating diseases and disorders caused by or related to PAR-2 activation.

[0007] The present invention includes antibodies which interact with the N-terminal region of PAR-2 and block proteolytic cleavage at the activating PAR-2 protease cleavage site (as defined herein) but do not block proteolytic cleavage at one or more non-activating protease cleavage sites. According to certain embodiments, anti-PAR-2 antibodies which exhibit such proteolytic cleavage blocking properties interact with specific amino acids in the vicinity of the activating PAR-2 protease cleavage site. For example, the present invention provides anti-PAR-2 antibodies with protease cleavage blocking activity and which interact with Val-42 and Asp-43 of human PAR-2 (SEQ ID NO:851), and may further interact with one or more human PAR-2 residues selected from the group consisting of Ser-37, Leu-38, Ile-39, Gly-40, and Gly-44.

[0008] According to other embodiments, the anti-PAR-2 antibodies of the present invention specifically bind to human PAR-2 and monkey PAR-2 but do not bind to at least one member selected from the group consisting of mouse, rat, rabbit, dog and pig PAR-2. The present invention also includes antibodies that are capable of inhibiting or attenuating proteolytic activation of PAR-2 but do not block proteolytic cleavage of PAR-2. Exemplary methods for measuring/assessing an antibody's ability to block PAR-2 cleavage or proteolytic activation are described herein.

[0009] The antibodies of the invention 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 affect functionality, e.g., to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933).

[0010] The present invention provides an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) having an amino acid 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, 162, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258, 262, 266, 282, 286, 290, 306, 310, 314, 330, 334, 338, 354, 358, 362, 378, 382, 386, 402, 406, 410, 426, 430, 434, 450, 454, 458, 474, 478, 482, 498, 502, 506, 522, 526, 530, 546, 550, 554, 570, 574, 578, 594, 598, 602, 618, 622, 626, 642, 646, 650, 666, 670, 674, 690, 694, 698, 714, 718, 722, 738, 742, 746, 762, 766, 770, 786, 790, 794, 810, 814, 818, 834, and 838, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. According to certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 98, 146, 338, and 714.

[0011] The present invention also provides an antibody or antigen-binding fragment of an antibody comprising a light chain variable region (LCVR) having an amino acid 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, 164, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260, 264, 274, 284, 288, 298, 308, 312, 322, 332, 336, 346, 356, 360, 370, 380, 384, 394, 404,

408, 418, 428, 432, 442, 452, 456, 466, 476, 480, 490, 500, 504, 514, 524, 528, 538, 548, 552, 562, 572, 576, 586, 596, 600, 610, 620, 624, 634, 644, 648, 658, 668, 672, 682, 692, 696, 706, 716, 720, 730, 740, 744, 754, 764, 768, 778, 788, 792, 802, 812, 816, 826, 836, and 840, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. According to certain embodiments, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 106, 154, 346, and 692.

[0012] The present invention also provides an antibody or antigen-binding fragment thereof comprising a HCVR and LCVR (HCVR/LCVR) sequence pair 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, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, 502/504, 506/514, 522/524, 526/528, 530/538, 546/548, 550/552, 554/562, 570/572, 574/576, 578/586, 594/596, 598/600, 602/610, 618/620, 622/624, 626/634, 642/644, 646/648, 650/658, 666/668, 670/672, 674/682, 690/692, 694/696, 698/706, 714/716, 714/692, 718/720, 722/730, 738/740, 742/744, 746/754, 762/764, 766/768, 770/778, 786/788, 790/792, 794/802, 810/812, 814/816, 818/826, 834/836, and 838/840. According to certain embodiments, the antibody or fragment thereof comprises a HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO: 98/106, 146/154, 338/346, and 714/692.

[0013] The present invention also provides an antibody or antigen-binding fragment of an antibody comprising a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 32, 56, 80, 104, 128, 152, 176, 200, 224, 248, 272, 296, 320, 344, 368, 392, 416, 440, 464, 488, 512, 536, 560, 584, 608, 632, 656, 680, 704, 728, 752, 776, 800, and 824, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 40, 64, 88, 112, 136, 160, 184, 208, 232, 256, 280, 304, 328, 352, 376, 400, 424, 448, 472, 496, 520, 544, 568, 592, 616, 640, 664, 688, 712, 736, 760, 784, 808, and 832, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0014] In certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 32/40, 56/64, 80/88, 104/112, 128/136, 152/160, 176/184, 200/208, 224/232, 248/256, 272/280, 296/304, 320/328, 344/352, 368/376, 392/400, 416/424, 440/448, 464/472, 488/496, 512/520, 536/544, 560/568, 584/592, 608/616, 632/640, 656/664, 680/688, 704/712,

728/736, 752/760, 776/784, 800/808, and 824/832. According to certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 104/112, 152/160, 344/352 and 704/712. Non-limiting examples of anti-PAR-2 antibodies having these HCDR3/LCDR3 pairs are the antibodies designated H4H588N, H4H591N, H4H618N, and H4H581P, respectively.

[0015] The present invention also provides an antibody or fragment thereof further comprising a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 28, 52, 76, 100, 124, 148, 172, 196, 220, 244, 268, 292, 316, 340, 364, 388, 412, 436, 460, 484, 508, 532, 556, 580, 604, 628, 652, 676, 700, 724, 748, 772, 796, and 820, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 30, 54, 78, 102, 126, 150, 174, 198, 222, 246, 270, 294, 318, 342, 366, 390, 414, 438, 462, 486, 510, 534, 558, 582, 606, 630, 654, 678, 702, 726, 750, 774, 798, and 822, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 36, 60, 84, 108, 132, 156, 180, 204, 228, 252, 276, 300, 324, 348, 372, 396, 420, 444, 468, 492, 516, 540, 564, 588, 612, 636, 660, 684, 708, 732, 756, 780, 804, and 828, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 38, 62, 86, 110, 134, 158, 182, 206, 230, 254, 278, 302, 326, 350, 374, 398, 422, 446, 470, 494, 518, 542, 566, 590, 614, 638, 662, 686, 710, 734, 758, 782, 806, and 830, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0016] Certain non-limiting, exemplary antibodies and antigen-binding fragments of the invention comprise HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 domains, respectively, selected from the group consisting of: (i) SEQ ID NO: 100, 102, 104, 108, 110 and 112 (*e.g.*, H4H588N); (ii) SEQ ID NO: 148, 150, 152, 156, 158 and 160 (*e.g.*, H4H591N); (iii) SEQ ID NO: 340, 342, 344, 348, 350 and 352 (*e.g.*, H4H618N); and (iv) SEQ ID NO: 700, 702, 704, 708, 710 and 712 (*e.g.*, H4H581P). The amino acid sequences of these exemplary CDRs are depicted in Figs. 2 and 3.

[0017] In a related embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody which specifically binds PAR-2, wherein the antibody or fragment comprises the heavy and light chain CDR domains contained within heavy and light chain sequences 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, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216,

218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, 502/504, 506/514, 522/524, 526/528, 530/538, 546/548, 550/552, 554/562, 570/572, 574/576, 578/586, 594/596, 598/600, 602/610, 618/620, 622/624, 626/634, 642/644, 646/648, 650/658, 666/668, 670/672, 674/682, 690/692, 694/696, 698/706, 714/716, 714/692, 718/720, 722/730, 738/740, 742/744, 746/754, 762/764, 766/768, 770/778, 786/788, 790/792, 794/802, 810/812, 814/816, 818/826, 834/836, and 838/840. According to certain embodiments, the antibody or fragment thereof comprises the CDR sequences contained within HCVRs and LCVRs selected from the amino acid sequence pairs of SEQ ID NO: 98/106, 146/154, 338/346, and 714/692. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0018] In another aspect, the invention provides nucleic acid molecules encoding anti-PAR-2 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0019] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid 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, 161, 165, 169, 185, 189, 193, 209, 213, 217, 233, 237, 241, 257, 261, 265, 281, 285, 289, 305, 309, 313, 329, 333, 337, 353, 357, 361, 377, 381, 385, 401, 405, 409, 425, 429, 433, 449, 453, 457, 473, 477, 481, 497, 501, 505, 521, 525, 529, 545, 549, 553, 569, 573, 577, 593, 597, 601, 617, 621, 625, 641, 645, 649, 665, 669, 673, 689, 693, 697, 713, 717, 721, 737, 741, 745, 761, 765, 769, 785, 789, 793, 809, 813, 817, 833, and 837, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. According to certain embodiments, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 97, 145, 337, and 713.

[0020] The present invention also provides an antibody or fragment thereof comprising a LCVR encoded by a nucleic acid 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, 163, 167, 177, 187, 191, 201, 211, 215, 225, 235, 239, 249, 259, 263, 273, 283, 287, 297, 307, 311, 321, 331, 335, 345, 355, 359, 369, 379, 383, 393, 403, 407, 417, 427, 431, 441, 451, 455, 465, 475, 479, 489, 499, 503, 513, 523, 527, 537, 547, 551, 561, 571, 575, 585, 595, 599, 609, 619, 623, 633, 643, 647, 657, 667, 671, 681, 691, 695, 705, 715, 719, 729, 739, 743, 753, 763, 767, 777, 787, 791, 801, 811, 815, 825, 835, and 839, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. According to certain embodiments, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 105, 153, 345, and 715.

[0021] The present invention also provides an antibody or antigen-binding fragment of an antibody comprising 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, 247, 271, 295, 319, 343, 367, 391, 415, 439, 463, 487, 511, 535, 559, 583, 607, 631, 655, 679, 703, 727, 751, 775, 799, and 823, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a 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, 255, 279, 303, 327, 351, 375, 399, 423, 447, 471, 495, 519, 543, 567, 591, 615, 639, 663, 687, 711, 735, 759, 783, 807, and 831, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. According to certain embodiments, the antibody or fragment thereof comprises HCDR3 and LCDR3 sequences encoded by the nucleic acid sequence pairs selected from the group consisting of SEQ ID NO: 103/111, 151/159, 343/351, and 703/711.

[0022] The present invention also provides an antibody or fragment thereof which further comprises 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, 243, 267, 291, 315, 339, 363, 387, 411, 435, 459, 483, 507, 531, 555, 579, 603, 627, 651, 675, 699, 723, 747, 771, 795, and 819, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% 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, 245, 269, 293, 317, 341, 365, 389, 413, 437, 461, 485, 509, 533, 557, 581, 605, 629, 653, 677, 701, 725, 749, 773, 797, and 821, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% 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, 251, 275, 299, 323, 347, 371, 395, 419, 443, 467, 491, 515, 539, 563, 587, 611, 635, 659, 683, 707, 731, 755, 779, 803, and 827, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and 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, 253, 277, 301, 325, 349, 373, 397, 421, 445, 469, 493, 517, 541, 565, 589, 613, 637, 661, 685, 709, 733, 757, 781, 805, 829, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0023] According to certain embodiments, the antibody or fragment thereof comprises the heavy and light chain CDR sequences encoded by the nucleic acid sequences of SEQ ID NO: 97 and 105; SEQ ID NO: 145 and 153; SEQ ID NO: 337 and 345; or SEQ ID NO: 713 and 715.

[0024] The present invention also provides an isolated antibody or antigen-binding fragment of an antibody that specifically binds PAR-2, comprising a HCDR3 and a LCDR3, wherein 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} (SEQ ID NO:843) wherein X^1 is Ala or Val, X^2 is Lys, X^3 is Gly or Glu, X^4 is Asp or Gly, X^5 is Phe or Asp, X^6 is Trp or Ser, X^7 is Ser or Gly, X^8 is Gly or Tyr, X^9 is Tyr or Asp, X^{10} is Phe or Leu, X^{11} is Asp or Ala, and X^{12} is Tyr; 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:846) wherein X^1 is Met or Gln, X^2 is Gln, X^3 is Ala or Tyr, X^4 is Thr or Lys, X^5 is Gln, Ser or Ile, X^6 is Phe or Ser, X^7 is Pro, X^8 is Thr or Leu, and X^9 is Thr or absent.

[0025] In a more specific embodiment, the invention features an isolated antibody or fragment thereof that specifically binds PAR-2, comprising a HCDR1 sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO:841), wherein X^1 is Gly, X^2 is Phe, X^3 is Thr, X^4 is Phe, X^5 is Ser or Arg, X^6 is Ser or Arg, X^7 is Tyr, and X^8 is Gly, Ala or Thr; a HCDR2 sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO:842), wherein X^1 is Ile, X^2 is Ser, Gly or Thr, X^3 is Tyr, Gly or Asp, X^4 is Asp, Gly or Ser, X^5 is Gly or Arg, X^6 is Ile, Gly or Ala, X^7 is Asn, Ser, Arg or Gly, and X^8 is Lys, Ala or Thr; a HCDR3 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} (SEQ ID NO:843) wherein X^1 is Ala or Val, X^2 is Lys, X^3 is Gly or Glu, X^4 is Asp or Gly, X^5 is Phe or Asp, X^6 is Trp or Ser, X^7 is Ser or Gly, X^8 is Gly or Tyr, X^9 is Tyr or Asp, X^{10} is Phe or Leu, X^{11} is Asp or Ala, and X^{12} is Tyr; a LCDR1 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:844) wherein X^1 is Gln, X^2 is Ser or Gly, X^3 is Leu or Ile, X^4 is Val or Ser, X^5 is His, Asn or Thr, X^6 is Ser, Asn or Tyr, X^7 is Asp or absent; X^8 is Gly or absent, X^9 is Asn or absent, X^{10} is Thr or absent, and X^{11} is Tyr or absent; a LCDR2 sequence of the formula $X^1 – X^2 – X^3$ (SEQ ID NO:845) wherein X^1 is Lys or Ala, X^2 is Ile, Ala or Thr, and X^3 is Ser; and a 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:846) wherein X^1 is Met or Gln, X^2 is Gln, X^3 is Ala or Tyr, X^4 is Thr or Lys, X^5 is Gln, Ser or Ile, X^6 is Phe or Ser, X^7 is Pro, X^8 is Thr or Leu, and X^9 is Thr or absent.

[0026] The invention encompasses anti-PAR-2 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 galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0027] In another aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds PAR-2 and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of a PAR-2 inhibitor and a second therapeutic agent. In one embodiment, the PAR-2 inhibitor is an antibody or fragment thereof. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with a PAR-2 inhibitor. Exemplary agents that may be advantageously combined with a PAR-2 inhibitor include, without limitation, other agents that inhibit PAR-2 activity (including other antibodies or antigen-binding fragments thereof, peptide inhibitors, small molecule antagonists, etc) and/or agents which interfere with PAR-2 upstream or downstream signaling.

[0028] In yet another aspect, the invention provides methods for inhibiting PAR-2 activity using the anti-PAR-2 antibody or antigen-binding portion of an antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of PAR-2 activity. The anti-PAR-2 antibody or antibody fragment of the invention may function to block the interaction between PAR-2 and a protease (*e.g.*, trypsin or trypsin-like serine proteases) or otherwise inhibit protease-mediated activation of PAR-2. Alternatively, or additionally, the anti-PAR-2 antibodies of the invention may interfere with the interaction between the PAR-2 tethered ligand and one or more of the PAR-2 extracellular loops (*see, e.g.*, MacFarlane et al., 2001, Pharmacological Reviews 53:245-282 for a general discussion of PAR-2 proteolytic cleavage and activation). The antibody or antibody fragment may be used alone or in combination with one or more additional therapeutic agents.

[0029] The present invention also includes the use of an anti-PAR-2 antibody or antigen binding portion of an antibody of the invention in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by PAR-2 activity in a patient.

[0030] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0031] **Fig. 1.** Sequence comparison table of heavy chain variable regions and CDRs of antibodies H4H581P, H4H588N, H4H591N and H4H618N.

[0032] **Fig. 2.** Sequence comparison table of light chain variable regions and CDRs of antibodies H4H581P, H4H588N, H4H591N and H4H618N.

[0033] **Fig. 3.** The top panel (A) shows C- and N-terminal biotin labeled peptides corresponding to the sequence surrounding the activating PAR-2 protease cleavage site (GTNRSSKGRSLIGKVDGT; SEQ ID NO:852). The protease cleavage sites are designated by number 1 (an upstream, non-activating protease cleavage site) and number 2 (the activating PAR-2 protease cleavage site). The expected sizes of the uncleaved and cleaved fragments are indicated in the top table. The bottom panel (B) shows the fragment sizes that were observed following 0, 5, and 15 minutes of trypsin treatment in the presence of anti-PAR-2 antibodies or negative control.

[0034] **Fig. 4.** The top panel (A) shows the mouse, rat and human peptides corresponding to the sequence surrounding the activating PAR-2 protease cleavage sites (SEQ ID NOs:883, 858 and 852, respectively). The protease cleavage sites are designated by numbers 1 and 2 (upstream, non-activating protease cleavage sites) and number 3 (the activating PAR-2 protease cleavage site). The expected sizes of the uncleaved and cleaved fragments are indicated in the top table. The bottom panel (B) shows the fragment sizes that were observed following 0 and 5 minutes of trypsin treatment in the presence of anti-PAR-2 antibodies or negative control.

[0035] **Fig. 5.** Depiction of alanine scanning epitope mapping results for antibody binding to the sequence surrounding the PAR-2 activating protease cleavage site (SEQ ID NO:852). Open triangles represent protease cleavage sites located upstream from the activating PAR-2 protease cleavage site. The activating PAR-2 protease cleavage site is designated by a closed triangle. The numbers in parentheses indicate the amino acid numbering in the full-length human PAR-2 sequence (SEQ ID NO:851). Numbers in circles under the amino acid residues indicate the percent of T½ of antibody binding to alanine-scan mutant peptide relative to the T½ of antibody binding to wild-type peptide, as shown in Tables 24-26 and 28. If duplicate experiments were conducted, the average T½ percentage is shown in the circle. Black circles with white numbers indicate amino acids that, when changed to alanine, reduce the T½ of antibody binding to 30% or less of the T½ of antibody binding to wild-type peptide. Such amino acids are defined herein as residues with which the antibody interacts.

DETAILED DESCRIPTION

[0036] Before the present invention is 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.

[0037] 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. As used herein, the term "about," when used in reference to a particular recited

numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0038] 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] As used herein, the terms "proteinase-activated receptor-2," "protease-activated receptor-2," and "PAR-2", refer to full-length PAR-2 protein. Human PAR-2 is encoded by the nucleic acid sequence shown in SEQ ID NO:850 and has the amino acid sequence of SEQ ID NO:851. Amino acid sequences of PAR-2 molecules from non-human species (*e.g.*, mouse, monkey, rabbit, dog, pig, etc.) are available from public sources.

[0040] The term "PAR-2 fragment," as used herein, means a peptide or polypeptide comprising 4, 5, 6, 7, 8, 9, 10 or more contiguous amino acids located upstream from (*i.e.*, N-terminal to) the activating PAR-2 protease cleavage site (as defined herein below) and/or 4, 5, 6, 7, 8, 9, 10 or more contiguous amino acids located downstream from (*i.e.*, C-terminal to) the activating PAR-2 protease cleavage site. Exemplary PAR-2 fragments are illustrated in Example 2, Table 2 (designated Peptides "A" through "J"; *i.e.*, SEQ ID NOs:852 through 861, respectively).

[0041] The expressions "PAR-2" and "PAR-2 fragment," as used herein refer to the human PAR-2 protein or fragment unless specified as being from a non-human species (*e.g.*, "mouse PAR-2," mouse PAR-2 fragment," "monkey PAR-2," "monkey PAR-2 fragment," etc.).

[0042] As used in the context of the present disclosure, the expression "activating PAR-2 protease cleavage site" means the junction of residues Arg-36 and Ser-37 of human PAR-2 (SEQ ID NO:851). The activating PAR-2 protease cleavage site is the site which, when cleaved, results in the formation of the PAR-2 tethered ligand in the naturally occurring protein.

[0043] The term "PAR-2 protease," as used herein, means an enzyme which is capable of cleaving a PAR-2 or PAR-2 fragment at the activating PAR-2 protease cleavage site. Exemplary PAR-2 proteases include trypsin, cathepsin G, acrosin, tissue factor VIIa, tissue factor Xa, human airway trypsin-like protease, tryptase, membrane-type serine protease-1 (MT-SP1), TMPRSS2, protease-3, elastase, kallikrein-5, kallikrein-6, kallikrein-14, activated protein C, duodenase, gingipains-R, Der p1, Der p3, Der p9, thermolysin, serralysin, and *T. denticla* protease.

[0044] 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, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3.

Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L 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. In different embodiments of the invention, the FRs of the anti-Ang-2 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0045] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0046] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) $F(ab')_2$ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR)). Other engineered molecules, such as diabodies, triabodies, tetrabodies and minibodies, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0047] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0048] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0049] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0050] 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.

[0051] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0052] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse)

that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0053] Human antibodies 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 inter-chain 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.

[0054] 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. 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 which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0055] An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, tissue or cell in which the antibody naturally exists or is naturally produced is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell, as well as an antibody that has been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0056] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by a dissociation constant of $1x10^{-6}$ M or less. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For

example, an antibody that "specifically binds" human PAR-2, as used in the context of the present invention, includes antibodies that bind human PAR-2 or portion thereof (*e.g.*, a PAR-2 fragment comprising the activating protease cleavage site) with a K_D of less than about 1000 nM, less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. (See, *e.g.*, Example 4, herein). An isolated antibody that specifically binds human PAR-2 may, however, have cross-reactivity to other antigens, such as PAR-2 molecules from other species.

[0057] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to PAR-2: (i) interferes with the interaction between PAR-2 or a PAR-2 fragment and one or more proteases, (ii) prevents cleavage of PAR-2 or a PAR-2 fragment by a PAR-2 protease, (iii) inhibits the interaction between the PAR-2 tethered ligand and a PAR-2 extracellular loop, and/or (iv) results in inhibition of at least one biological function of PAR-2. The inhibition caused by a PAR-2 neutralizing or blocking antibody need not be complete so long as it is detectable using an appropriate assay. Exemplary assays for detecting PAR-2 inhibition are described herein.

[0058] The fully-human anti-PAR-2 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are back-mutated to the corresponding germline residue(s) or to a conservative amino acid substitution (natural or non-natural) of the corresponding germline residue(s) (such sequence changes are referred to herein as "germline back-mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline back-mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the germline sequence. In other embodiments, only certain residues are mutated back to the germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. Furthermore, the antibodies of the present invention may contain any combination of two or more germline back-mutations

within the framework and/or CDR regions, *i.e.*, wherein certain individual residues are mutated back to the germline sequence while certain other residues that differ from the germline sequence are maintained. Once obtained, antibodies and antigen-binding fragments that contain one or more germline back-mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0059] The present invention also includes anti-PAR-2 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-PAR-2 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. In one embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:698 with 8 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:698 with 6 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:698 with 4 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:698 with 2 or fewer conservative amino acid substitutions. In one embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:706 with 8 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:706 with 6 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:706 with 4 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:706 with 2 or fewer conservative amino acid substitutions.

[0060] 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 (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0061] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0062] The term "epitope" refers to 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. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. 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 sulfonyl groups on the antigen.

[0063] 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. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0064] 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: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0065] 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

[0066] Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human PAR-2.

[0067] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to PAR-2 are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0068] The anti-PAR-2 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind human PAR-2. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-PAR-2 antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-PAR-2 antibody or antibody fragment that is essentially bioequivalent to an anti-PAR-2 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences

are discussed above.

[0069] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, e.g., chronic use, and are considered medically insignificant for the particular drug product studied.

[0070] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0071] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0072] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0073] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, e.g., (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0074] Bioequivalent variants of anti-PAR-2 antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-PAR-2 antibody variants comprising amino acid changes which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Biological Characteristics of the Antibodies

[0075] The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See*, *e.g.*, U.S. 5,500,362 and 5,821,337, and Clynes *et al.* (1998) Proc. Natl. Acad. Sci. (USA) *95*:652-656).

[0076] Alternatively, or additionally, the antibodies of the invention may be therapeutically useful in blocking a PAR-2 interaction or inhibiting receptor component interaction. In the case of the PAR-2 antibodies of the present invention, the antibodies may function by, *inter alia*, blocking or obscuring the activating PAR-2 protease cleavage site. Alternatively, the antibodies of the invention may function by interfering with the interaction between the tethered ligand and one or more extracellular loops (*e.g.*, loop-1, loop-2 and/or loop-3).

[0077] More specifically, the anti-PAR-2 antibodies of the invention may exhibit one or more of the following characteristics: (1) ability to bind to a human PAR-2 or human PAR-2 fragment and to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-2 or PAR-2 fragment; (2) ability to bind to a human PAR-2 or human PAR-2 fragment but not to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-2 or PAR-2 fragment; (3) ability to bind to a human PAR-2 or human PAR-2 fragment and to a monkey PAR-2 or monkey PAR-2 fragment, but not to a mouse, rat, rabbit, dog or pig PAR-2 or PAR-2 fragment; (4) ability to bind to a human PAR-2 or human PAR-2 fragment and to a human PAR-1, PAR-3 or PAR-4 or fragment thereof; (5) ability to bind to a human PAR-2 or human PAR-2 fragment but not to a human PAR-1, PAR-3, or PAR-4 or fragment thereof; (6) ability to bind to a human PAR-2 or human PAR-2 fragment and to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-1, PAR-3 or PAR-4 or fragment thereof; (7) ability to bind to a human PAR-2 or human PAR-2 fragment but not to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-1, PAR-3 or PAR-4 or fragment thereof; (8) ability to block proteolytic cleavage of a PAR-2 or a PAR-2 fragment; (9) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment and a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-2 or PAR-2 fragment; (10) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment but not a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-2 or PAR-2 fragment; (11) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment and a human PAR-1, PAR-3 or PAR-4 or fragment thereof; (12) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment but not a human PAR-1, PAR-3 or PAR-4 or fragment thereof; (13) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment

and a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-1, PAR-3 or PAR-4 or fragment thereof; and/or (14) ability to block proteolytic cleavage of a human PAR-2 or human PAR-2 fragment but not a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.) PAR-1, PAR-3 or PAR-4 or fragment thereof.

[0078] As used in items (8) – (14) above, the term "proteolytic cleavage" means cleavage of a PAR molecule (PAR-1, PAR-2, PAR-3 or PAR-4) or fragment thereof by a PAR-2 protease or other enzyme that is capable of cleaving PAR-2 at the activating PAR-2 protease cleavage site.

[0079] The N-terminal region of human PAR-2 has at least two "non-activating" protease cleavage sites, *i.e.*, sites that are capable of being cleaved by trypsin but do not result in activation of the receptor. The N-terminal non-activating protease cleavage sites are located: (a) at the junction of residues Arg-31 and Ser-32 of human PAR-2 (SEQ ID NO:851); and (b) at the junction of residues Lys-34 and Gly-35 of human PAR-2 (SEQ ID NO:851). The activating and non-activating cleavage sites at the N-terminus of PAR-2 are illustrated in Fig. 5 (white triangles indicate the non-activating protease cleavage sites and the black triangle indicates the activating PAR-2 protease cleavage site); *see also* Fig. 4. The present invention includes anti-PAR-2 antibodies that block the activating PAR-2 protease cleavage site but do not block one or both of the non-activating protease cleavage sites. Whether a candidate antibody blocks or does not block a particular protease cleavage site can be determined by a person of ordinary skill in the art using any suitable assay such as the exemplary *in vitro* blocking assays set forth in Example 8 herein. As illustrated in Example 8, the exemplary antibody H4H581P was shown to block trypsin cleavage at the activating PAR-2 protease cleavage site and at the non-activating protease cleavage site located at the junction of residues Lys-34 and Gly-35 of human PAR-2 (SEQ ID NO:851), but did not block cleavage at the non-activating protease cleavage site located at the junction of residues Arg-31 and Ser-32 of human PAR-2 (SEQ ID NO:851). By contrast, the comparator antibody used in Example 8 blocked cleavage at the activating PAR-2 protease cleavage site and at both non-activating sites. The differential blocking capabilities of these exemplary anti-PAR-2 antibodies most likely reflects differences in the particular regions of the PAR-2 molecule with which these antibodies bind (*see, e.g.*, Example 9 herein).

[0080] As used herein, an antibody "does not bind" to a specified target molecule (*e.g.*, mouse PAR-2, rat PAR-2, rabbit PAR-2, dog PAR-2, pig PAR-2, or fragment thereof) if the antibody, when tested for binding to the target molecule at 25°C in a surface plasmon resonance assay, exhibits a K_D of greater than 500 nM, or if tested for binding to the target molecule at 25°C in an enzyme-linked immunosorbent assay (ELISA) exhibits an EC_{50} of greater than 50 nM, or fails to exhibit any binding in either type of assay or equivalent thereof.

[0081] Certain anti-PAR-2 antibodies of the present invention are able to inhibit or attenuate PAR-2 activation in an *in vitro* cellular assay. A non-limiting, exemplary *in vitro* cellular assay for PAR-2 activation is illustrated in Example 6, herein. In this Example, cells are used which

express PAR-2 and harbor a construct comprising NF-κB fused to a reporter molecule (*e.g.*, luciferase). Briefly, such cells are combined with an anti-PAR-2 antibody, followed by treatment with a PAR-2 protease. Cells that are treated with the protease in the presence of an inhibitory anti-PAR-2 antibody will exhibit significantly less or no reporter signal as compared to cells treated with the protease in the absence of an inhibitory anti-PAR-2 antibody. The concentration of antibody necessary to achieve half-maximal inhibition of reporter signal (IC_{50}) can be calculated using such an assay. The present invention includes inhibitory anti-PAR-2 antibodies that exhibit an IC_{50} of less than 300 nM when tested in an *in vitro* cellular assay for PAR-2 activation as described above. For example, the invention includes anti-PAR-2 antibodies with an IC_{50} of less than 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nM when tested in an *in vitro* cellular assay for PAR-2 activation as described above in which the cells are incubated with the antibody for 1 h at 37°C followed by treatment with 20 nM trypsin (or other PAR-2 protease) for 5 h at 37°C.

[0082] The present invention includes anti-PAR-2 antibodies and antigen binding fragments thereof which bind to one or more of the following peptides: Peptide A (GTNRSSKGRSLIGKVDGT, SEQ ID NO:852); Peptide B (SLIGKVDGTSHVTG, SEQ ID NO:853); Peptide C (SLIGKV, SEQ ID NO:854); Peptide D (N-terminal domain of human PAR-2 – mouse IgG, SEQ ID NO:855); Peptide E (LAPGRNNSKGRSLIGRLETQ, SEQ ID NO:856); Peptide F (GTNRSSKGRSLIGRVDGT, SEQ ID NO:857); Peptide G (GPNSKGRSLIGRLDTP, SEQ ID NO:858); Peptide H (GTNKTSKGRSLIGRNTGS, SEQ ID NO:859); Peptide I (GTNRTSKGRSLIGKTDSS, SEQ ID NO:860); Peptide J (GTSRPSKGRSLIGKADNT, SEQ ID NO:861); Peptide K (ATNATLDPRSFLLRNPND, SEQ ID NO:862); Peptide L (DTNNLAKPTLPIKTFRGA, SEQ ID NO:863); or Peptide M (ESGSTGGGDDSTPSILPAP, SEQ ID NO:864). Additional information regarding these peptides can be found in Example 3 herein. These peptides may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

[0083] With regard to the aforementioned Peptides, the present invention includes anti-PAR-2 antibodies with one or more of the following binding profiles: (1) binding to Peptides A and B, but not binding to Peptide C; (2) binding to Peptides A, B and D, but not binding to Peptide C; (3) binding to Peptides A, B, D and F, but not binding to Peptide C; (4) binding to Peptides A, B, D and F, but not binding to either of Peptides C or E; (5) binding to Peptides A, B and D, but not binding to any of Peptides K, L or M; (6) binding to Peptides A, B and F, but not binding to any of Peptides K, L or M; (7) binding to Peptides A, B, D and F, but not binding to any of Peptides

K, L or M; and/or (8) binding to at least three of Peptides A, B, C, D, E, F, G, I and J, but not binding to Peptide H. Other binding profiles of the antibodies of the invention will be evident from the examples herein.

Epitope Mapping and Related Technologies

[0084] To screen for antibodies that bind to a particular epitope (e.g., those which block binding of IgE to its high affinity receptor), a routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke, 2004, Methods Mol Biol 248:443-463), 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).

[0085] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0086] 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 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with 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 that produce mAbs having the desired characteristics. MAP may be used to sort the anti-PAR-2 antibodies of the invention into groups of antibodies binding different epitopes.

[0087] The present invention includes anti-PAR-2 antibodies which bind to an epitope at or near (*e.g.*, within 5, 10, 15 or 20 amino acids of) the activating PAR-2 protease cleavage site. In certain embodiments, the anti-PAR-2 antibodies bind to an epitope located upstream from (*i.e.*, N-terminal to) the activating PAR-2 protease cleavage site. In certain other embodiments of the invention, the anti-PAR-2 antibodies bind to an epitope located downstream from (*i.e.*, C-terminal to) the activating PAR-2 protease cleavage site. In yet other embodiments, the anti-PAR-2 antibodies of the invention may bind an epitope that includes both amino acid sequences located upstream from the activating PAR-2 protease cleavage site and amino acid sequences located downstream from the activating PAR-2 protease cleavage site.

[0088] Alternatively, the anti-PAR-2 antibodies of the invention may, in certain embodiments, bind to an epitope located on one or more extracellular loops of the PAR-2 protein (*e.g.*,

extracellular loop 1, extracellular loop 2 and/or extracellular loop 3).

[0089] The present invention includes isolated human antibodies or antigen-binding fragments thereof that interact with certain amino acid residues located downstream from the activating PAR-2 protease cleavage site. For example, the present invention includes isolated human antibodies or antigen-binding fragments thereof that interact with Val-42 and Asp-43 of human PAR-2 (SEQ ID NO:851). In addition to these two residues, the isolated human antibodies or antigen-binding fragments thereof may also interact with one or more of the following residues located downstream from the activating PAR-2 protease cleavage site: Ser-37, Leu-38, Ile-39, Gly-40 or Gly-44 of human PAR-2 (SEQ ID NO:851). In certain embodiments, the isolated human antibody or antigen binding fragment thereof does not interact with Lys-41 of human PAR-2 (SEQ ID NO:851). For example, the present invention includes isolated human antibodies or antigen-binding fragments thereof that interact with Ser-37, Leu-38, Ile-39, Gly-40, Val-42 and Asp-43 of human PAR-2 (SEQ ID NO:851), and do not interact with Lys-41 of human PAR-2 (SEQ ID NO:851). The experimental procedures illustrated in Example 9 can be used to determine if a candidate anti-PAR-2 antibody "interacts with" or "does not interact with" a particular amino acid residue of PAR-2. For example, if a candidate antibody is tested for binding to a peptide having SEQ ID NO:879 (corresponding to the N-terminal region of PAR-2 wherein Val-42 of PAR-2 is mutated to alanine, *see, e.g.,* Tables 24-28) using the procedure of Example 9, and the $T_{1/2}$ of the antibody is less than 30% the $T_{1/2}$ observed when the candidate antibody is tested for binding to the wild-type peptide (SEQ ID NO:871), then for purposes of the present disclosure, the candidate antibody is deemed to "interact with" the amino acid that was mutated to alanine (in this case, Val-42); that is, binding of the candidate antibody is substantially reduced when the amino acid corresponding to Val-42 is mutated to alanine (such residues are depicted by black circles in Figure 5). On the other hand, if a candidate antibody is tested for binding to a peptide having SEQ ID NO:878 (corresponding to the N-terminal region of PAR-2 wherein Lys-41 of PAR-2 is mutated to alanine, *see, e.g.,* Tables 24-28) using the procedure of Example 9, and the $T_{1/2}$ of the antibody is greater than or equal to 30% the $T_{1/2}$ observed when the candidate antibody is tested for binding to the wild-type peptide (SEQ ID NO:871), then for purposes of the present disclosure, the candidate antibody is deemed to "not interact with" the amino acid that was mutated to alanine (in this case, Lys-41); that is, binding of the candidate antibody is not substantially reduced when the amino acid corresponding to Lys-41 is mutated to alanine (such residues are depicted by white circles in Figure 5).

[0090] The present invention includes anti-PAR-2 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H4H581P, H4H588N, H4H591N or H4H618N). Likewise, the present invention also includes anti-PAR-2 antibodies that cross-compete for binding to PAR-2 or a PAR-2 fragment with any of the specific exemplary antibodies described herein (*e.g.*, H4H581P, H4H588N, H4H591N or H4H618N).

[0091] One can easily determine whether an antibody binds to the same epitope as, or

competes for binding with, a reference anti-PAR-2 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-PAR -2 antibody of the invention, the reference antibody is allowed to bind to a PAR-2 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the PAR-2 molecule is assessed. If the test antibody is able to bind to PAR-2 following saturation binding with the reference anti-PAR-2 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-PAR-2 antibody. On the other hand, if the test antibody is not able to bind to the PAR-2 molecule following saturation binding with the reference anti-PAR-2 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-PAR-2 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0092] To determine if an antibody competes for binding with a reference anti-PAR-2 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a PAR-2 molecule under saturating conditions followed by assessment of binding of the test antibody to the PAR-2 molecule. In a second orientation, the test antibody is allowed to bind to a PAR-2 molecule under saturating conditions followed by assessment of binding of the reference antibody to the PAR-2 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the PAR-2 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to PAR-2. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Species Selectivity and Species Cross-Reactivity

[0093] According to certain embodiments of the invention, the anti-PAR-2 antibodies bind to

human PAR-2 but not to PAR-2 from other species. Alternatively, the anti-PAR-2 antibodies of the invention, in certain embodiments, bind to human PAR-2 and to PAR-2 from one or more non-human species. For example, the anti-PAR-2 antibodies of the invention may bind to human PAR-2 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomologous, marmoset, rhesus or chimpanzee PAR-2.

Immunoconjugates

[0094] The invention encompasses anti-PAR-2 monoclonal antibodies conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxic agents include any agent that is detrimental to cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming immunoconjugates are known in the art, see for example, WO 05/103081).

Multispecific Antibodies

[0095] The antibodies of the present invention may be monospecific, bi-specific, 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; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-PAR-2 antibodies of the present invention 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 bi-specific or a multispecific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for human PAR-2 or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety such as a trypsin inhibitor.

[0096] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and

V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

Therapeutic Formulation and Administration

[0097] The invention provides therapeutic compositions comprising the anti-PAR-2 antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention 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.

[0098] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When an antibody of the present invention is used for treating a condition or disease associated with PAR-2 activity in an adult patient, it may be advantageous to intravenously administer the antibody of the present invention 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. Effective dosages and schedules for administering PAR-2 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351).

[0099] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, 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.

[0100] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0101] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0102] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, 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.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, 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, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0103] 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.

[0104] 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 about 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 about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0105] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with PAR-2 activity, including diseases or disorders associated with the proteolytic activation of PAR-2. Exemplary diseases and disorders that can be treated with the anti-PAR-2 antibodies of the present invention include pain conditions such as nociceptive pain and visceral pain, as well as pain associated with conditions such as inflammation, post-operative incision, neuropathy, bone fracture, burn, osteoporotic fracture, bone cancer, gout, migraine headache, fibromyalgia, etc. The antibodies of the invention may also be used to treat, prevent and/or ameliorate inflammatory conditions such as joint inflammation, airway inflammation (*e.g.*, asthma), skin inflammation, dermatitis (*e.g.*, atopic dermatitis, allergic contact dermatitis, etc.), inflammatory bowel disease (IBD), glomerulonephritis, interstitial cystitis, bladder inflammation, hyperalgesia, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, multiple sclerosis, anti-phospholipid syndrome, alpha-1-antitrypsin deficiency, etc. The antibodies of the present invention may be used to treat fibrotic conditions, including, *e.g.*, scleroderma, biliary cirrhosis, post-transplant fibrosis, renal fibrosis, lung fibrosis, liver fibrosis, pancreatic fibrosis, testicular fibrosis, hypertrophic scarring and cutaneous keloids. In certain embodiments, the antibodies of the invention are useful for the treatment of gastrointestinal conditions (*e.g.*, celiac disease, Crohn's disease, ulcerative colitis, idiopathic gastroparesis, pancreatitis, irritable bowel syndrome (IBS) and ulcers (including

gastric and duodenal ulcers)); acute lung injury; acute renal injury; and sepsis. The anti-PAR-2 antibodies of the present invention are also useful for the treatment of pruritus; *e.g.*, dermal/pruritoceptive, neuropathic, neurogenic, and psychogenic itch, as well as pruritus associated with atopic dermatitis, psoriasis, burn scarring (burn-related itch), hypertrophic scarring, keloids, renal failure and hepatic failure. Other therapeutic uses of the anti-PAR-2 antibodies of the present invention include the treatment, prevention and/or amelioration of Alzheimer's disease, Netherton's disease, pathological angiogenesis, chronic urticaria, angioedema, mastocytosis, endometriosis, infertility (*e.g.*, male infertility associated with testicular fibrosis), mast cell-mediated diseases, *Clostridium difficile* Toxin-A induced enteritis, and cancer (*e.g.*, blood cell cancer, brain cancer, breast cancer, colon cancer, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, etc.).

Combination Therapies

[0106] The present invention includes therapeutic administration regimens which comprise administering an anti-PAR-2 antibody of the present invention in combination with at least one additional therapeutically active component. Non-limiting examples of such additional therapeutically active components include other PAR-2 antagonists (*e.g.*, anti-PAR-2 antibody or small molecule inhibitor of PAR-2 (*e.g.*, N1-3-methylbutyryl-N4-6-aminohexanoyl-piperazine; ENMD-1068)), cytokine inhibitors (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept or anakinra, a small molecule IL-1 antagonist, or an anti-IL-1 antibody); IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); IL-4 inhibitor (such as a small molecule IL-4 antagonist, an anti-IL-4 antibody or an anti-IL-4 receptor antibody); IL-6 inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti-IL-6 receptor antibody); antiepileptic drugs (*e.g.*, gabapentain); nerve growth factor (NGF) inhibitors (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody); low dose cochicine; aspirin; NSAIDs; steroids (*e.g.*, prednisone, methotrexate, etc.); low dose cyclosporine A; tumor necrosis factor (TNF) or TNF receptor inhibitors (*e.g.*, a small molecule TNF or TNFR antagonist or an anti-TNF or TNFR antibody); uric acid synthesis inhibitors (*e.g.*, allopurinol); uric acid excretion promoters (*e.g.*, probenecid, sulfinpyrazone, benzbromarone, etc.); other inflammatory inhibitors (*e.g.*, inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, etc.); and/or corticosteroids. The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-PAR-2 antibody of the present invention.

Diagnostic Uses of the Antibodies

[0107] The anti-PAR-2 antibodies of the present invention may also be used to detect and/or measure PAR-2 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-PAR-2 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of PAR-

2. Exemplary diagnostic assays for PAR-2 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-PAR-2 antibody of the invention, wherein the anti-PAR-2 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-PAR-2 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, ß-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure PAR-2 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0108] Samples that can be used in PAR-2 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of PAR-2 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of PAR-2 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal PAR-2 levels or activity) will be measured to initially establish a baseline, or standard, level of PAR-2. This baseline level of PAR-2 can then be compared against the levels of PAR-2 measured in samples obtained from individuals suspected of having a PAR-2 related disease or condition.

EXAMPLES

[0109] 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 PAR-2

[0110] An immunogen comprising human PAR-2 peptide having the amino acid sequence GTNRSSKGRSLIGKVDGT (SEQ ID NO:852) was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a PAR-2-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce PAR-2-specific antibodies. Using this technique several anti-PAR-2 chimeric antibodies (*i.e.*, antibodies possessing human variable domains

and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H2M588, H2M589, H1M590, H2M591, H1M592, H1M595, H2M609, H2M610, H2M611, H1M612, H1M613, H2M614, H1M615, H1M616, H3M617, H2M618, H1M619, and H3M620.

[0111] Anti-PAR-2 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1. Using this method, several fully human anti-PAR-2 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1H571, H1H572, H1H573, H1H574, H1H575, H1H576, H1H577, H1H578, H1H579, H1H580, H1H581, H1H583, H1H584, H1H585, H1H586, and H1H587.

[0112] The biological properties of the exemplary anti-PAR-2 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0113] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected anti-PAR-2 antibodies and their corresponding antibody identifiers. The N, P and G designations refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (i.e., in the framework regions). Thus, N, P and G variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but differ from one another within their framework regions.

Table 1

Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs
H1H571N	458/466	H1H571P	474/476	H1H571G	478/480
H1H572N	482/490	H1H572P	498/500	H1H572G	502/504
H1H573N	506/514	H1H573P	522/524	H1H573G	526/528
H1H574N	530/538	H1H574P	546/548	H1H574G	550/552
H1H575N	554/562	H1H575P	570/572	H1H575G	574/576
H1H576N	578/586	H1H576P	594/596	H1H576G	598/600
H1H577N	602/610	H1H577P	618/620	H1H577G	622/624
H1H578N	626/634	H1H578P	642/644	H1H578G	646/648
H1H579N	650/658	H1H579P	666/668	H1H579G	670/672
H1H580N	674/682	H1H580P	690/692	H1H580G	694/696
H1H581N	698/706	H1H581P	714/692	H1H581G	718/720
H1H583N	722/730	H1H583P	738/740	H1H583G	742/744
H1H584N	746/754	H1H584P	762/764	H1H584G	766/768
H1H585N	770/778	H1H585P	786/788	H1H585G	790/792
H1H586N	794/802	H1H586P	810/812	H1H586G	814/816

H1H587N	818/826	H1H587P	834/836	H1H587G	838/840
H2M588N	98/106	H2M588P	114/116	H2M588G	118/120
H2M589N	122/130	H2M589P	138/140	H2M589G	142/144
H1M590N	218/226	H1M590P	234/236	H1M590G	238/240
H2M591N	146/154	H2M591P	162/164	H2M591G	166/168
H1M592N	242/250	H1M592P	258/260	H1M592G	262/264
H1M595N	266/274	H1M595P	282/284	H1M595G	286/288
H2M609N	170/178	H2M609P	186/188	H2M609G	190/192
H2M610N	194/202	H2M610P	210/212	H2M610G	214/216
H2M611N	290/298	H2M611P	306/308	H2M611G	310/312
H1M612N	2/10	H1M612P	18/20	H1M612G	22/24
H1M613N	410/418	H1M613P	426/428	H1M613G	430/432
H2M614N	314/322	H2M614P	330/332	H2M614G	334/336
H1M615N	26/34	H1M615P	42/44	H1M615G	46/48
H1M616N	50/58	H1M616P	66/68	H1M616G	70/72
H3M617N	362/370	H3M617P	378/380	H3M617G	382/384
H2M618N	338/346	H2M618P	354/356	H2M618G	358/360
H1M619N	74/82	H1M619P	90/92	H1M619G	94/96
H3M620N	386/394	H3M620P	402/404	H3M620G	406/408
FP3B12F6N	434/442	FP3B12F6P	450/452	FP3B12F6G	454/456

Example 3. Antibody Binding to PAR-2 Peptides

[0114] Synthetic peptides (Celtek Bioscience, Nashville, TN) of PAR-2 and PAR-2 related sequences were generated to characterize the binding profiles of anti-PAR-2 antibodies. Both biotinylated and unbiotinylated forms for the various peptides were generated for the examples set forth below. For biotinylated forms, biotin moieties were covalently attached to the peptide at either the C-terminus or the N-terminus via a G_4S linker. Table 2 sets forth the sequence and derivation of these peptides.

Table 2

Designation	Species	Gene	Sequence	SEQ ID NO:
Peptide A	Human	PAR-2	GTNRSSKGRSLIGKVDGT	852
Peptide B	Human	PAR-2	SLIGKVDGTSHVTG	853
Peptide C	Human	PAR-2	SLIGKV	854
Peptide D	Human	PAR-2	SLIGKVDGTSHVTGKGVTVE TVFSVDEFSASVLTGKLTTVF LP – mouse IgG2a	855
Peptide E	Mouse (*Mus musculus*)	PAR-2	LAPGRNNSKGRSLIGRLETQ	856
Peptide F	Monkey (*Macaca mulatta*)	PAR-2	GTNRSSKGRSLIGRVDGT	857
Peptide G	Rat (*Rattus norvegicus*)	PAR-2	GPNSKGRSLIGRLDTP	858

Peptide H	Rabbit (*Oryctolagus cuniculus*)	PAR-2	GTNKTSKGRSLIGRNTGS	859
Peptide I	Dog (*Canis familiaris*)	PAR-2	GTNRTSKGRSLIGKTDSS	860
Peptide J	Pig (*Sus scrofa*)	PAR-2	GTSRPSKGRSLIGKADNT	861
Peptide K	Human	PAR-1	ATNATLDPRSFLLRNPND	862
Peptide L	Human	PAR-3	DTNNLAKPTLPIKTFRGA	863
Peptide M	Human	PAR-4	ESGSTGGGDDSTPSILPAP	864

[0115] Anti-PAR-2 antibodies were tested for their ability to bind to the PAR-2 peptides. Various PAR-2 Peptides (Table 3) were coated onto 96-well plates at a concentration of 2 µg/ml and incubated overnight followed by blocking in a suitable blocking agent for one hour. In a similar fashion, for biotinylated peptides (N-Term: N-terminal biotinylated; C-Term: C-terminal biotinylated), avidin was coated on plates at 2 µg/ml followed by incubation with biotinylated PAR-2 peptides at a concentration of 0.2 µg/ml and incubated for one hour. Purified anti-PAR-2 antibodies were added to the plate coated with PAR-2 peptides to a final concentration ranging from 0.2 to 2.0 µg/ml and incubated for one hour at room temperature. Detection of bound antibodies was determined with Horse-Radish Peroxidase (HRP) conjugated anti-mouse or human IgG (Jackson Immuno Research Lab, West Grove, PA) and developed by standard colorimetric response using tetramethylbenzidine (TMB) substrate. Absorbance was read at OD_{450} for 0.1 second.

[0116] Relative binding (+++, ++, +) to unbiotinylated (No Biotin) or biotinylated Peptides A, B and C as compared to no binding (-) for each anti-PAR-2 antibody tested according to the observed OD_{450} value (1.0 – 4.0, 0.50 – 0.99, 0.1 – 0.49, 0.0 – 0.09, respectively) is shown in Table 3. Control: "Sam11," a commercially available mouse monoclonal antibody that binds human PAR-2 (Santa Cruz Biotechnology, Santa Cruz, CA).

Table 3

Antibody	Peptide A			Peptide B			Peptide C	
	No Biotin	N-Term	C-Term	No Biotin	N-Term	C-Term	N-Term	C-Term
H2M588N	+++	+++	+++	+++	+++	+++	-	-
H2M589N	+++	+++	+++	+++	+++	+++	-	-
H1M590N	+++	+++	+++	-	-	-	-	-
H2M591N	+++	+++	+++	+++	+++	+++	-	-
H1M592N	+++	+++	+++	-	++	+++	+++	+++
H1M595N	+++	+++	+++	-	-	-	++	-
H2M609N	+++	+++	+++	-	-	-	-	-
H2M610N	+++	+++	+++	-	-	-	-	-
H2M611N	+++	+++	+++	+++	+++	+++	-	-
H1M612N	+++	+++	+++	-	-	-	-	-

H1M613N	+++	+++	+++	-	++	+++	+++	+++
H2M614N	++	+++	+++	-	-	-	-	-
H1M615N	+++	+++	+++	-	-	-	-	-
H1M616N	+++	+++	+++	-	-	-	-	-
H2M618N	+++	+++	+++	+++	+++	+++	-	-
H1M619N	+++	+++	+++	-	-	-	-	-
H3M620N	+++	++	+++	-	-	-	-	-
Control	-	-	-	+++	+++	+++	-	-

[0117] In a similar experiment, selected anti-PAR-2 antibodies cloned onto a mutant human IgG4 (SEQ ID NO:849) were tested for their ability to bind unbiotinylated and biotinylated forms of human PAR-2 peptides (as described above). Results are shown in Table 4.

Table 4

Antibody	Peptide A			Peptide B			Peptide C	
	No Biotin	N-Term	C-Term	No Biotin	N-Term	C-Term	N-Term	C-Term
H4H572P	-	-	-	-	-	-	-	-
H4H573P	+++	+++	+++	-	-	-	-	-
H4H576P	+	-	-	-	-	-	-	-
H4H578P	+++	+++	+++	-	-	-	-	-
H4H579P	+++	+++	+++	+++	+++	+++	-	-
H4H580P	+++	+++	+++	+++	+++	+++	-	-
H4H581P	+++	+++	+++	+++	+++	+++	++	+
H4H583P	+++	+++	+++	-	-	-	-	-
H4H584P	+++	+++	+++	+++	+++	+++	-	-
H4H585P	+++	+++	+++	+++	+++	+++	+	+
H4H587P	-	-	-	-	-	-	-	+
H4H588N	+++	+++	+++	+++	+++	+++	-	-
H4H591N	+++	+++	+++	+++	+++	+++	-	-
H4H618N	+++	+++	+++	+++	+++	+++	-	-
Control	-	-	-	+++	+++	+++	-	-

[0118] In another experiment, selected anti-PAR-2 antibodies were tested for binding to unbiotinylated Peptides D, K, L and M (as described above). Results for chimeric antibodies (e.g. H2M588N) and fully human antibodies (e.g. H4H572P) are shown in Tables 5 and 6, respectively. For Peptide D, detection of bound antibodies was determined with Horse-Radish Peroxidase (HRP) conjugated anti-mouse κ (Southern Biotech, Birmingham, AL).

Table 5

Antibody	Peptide D	Peptide K	Peptide L	Peptide M
H2M588N	+++	-	-	-
H2M589N	+++	-	-	-
H1M590N	-	-	-	-
H2M591N	+++	-	-	-
H1M592N	+	-	-	-
H1M595N	-	-	-	-
H2M609N	+	-	-	-
H2M610N	-	-	-	-
H2M611N	+	-	-	-
H1M612N	-	-	-	-
H1M613N	+	-	-	-
H2M614N	-	-	-	-
H1M615N	-	-	-	-
H1M616N	-	-	-	-
H2M618N	+++	-	-	-
H1M619N	-	-	-	-
H3M620N	-	-	-	-
Control	+++	-	-	-

Table 6

Antibody	Peptide D	Peptide K	Peptide L	Peptide M
H4H572P	+	-	-	-
H4H573P	+	-	-	-
H4H576P	+	-	-	-
H4H578P	+	-	-	-
H4H579P	+++	-	-	-
H4H580P	+++	-	-	-
H4H581P	+++	+	+	+
H4H583P	++	-	-	-
H4H584P	+++	-	-	-
H4H585P	+++	+	+	+
H4H587P	+	-	-	-
H4H588N	+++	-	-	-
H4H591N	+++	+	+	+
H4H618N	+++	-	-	-
Control	+++	-	-	-

[0119] In another experiment, selected anti-PAR-2 antibodies were tested for binding to N-

terminal biotinylated mouse (Peptide E N-Term) and monkey (Peptide F N-Term) PAR-2 peptides (as described above).

Table 7

Antibody	Peptide E N-Term	Peptide F N-Term
H2M588N	-	+++
H2M589N	-	+++
H1M590N	+++	+++
H2M591N	-	+++
H1M592N	-	-
H1M595N	+++	+++
H2M609N	+++	+++
H2M610N	+++	+++
H2M611N	-	+++
H1M612N	+++	+++
H1M613N	-	-
H2M614N	+++	+++
H1M615N	+++	+++
H1M616N	+++	+++
H2M618N	-	+++
H1M619N	+++	+++
H3M620N	+++	++
Control	-	-

[0120] In another experiment, selected anti-PAR-2 antibodies cloned onto human IgG4 were tested for binding to unbiotinylated and biotinylated forms of Peptides E through J (as described above). Results are shown in Table 8.

Table 8

Antibody	Peptide E		Peptide F N-Term	Peptide G			Peptide H	Peptide I	Peptide J
	No Biotin	N-Term		No Biotin	N-Term	C-Term			
H4H572P	+	-	-	-	-	+	-	-	-
H4H573P	+++	+++	+++	+++	+++	++	+++	+++	+++
H4H576P	+	-	-	-	-	+	-	-	-
H4H578P	+++	+++	+++	+++	+++	+	+++	+++	+++
H4H579P	+++	+++	+++	+++	+++	+++	+	+++	+
H4H580P	+++	+++	+++	+++	+++	+++	-	+++	+
H4H581P	+++	+++	+++	+++	+++	+++	-	+++	+++
H4H583P	+++	+++	+++	+++	+++	+++	+++	+++	+++
H4H584P	+++	+++	+++	+++	+++	+++	-	+++	++
H4H585P	+++	+++	+++	+++	+++	+++	+	+++	+++

H4H587P	-	-	-	-	+	-	-	-	+
H4H588N	-	-	+++	-	+	-	-	-	-
H4H591N	+	+	+++	+	+	+	+	-	+
H4H618N	-	-	+++	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-

[0121] In another experiment, selected anti-PAR-2 antibodies cloned onto human IgG4 were tested for binding to unbiotinylated and biotinylated forms of PAR-2 peptides (Peptides A, E, F and G; as described above). In this experiment, anti-PAR-2 antibodies, serially diluted three-fold from 13.3 nM to 0.22 pM, were incubated on the peptide-coated plates for one hour at room temperature. Absorbance values at 450 nm were analyzed using a sigmoidal dose-response model in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) and EC_{50} values were reported (Table 9). EC_{50} values are defined as the antibody concentration required to achieve 50% maximal binding to PAR-2 peptide.

Table 9

Antibody	EC_{50} (nM)								
	Peptide A			Peptide E		Peptide F N-Term	Peptide G		
	No Biotin	C-Term	N-Term	No Biotin	N-Term		No Biotin	C-Term	N-Term
H4H572P	>50	>50	>50	>50	>50	>50	>50	>50	>50
H4H573P	0.093	0.022	0.093	0.355	0.016	0.018	0.014	10.130	0.017
H4H576P	>50	>50	>50	>50	>50	>50	>50	>50	>50
H4H578P	0.034	0.040	0.062	2.778	0.090	0.036	0.046	>50	0.081
H4H579P	0.038	0.076	0.077	0.713	0.540	0.061	0.055	0.044	0.059
H4H580P	0.090	0.202	0.160	2.533	0.932	0.148	0.139	0.100	0.142
H4H581P	0.012	0.028	0.020	0.029	0.032	0.020	0.020	0.013	0.019
H4H583P	0.008	0.018	0.015	0.019	0.014	0.013	0.012	0.630	0.015
H4H584P	0.012	0.015	0.019	2.152	0.511	0.012	0.013	0.015	0.012
H4H585P	0.017	0.021	0.026	0.308	0.189	0.017	0.018	0.021	0.017
H4H587P	>50	>50	>50	>50	>50	>50	>50	>50	>50
H4H588N	0.010	0.016	0.019	>50	>50	0.012	>50	>50	>50
H4H591N	0.010	0.016	0.022	>50	>50	0.011	>50	>50	>50
H4H618N	0.009	0.016	0.021	>50	>50	0.011	>50	>50	>50
Control	>50	>50	>50	>50	>50	>50	>50	>50	>50

[0122] As indicated by the foregoing experiments, antibodies H4H581P, H4H588N, H4H591N or H4H618N all show substantial binding to human Peptides A, B and D, which comprise the sequence SLIGKVDGT (amino acids 10-18 of SEQ ID NO:852), as well as to monkey Peptide F, which comprises the sequence SLIGRVDGT (amino acids 10-18 of SEQ ID NO:857). For antibodies H4H588N, H4H591N and H4H618N, the sequence VDGT, located downstream from

the activating PAR-2 protease cleavage site, appears to be particularly important for binding since changes to this sequence resulted in substantially reduced or no binding by these antibodies (see, e.g., binding data for Peptides E (mouse), G (rat), H (rabbit), I (dog) and J (pig)).

Example 4. Antigen Binding Affinity Determination

[0123] Equilibrium dissociation constants (K_D values) for antigen binding to selected purified PAR-2 antibodies were determined by surface kinetics using a real-time surface plasmon resonance biosensor assay. Antibody was captured on either a rabbit anti-mouse IgG polyclonal antibody (GE Healthcare, Piscataway, NJ) surface or a goat anti-human IgG polyclonal antibody (Jackson Immuno Research Lab, West Grove, PA) surface created through direct amine chemical coupling to a BIACORE™ CM5 sensor chip to form a captured antibody surface. Various concentrations (ranging from 15.6 to 250 nM) of monomeric human PAR-2 peptides (Peptides A and B) were injected at a rate of 100 µl/min over the captured antibody surface for 90 seconds. Antigen-antibody binding and dissociation were monitored in real time at room temperature. Kinetic analysis was performed to calculate K_D and half-life of antigen/antibody complex dissociation (Table 10). For those antibodies where no $T_{1/2}$ value is shown, steady state analysis was used to calculate the K_D value. NB: no binding observed under current experimental conditions. ND: not determined.

Table 10

Antibody	Peptide A		Peptide B	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H4H572P	NB	-	ND	-
H4H573P	749	-	ND	-
H4H576P	NB	-	ND	-
H4H578P	245	0.17	ND	-
H4H579P	15	4	>500	-
H4H580P	103	0.88	ND	-
H4H581P	9.44	1.4	>500	-
H4H583P	37.1	0.37	NB	-
H4H584P	22	0.73	>500	-
H4H585P	8.78	3.7	>500	-
H4H587P	NB	-	ND	-
H1M590N	173	-	ND	-
H1M592N	2100	-	ND	-
H1M595N	510	-	ND	-
H1M612N	180	-	ND	-
H1M613N	2100	-	ND	-
H1M615N	162	-	ND	-

H1M616N	144	-	ND	-
H1M619N	164	-	ND	-
H2M588N	4.47	60.8	61.7	7.7
H2M589N	3.75	51.3	128	7.3
H2M591N	4.22	42.7	151	8.1
H2M610N	71.3	-	360	0.8
H2M611N	72.8	4.1	1090	1.2
H2M614N	470	-	ND	-
H2M618N	9.62	6.7	126	3.5
Control	NB	-	149	0.1

[0124] In a similar experiment, K_D values for binding to unbiotinylated monomeric mouse (Peptide E) and N-terminal biotinylated monkey (Peptide F N-Term) PAR-2 peptides of selected antibodies cloned onto human IgG4 were determined (as described above) (Table 11). Antibodies H4H588N, H4H591N, and H4H618N did not bind Peptide E, while the Control antibody did not bind to either Peptide E or F.

Table 11

Antibody	Peptide F N-Term	
	K_D (nM)	$T_{1/2}$ (min)
H4H588N	7.10	24
H4H591N	8.54	21
H4H618N	13.8	5

[0125] In another series of experiments, equilibrium dissociation constants (K_D values) for purified antibody binding to selected biotinylated and unbiotinylated forms of PAR-2 peptides were determined by surface kinetics using a real-time surface plasmon resonance biosensor assay. Neutravidin (Pierce, Rockford, IL) was covalently coupled to the surface of a Biacore™ C1 chip or CM5 chip using amine coupling chemistry. Biotinylated (N-Term or C-Term) PAR-2 peptides (Peptides A and B) were immobilized on the surface via the high affinity binding interaction between biotin and the amine coupled Neutravidin.

[0126] In a first experiment using this format, varying concentrations (ranging from 5 to 100 µg/ml) of purified antibody were injected at a rate of 50 µl/min over a surface coated with immobilized peptide at low-density (<1RU) for 300 seconds. Antibody-peptide binding and dissociation was monitored in real time 25°C (Table 12).

Table 12

Antibody	Peptide A C-Term		Peptide B C-Term	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H2M588N	0.826	169	1.49	140
H1M590N	1.27	13	NB	-
H2M591N	0.545	209	1.82	94
H2M618N	1.8	79	2.32	60
Control	NB	-	0.99	19

[0127] In another similar experiment, K_D values for binding to a low-density surface (<1RU) of biotinylated forms of Peptides A, B, C, E, F and G of selected antibodies cloned onto human IgG4 were determined (as described above). Results for binding to C-terminal and N-terminal biotinylated PAR-2 peptides are shown in Tables 13-14, respectively. In this experiment, only antibody H4H581P demonstrated affinity for N-terminal biotinylated Peptide C (K_D of >100 nM), while all other antibodies tested, including the control, showed no binding to this peptide.

Table 13

Antibody	Peptide A C-Term		Peptide B C-Term	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H4H579P	0.143	479	0.453	96
H4H581P	0.237	134	0.346	49
H4H583P	0.898	34	NB	-
H4H584P	0.688	55	1.66	15
H4H585P	0.151	493	0.376	123
H4H588N	0.517	141	1.03	70
H4H590N	3.56	3	NB	-
Control	NB	-	0.935	14

Table 14

Antibody	Peptide A N-Term		Peptide B N-Term		Peptide E N-Term		Peptide F N-Term		Peptide G N-Term	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H4H579P	1.61	46	5.44	55	3.40	20	0.796	70	1.16	38
H4H581P	0.498	41	2.72	38	1.13	21	0.272	78	0.354	62
H4H583P	0.412	46	NB	-	1.68	11	0.291	73	1.49	13
H4H584P	0.525	43	3.20	30	>100	-	0.565	40	1.39	15
H4H585P	1.69	48	5.41	63	>100	-	0.899	66	1.36	35

H4H588N	1.05	189	0.0272	>1155	NB	-	1.81	94	NB	-
H4H590N	1.52	3	NB	-	1.28	7	1.84	2	2.94	2
Control	NB	-	0.279	32	NB	-	NB	-	NB	-

[0128] In a similar experiment, K_D values for binding to monomeric biotinylated and unbiotinylated forms of PAR-2 peptides (Peptides A, B, E – M) for selected antibodies cloned onto human IgG4 were determined (as described above for the captured antibody surface). Results are shown in Tables 15-16. None of the antibodies tested showed binding to Peptides K, L or M.

Table 15

Antibody	Peptide A		Peptide B		Peptide E	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H4H579P	15	4.1	>500	-	>500	-
H4H581P	9.44	1.4	>500	-	>500	-
H4H583P	37.1	0.37	NB	-	>500	-
H4H584P	22	0.73	NB	-	NB	-
H4H585P	8.78	3.7	>500	-	>500	-
H4H588N	4.95	25.1	84	9	NB	-
H4H590N	>500	-	NB	-	>500	-
Control	ND	-	149	0.1	ND	-

Table 16

Antibody	Peptide F N-Term		Peptide G		Peptide H		Peptide I		Peptide J	
	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)	K_D (nM)	$T_{1/2}$ (min)
H4H579P	49.4	2	134	0.45	NB	-	311	0.26	NB	-
H4H581P	36.6	0.53	40.2	0.63	NB	-	143	0.07	NB	-
H4H583P	112	0.47	530	0.07	189	0.17	320	0.16	190	0.16
H4H584P	93.3	0.23	246	0.13	NB	-	>500	-	NB	-
H4H585P	46.2	2	162	0.33	NB	-	372	0.19	NB	-
H4H588N	10.8	20	NB	-	NB	-	NB	-	NB	-
H4H590N	>500	-	>500	-	>500	-	>500	-	>500	-

Example 5. Antibody Binding to Cells Engineered to Express PAR-2

[0129] To further characterize anti-PAR-2 antibodies, cells of the human embryonic kidney 293 cell line (HEK293) were genetically engineered to overexpress either full length human (SEQ ID NO:851) or mouse (SEQ ID NO:866) PAR-2.

[0130] HEK293 cells were transfected with an NF-κB-luciferase-IRES-eGFP reporter plasmid.

Stability of transfected cells was demonstrated by response to IL-1β as detected by eGFP expression through flow cytometry and luciferase activity. A clonal cell line, named D9, having low background levels of luciferase activity and high levels of eGFP when induced with IL-1β was made by a series of successive sorts of cell populations using flow cytometry. The 293/D9 cell line was then separately transfected with human PAR-2 or mouse PAR-2 to create the stable cell lines 293/D9/hPAR-2rec and 293/D9/mPAR-2rec, respectively.

[0131] Binding of anti-PAR-2 antibodies to 293/D9/hPAR-2rec cells was determined by ELISA. 293/D9 and 293/D9/hPAR-2rec cells were plated at a density of $5X10^4$ cells/well in media and incubated overnight at 37°C and 5% CO_2. Purified antibody was added to the cells to a final concentration of 10 μg/ml and incubated at room temperature for one hour. Cells were then fixed and washed before detection of bound antibodies with HRP conjugated anti-mouse IgG and developed by standard colorimetric response using TMB substrate. Absorbance was read at OD_{450} for 0.1 second. The A_{450} ratio of antibody binding to 293/D9/hPAR-2rec cells compared to 293/D9 cells is shown in Table 17.

Table 17

Antibody	A_{450} ratio
H2M588N	2.50
H2M589N	2.03
H1M590N	1.81
H2M591N	2.25
H1M592N	1.25
H1M595N	1.80
H2M609N	1.54
H2M610N	1.66
H2M611N	1.76
H1M612N	1.90
H1M613N	1.27
H2M614N	1.17
H1M615N	2.18
H1M616N	2.78
H2M618N	2.44
H1M619N	1.51
H3M620N	1.24
Control	1.49

[0132] In a similar experiment, anti-PAR-2 antibodies were tested for binding to 293/D9, 293/D9/hPAR-2rec and 293/D9/mPAR-2rec cells using electro-chemiluminescence technology (Meso Scale Discovery, MSD, Gaithersburg, MD). Cells were plated on MSD high-bind 96 well

plates at a density of $4X10^4$ cells/well in PBS and incubated for one hour at room temperature. Cells were then blocked in PBS with 2% BSA and incubated at room temperature for one hour. Anti-PAR-2 antibodies (ranging from 100 nM to 0.098 nM) were serially diluted two-fold in PBS with 0.5% BSA and incubated with the cells for one hour at room temperature followed by washing in PBS with 0.5% BSA. Sulfo-tagged anti-human IgG antibody (MSD) at a concentration of 0.1 µg/ml was then added to the cell/antibody mixture and incubated at room temperature for an additional hour. After another wash, a 1X non-surfactant containing read buffer was added and electro-chemiluminescent signal was read on the MSD Sector Imager. Signal of antibody binding to 293/D9 cells was subtracted from signal to 293/D9/hPAR-2rec or 293/D9/mPAR-2rec cells. Subtracted data were analyzed using a sigmoidal dose-response model in GraphPad Prism and EC_{50} and B_{max} values were reported (Table 18). EC_{50} values are defined as the antibody concentration required to achieve 50% maximal binding (B_{max}) to cells.

Table 18

Antibody	293/D9/hPAR-2rec		293/D9/mPAR-2rec	
	EC_{50} (nM)	B_{max} (MSD Unit)	EC_{50} (nM)	B_{max} (MSD Unit)
H4H579P	1.86	25049	NB	-
H4H580P	8.69	30177	NB	-
H4H581P	0.652	28224	NB	-
H4H583P	2.12	13252	9.28	7219
H4H584P	1.45	26044	NB	-
H4H585P	1.95	22540	NB	-
H4H588N	5.95	27549	NB	-
H4H590N	9.72	6554	5.76	13255

Example 6. *In vitro* Blocking of Human PAR-2 Activation By Anti-PAR-2 Antibodies

[0133] Blocking of PAR-2 activation (signaling) was determined by binding of selected purified anti-PAR-2 antibodies to 293/D9/hPAR-2rec cells (see Example 5) by a luciferase assay. 293/D9/hPAR-2rec cells were plated at a concentration ranging from $5X10^4$ to 10^5 cells/well in a 96 well plate in low serum media and incubated overnight at 37°C with 5% CO_2. The media was removed and purified anti-PAR-2 antibodies were added to the cells at various concentrations (ranging from 51 pM to 1 µM) and incubated for one hour at 37°C with 5% CO_2. Various concentrations of different serine proteases (Trypsin, Human Trypsin 1, Factor Xa and Lung Tryptase) were then added separately to the cell/antibody mixture and incubated for five hours at 37°C with 5% CO_2. Proteolytic cleavage of PAR-2 in this assay leads to expression of the NF-κB-luciferase reporter construct, whereas a reduced or attenuated level of luciferase signal indicates inhibition of PAR-2 cleavage. IC_{50} values are shown in Table 19. ND: not determined.

Table 19

Antibody	IC_{50} (nM)							
	Trypsin					10 nM Human Trypsin 1	200 nM Factor Xa	750 nM Lung Tryptase
	250 nM	75 nM	30 nM	20 nM	10 nM			
H2M588N	2.3	5.7	33.0	26.8	10.4	164.9	45.3	1.0
H2M589N	6.3	3.7	ND	ND	ND	ND	ND	ND
H1M591N	4.5	ND	ND	121.8	24.2	149.6	51.5	88.1
H2M618N	6.6	7.8	ND	70	12.4	153.5	54.2	7.0

[0134] As shown in Table 20, antibodies H4H581P, H4H588N, H4H591N and H4H618N were able to significantly block protease activation of PAR-2 in reporter cells. By contrast, anti-PAR-2 antibodies H4H592N, H4H595N, H4H611N, H4H613N, H4H614N, H4H615N, H4H616N, H4H617N and H4H619N did not demonstrate any measurable blocking of PAR-2 cleavage/activation in this assay (data not shown).

Table 20

Antibody	20 nM Trypsin IC_{50} (nM)
H4H579P	4.1
H4H580P	3.5
H4H581P	1.1
H4H583P	14
H4H584P	5.6
H4H585P	8.9
H4H588N	16.9
H4H591N	204.1
H4H618N	50.2

[0135] Under the experimental conditions used in this Example, no blocking of PAR-2 signaling was observed for antibodies H4H572P, H4H573P, H4H576P, H4H578P or H4H587P, whereas significant blockage (to varying degrees) was observed with antibodies H4H579P, H4H580P, H4H581P, H4H583P, H4H584P, H4H585P, H4H588N, H4H591N and H4H618N.

[0136] In another similar experiment, antibody blocking of PAR-2 signaling mediated by human Trypsin 1, Factor Xa and Lung Tryptase was determined for selected purified anti-PAR-2 antibodies cloned onto human IgG4 (as described above). Results are shown in Table 21.

Table 21. IC_{50} (nM)

Antibody	10 nM Human Trypsin 1	200 nM Factor Xa	750 nM Lung Tryptase
H4H581P	7.4	5.3	1.1
H4H588N	42.7	17.1	1.6
H4H591N	104.7	74.6	62.9
H4H618N	92.2	47.2	8.5

[0137] HEK293/NFκB-luciferase cells expressing human, monkey, mouse or rat PAR-2 were treated with various proteases after preincubation with increasing amounts of anti-PAR-2 antibody H4H581P, and the IC_{50} was determined. Results are summarized in Table 22.

Table 22

Activator (6h)	PAR-2 Species	EC_{50} (nM)	H4H581P IC_{50} (nm)
Human Pancreatic Trypsin	Human	0.8	1.9
	mouse	1.4	491.0
	monkey	2.4	5.4
	rat	6.0	700.0
Human Kallikrein 5	Human	28.0	0.9
	mouse	ND	ND
	monkey	16.8	11
Human Kallikrein 14	Human	5.0	1.2
	mouse	8.0	256.0
	monkey	6.3	11.6
Bovine Factor Xa	Human	27.2	2.5
	mouse	46.3	340.4
	monkey	58.2	0.9
Human Factor Xa	Human	46.3	1.1
	mouse	78.7	109
	monkey	ND	ND
Tryptase	Human	61.1	3.5
	mouse	ND	ND
	monkey	ND	ND

[0138] Under the particular experimental conditions used, the H4H581P antibody effectively inhibited protease-activation of human and monkey PAR-2, but not mouse or rat PAR-2.

Example 7. *In vitro* Antibody Blocking of Human PAR-2 Dependent Calcium Mobilization

[0139] Blocking of trypsin-stimulated PAR-2 activation (signaling) was determined by treating

HEK293 cells with selected purified anti-PAR-2 antibodies cloned onto human IgG4 in a calcium mobilization FLIPR assay (Molecular Devices, Sunnyvale, CA). Also tested in this assay was a non-PAR-2 specific control antibody.

[0140] Briefly, $8X10^4$ HEK293 cells were plated on Poly-D-Lysine plates (BD Biosciences, San Jose, CA) in low serum media (DME with 0.5% FBS) and incubated overnight at 37°C with 5% CO_2. The following day cells were incubated with various concentrations (ranging from 0 to 1 μM) of selected anti-PAR-2 antibodies, or a control antibody, followed by the addition of trypsin. Trypsin-mediated activation of PAR-2 is indicated by calcium mobilization. In-cell measurement of calcium signaling was measured using a Fluo-4 NW Calcium Assay Kit (Invitrogen, Carlsbad, CA) on a FlexStation 3 (Molecular Devices, Sunnyvale, CA). The antibody concentration necessary to cause half-maximal inhibition of trypsin-mediated calcium signaling (IC_{50}) was measured for each experimental and control antibody. Results are shown in Table 23 as IC_{50} (nM).

Table 23

Antibody	100 nM Trypsin
H4H581P	54.96
H4H588N	29.47
Non-Specific Control	> 1000

[0141] As shown in this Example, antibodies H4H581P and H4H588N each inhibited trypsin-stimulated calcium signaling to a significant extent as compared to the control antibody.

Example 8. *In vitro* Blocking of Trypsin-mediated Cleavage of PAR-2 Peptides

[0142] A Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) assay was developed to determine the ability of selected purified anti-PAR-2 antibodies to block Trypsin-mediated cleavage of human PAR-2 peptide (Peptide A, SEQ ID NO:852). Biotinylated versions of Peptide A (containing C-terminal or N-terminal biotin) were mixed with anti-PAR-2 antibody to achieve a 3:1 molar ratio of antibody to peptide, and then trypsin was added to the peptide-antibody mixture. Biotinlyated peptides were recovered by immuno-precipitation (IP) via mono-avidin and analyzed by MALDI-TOF.

[0143] In a typical experiment, selected purified anti-PAR-2 antibodies cloned onto human IgG4 (H4H581P and H4H588N) were tested for their ability to block Trypsin-mediated cleavage of the biotinylated PAR-2 peptides. A non-PAR-2-specific antibody of the same isotype was used as a negative control ("Neg Ctrl" in Fig. 3). For H4H581P and the negative control antibody, biotinylated peptides were used at a final concentration of 4.75 μM and antibodies were used at 14.25 μM. For H4H588N, biotinylated peptides were used at a final concentration of 2.45 μM and the antibody at 7.35 μM. Peptide and antibody were mixed in PBS and allowed to come to equilibrium for 1 hr at room temperature. Trypsin (96 ng) was then added and the

mixture incubated at 37°C for 0, 5, 10 and 15 minutes. At each time point an aliquot equaling 100 ng of biotinylated peptide was removed and mixed with 10 µl of monomeric avidin resin (Pierce) for 1 minute. The bound peptides were rinsed 3x with 200 µl of PBS and then eluted with 20 µl of 100 mM glycine pH 2.5. Salt was removed from the eluted peptide mixture using ZipTips (Millipore). The molecular weights of the major biotinylated PAR-2 peptides produced after trypsin cleavage, as revealed by MALDI-TOF analysis, are summarized in Fig. 3.

[0144] The PAR-2 peptides used in these experiments contain two R/S protease cleavage sites. The first site (designated site "(1)" in Fig. 3) is an "upstream" cleavage site located N-terminal to the activating PAR-2 protease cleavage site. The activating PAR-2 protease cleavage site (designated site "(2)" in Fig. 3) is the site which, when cleaved, results in the formation of the PAR-2 tethered ligand in the naturally occurring protein. The sizes of the peptides detected following cleavage at the different sites are shown in the top portion of Fig. 3 (panel A).

[0145] As indicated in Fig. 3 (panel B), the C-terminal biotin PAR-2 peptide, when treated with the isotype-matched control antibody, followed by trypsin incubation, produced a cleavage fragment of 1558 Da (containing residues 10-18 of SEQ ID NO:852). The 1558 Da fragment is the result of cleavage at the activating PAR-2 protease cleavage site (2). This cleavage pattern was also observed in the experiment using the H4H588N anti-PAR-2 antibody. Thus, according to this assay, neither the control antibody nor the H4H588N antibody inhibit trypsin cleavage at the activating PAR-2 protease cleavage site (2).

[0146] By contrast, the C-terminal biotin PAR-2 peptide, when treated with the H4H581P antibody, followed by trypsin incubation, produced a cleavage fragment of 2073 Da (containing residues 5-18 of SEQ ID NO:852). The 2073 Da fragment is the fragment produced by cleavage at the upstream cleavage site (1) only. Thus, cleavage at the activating PAR-2 protease cleavage site (2) was apparently blocked by the H4H581P antibody.

[0147] Experiments with the N-terminal biotin PAR-2 peptide showed trypsin cleavage at the upstream cleavage site (1) and thus produced a 988 Da N-biotinylated fragment (containing residues 1-4 of SEQ ID NO:852) in the presence of all antibodies tested. Therefore, none of the antibodies tested blocked cleavage at the upstream cleavage site (1) under these experimental conditions.

[0148] As shown in Examples 6 and 7 above, both H4H581P and H4H588N blocked PAR-2 activation by trypsin in cell-based assays. In the present Example, however, only H4H581P blocked trypsin cleavage at the activating PAR-2 protease cleavage site. Without being bound by any mechanistic theory, it therefore appears that H4H588N may exert its inhibitory effect(s) by interfering with the interaction between the tethered ligand and one or more extracellular loops (*e.g.*, loop 1, loop 2 and/or loop 3) of PAR-2. On the other hand, H4H581P may inhibit PAR-2 activity primarily by blocking protease cleavage but may also interfere with tethered ligand interactions as well.

[0149] To further investigate the protease cleavage-blocking properties of anti-PAR-2 antibodies, additional MALDI-TOF experiments were conducted using C-terminal biotinylated mouse, rat and human PAR-2 peptides. (See Fig. 4). The antibodies tested in these experiments were H4H581P, H4H588N, a comparator antibody having the heavy and light chain variable regions of the antibody referred to as "1A1" in WO 2009/005726 (referred to in Fig. 5 as "Comp. Ab"), and a negative control antibody (referred to in Fig. 4 as "Neg Ctrl"). The same experimental procedures that were used in the previous MALD-TOF experiment (described above) were used in this experiment as well.

[0150] The peptides used in these experiments each possess multiple sites capable of being cleaved by trypsin (designated "(1)," "(2)," and "(3)" in Fig. 5.) Site (3) for each peptide is the activating protease cleavage site. The sizes of the peptides produced following cleavage at the different sites are shown in the top portion (panel A) of Fig. 4.

[0151] As summarized in Fig. 4, the biotinylated human PAR-2 peptide, after being treated with H4H581P, and following trypsin incubation, produced a 2074 kDa peptide which corresponds to cleavage at site (1) only. Thus, H4H581P blocks cleavage at both site (2) and at site (3). By contrast, the human PAR-2 peptide, after being treated with the comparator antibody, and following trypsin incubation, remained at 2502 kDa which signifies no cleavage. Thus, the comparator antibody blocks all three protease cleavage sites in this assay, including the N-terminal-most site (1). When pre-treated with antibody H4H588N, the human PAR-2 peptide produces both a 1772 kDa and a 1558 kDa fragment following trypsin cleavage. This cleavage pattern suggests that H4H588N partially blocks cleavage at the activating site (3) but completely blocks the middle site (2).

[0152] This experiment was also conducted using a comparator antibody having the heavy and light chain variable regions of the antibody referred to as Sam-11 (Molino *et al.*, Arterioscler. Thromb. Vasc. Biol. 18:825-832 (1998)). As expected, this particular comparator antibody did not block cleavage at any of the protease cleavage sites (data not shown).

Example 9. Epitope Mapping by Alanine Scanning Mutagenesis of PAR-2 Peptide

[0153] In order to more particularly identify the amino acids of PAR-2 with which the PAR-2 antibodies interact, an alanine scanning study was carried out using peptides comprising the activating PAR-2 protease cleavage site. For these experiments, 11 separate C-terminal biotinylated peptides were synthesized in which each amino acid from position 35 through 45 of human PAR-2 (SEQ ID NO:851) was individually replaced with an alanine (SEQ ID NOs: 871-882). An additional set of C-terminal biotinylated peptides was also used which comprise the 14 amino acids located immediately C-terminal to the activating PAR-2 protease cleavage site, with Val-42 and/or Asp-43 changed to alanine (SEQ ID NOs: 884-887).

[0154] The ability of each peptide mutant to bind to PAR-2 antibodies was measured using biolayer interferometry (Octet Red; ForteBio). Each peptide (2.5µg/ml) was captured on streptavidin coated biosensor tips (Octet SA sensor) for 10 seconds. To measure binding and

dissociation between each peptide and PAR-2 antibody, the peptide-coated biosensors were contacted with 200nM solutions of PAR-2 antibodies for 5 minutes (binding) followed by transfer to buffer with no antibody for 10 min (dissociation). The binding of PAR-2 antibody to each peptide was expressed as percent native signal after dividing individual antibody binding signals by the original peptide loading signal observed for that peptide, to correct for slight variations in peptide loading on the individual biosensors. Dissociation half-lives ($T_{1/2}$) were calculated from the dissociation curves using the Scrubber version 2.0a curve-fitting software, and relative half-lives were calculated by dividing observed half-lives for an individual peptide by the half-life of the native peptide. The results are expressed as percent binding and percent $T_{1/2}$ relative to WT peptide (Tables 24-28). [Comparator 1 = an antibody having the heavy and light chain variable regions of the antibody referred to as "1A1" in WO 2009/005726; Comparator 2 = an antibody having the heavy and light chain variable regions of the antibody referred to as Sam-11 (Molino *et al.*, Arterioscler. Thromb. Vasc. Biol. 18:825-832 (1998)); and Comparator 3 = an antibody having the heavy and light chain variable regions of the antibody referred to as "PAR-B" in US 2010/0119506]. In certain cases, the binding experiments were repeated (indicated under the column headings Exp1 and Exp2). NB = no binding observed.

Table 24: H4H581P

SEQ ID NO:	SEQUENCE	% Rel Binding		% Rel $T_{½}$	
		Exp1	Exp2	Exp1	Exp2
871	GTNRSSKGRSLIGKVDGT------GGGGSK-B	100	100	100	100
872	GTNRSSK**A**RSLIGKVDGT------GGGGSK-B	78	97	118	117
873	GTNRSSKG**A**SLIGKVDGT------GGGGSK-B	87	105	122	90
874	GTNRSSKGR**A**LIGKVDGT------GGGGSK-B	45	56	7	3
875	GTNRSSKGRS**A**IGKVDGT------GGGGSK-B	9	19	0.4	0
876	GTNRSSKGRSL**A**GKVDGT------GGGGSK-B	8	14	0.2	0
877	GTNRSSKGRSLI**A**KVDGT------GGGGSK-B	101	92	33	15
878	GTNRSSKGRSLIG**A**VDGT------GGGGSK-B	113	109	63	49
879	GTNRSSKGRSLIGK**A**DGT------GGGGSK-B	36	46	1	1
880	GTNRSSKGRSLIGKV**A**GT------GGGGSK-B	18	5	36	4
881	GTNRSSKGRSLIGKVD**A**T------GGGGSK-B	67	102	117	124
882	GTNRSSKGRSLIGKVDG**A**------GGGGSK-B	65	91	129	94

884	SLIGKVDGTSHVTG-GGGGSK-B	---	100	---	100
885	SLIGKADGTSHVTG-GGGGSK-B	---	19	---	0
886	SLIGKVAGTSHVTG-GGGGSK-B	---	NB	---	NB
887	SLIGKAAGTSHVTG-GGGGSK-B	---	NB	---	NB

Table 25: H4H588N

SEQ ID NO:	SEQUENCE	% Rel Binding		% Rel $T_{½}$	
		Exp1	Exp2	Exp1	Exp2
871	GTNRSSKGRSLIGKVDGT------GGGGSK-B	100	100	100	100
872	GTNRSSKARSLIGKVDGT------GGGGSK-B	122	99	100	253
873	GTNRSSKGASLIGKVDGT------GGGGSK-B	208	115	100	180
874	GTNRSSKGRALIGKVDGT------GGGGSK-B	119	93	100	70
875	GTNRSSKGRSAIGKVDGT------GGGGSK-B	127	105	100	42
876	GTNRSSKGRSLAGKVDGT------GGGGSK-B	92	85	36	8
877	GTNRSSKGRSLIAKVDGT------GGGGSK-B	30	30	1	0
878	GTNRSSKGRSLIGAVDGT------GGGGSK-B	217	121	108	60
879	GTNRSSKGRSLIGKADGT------GGGGSK-B	74	52	1	0
880	GTNRSSKGRSLIGKVAGT------GGGGSK-B	35	8	4	5
881	GTNRSSKGRSLIGKVDAT------GGGGSK-B	81	67	1	1
882	GTNRSSKGRSLIGKVDGA------GGGGSK-B	125	121	94	33
884	SLIGKVDGTSHVTG-GGGGSK-B	---	100	---	100
885	SLIGKADGTSHVTG-GGGGSK-B	---	83	---	0
886	SLIGKVAGTSHVTG-GGGGSK-B	---	NB	---	NB
887	SLIGKAAGTSHVTG-GGGGSK-B	---	NB	---	NB

Table 26: Comparator 1

SEQ ID NO:	SEQUENCE	% Rel Binding		% Rel $T_{½}$	
		Exp1	Exp2	Exp1	Exp2
871	GTNRSSKGRSLIGKVDGT------GGGGSK-B	100	100	100	100
872	GTNRSSKARSLIGKVDGT------GGGGSK-B	111	111	29	27

873	GTNRSSKGASLIGKVDGT------GGGGSK-B	135	87	0.2	0
874	GTNRSSKGRALIGKVDGT------GGGGSK-B	126	102	223	148
875	GTNRSSKGRSAIGKVDGT------GGGGSK-B	147	105	2	1
876	GTNRSSKGRSLAGKVDGT------GGGGSK-B	156	108	47	41
877	GTNRSSKGRSLIAKVDGT------GGGGSK-B	139	108	31	24
878	GTNRSSKGRSLIGAVDGT------GGGGSK-B	118	84	3	2
879	GTNRSSKGRSLIGKADGT------GGGGSK-B	148	104	173	100
880	GTNRSSKGRSLIGKVAGT------GGGGSK-B	124	102	192	111
881	GTNRSSKGRSLIGKVDAT------GGGGSK-B	119	97	130	121
882	GTNRSSKGRSLIGKVDGA------GGGGSK-B	132	119	154	116
884	SLIGKVDGTSHVTG-GGGGSK-B	---	NB	---	NB
885	SLIGKADGTSHVTG-GGGGSK-B	---	NB	---	NB
886	SLIGKVAGTSHVTG-GGGGSK-B	---	NB	---	NB
887	SLIGKAAGTSHVTG-GGGGSK-B	---	NB	---	NB

Table 27: Comparator 2

SEQ ID NO:	SEQUENCE	% Rel Binding		% Rel $T_{½}$	
		Exp1	Exp2	Exp1	Exp2
871	GTNRSSKGRSLIGKVDGT------GGGGSK-B	---	NB	---	NB
872	GTNRSSKARSLIGKVDGT------GGGGSK-B	---	NB	---	NB
873	GTNRSSKGASLIGKVDGT------GGGGSK-B	---	NB	---	NB
874	GTNRSSKGRALIGKVDGT------GGGGSK-B	---	NB	---	NB
875	GTNRSSKGRSAIGKVDGT------GGGGSK-B	---	NB	---	NB
876	GTNRSSKGRSLAGKVDGT------GGGGSK-B	---	NB	---	NB
877	GTNRSSKGRSLIAKVDGT------GGGGSK-B	---	NB	---	NB
878	GTNRSSKGRSLIGAVDGT------GGGGSK-B	---	NB	---	NB
879	GTNRSSKGRSLIGKADGT------GGGGSK-B	---	NB	---	NB
880	GTNRSSKGRSLIGKVAGT------GGGGSK-B	---	NB	---	NB

881	GTNRSSKGRSLIGKVDAT------GGGGSK-B	---	NB	---	NB
882	GTNRSSKGRSLIGKVDGA------GGGGSK-B	---	NB	---	NB
884	SLIGKVDGTSHVTG-GGGGSK-B	---	100	---	100
885	SLIGKADGTSHVTG-GGGGSK-B	---	120	---	159
886	SLIGKVAGTSHVTG-GGGGSK-B	---	88	---	27
887	SLIGKAAGTSHVTG-GGGGSK-B	---	87	---	27

Table 28: Comparator 3

SEQ ID NO:	SEQUENCE	% Rel Binding		% Rel $T_{½}$	
		Exp1	Exp2	Exp1	Exp2
871	GTNRSSKGRSLIGKVDGT------GGGGSK-B	---	100	---	100
872	GTNRSSKARSLIGKVDGT------GGGGSK-B	---	101	---	109
873	GTNRSSKGASLIGKVDGT------GGGGSK-B	---	98	---	7
874	GTNRSSKGRALIGKVDGT------GGGGSK-B	---	106	---	21
875	GTNRSSKGRSAIGKVDGT------GGGGSK-B	---	115	---	79
876	GTNRSSKGRSLAGKVDGT------GGGGSK-B	---	92	---	7
877	GTNRSSKGRSLIAKVDGT------GGGGSK-B	---	101	---	8
878	GTNRSSKGRSLIGAVDGT------GGGGSK-B	---	109	---	95
879	GTNRSSKGRSLIGKADGT------GGGGSK-B	---	96	---	98
880	GTNRSSKGRSLIGKVAGT------GGGGSK-B	---	99	---	120
881	GTNRSSKGRSLIGKVDAT------GGGGSK-B	---	93	---	128
882	GTNRSSKGRSLIGKVDGA------GGGGSK-B	---	117	---	118
884	SLIGKVDGTSHVTG-GGGGSK-B	---	NB	---	NB
885	SLIGKADGTSHVTG-GGGGSK-B	---	NB	---	NB
886	SLIGKVAGTSHVTG-GGGGSK-B	---	NB	---	NB
887	SLIGKAAGTSHVTG-GGGGSK-B	---	NB	---	NB

[0155] The results from the alanine scanning experiments are summarized in Fig. 5, where the black circles indicate amino acids of PAR-2 which, when changed to alanine, substantially reduce binding by the corresponding antibody (*i.e.*, the T½ of antibody binding to the mutated peptide is less than 30% of the T½ of antibody binding to the wild-type peptide). (Open

triangles in Fig. 5 indicate non-activating upstream protease cleavage sites, and the black triangle indicates the activating protease cleavage site). As illustrated in Fig. 5, Comparators 1 and 3 were sensitive to mutations at residues on both sides of the activating protease cleavage site. By contrast, antibodies H4H581P and H4H588N are only sensitive to mutations at residues which are found C-terminal to the activating protease cleavage site. Thus, the H4H581P binding site on PAR-2 appears to be shifted by about 2-4 amino acids in the C-terminal direction relative to binding site of the Comparator 1 and 3 antibodies, and the H4H588N binding site is shifted by about 2-4 amino acids in the C-terminal direction from the H4H581P binding site. The Comparator 2 antibody only bound to the peptides comprising the 14 amino acids downstream from the activating PAR-2 protease cleavage site, *i.e.*, SLIGKVDGTSHVTG (residues 1-14 of SEQ ID NO:884), and was sensitive to mutations at the aspartic acid residue (Asp-43 of SEQ ID NO:851), but not to mutations at the valine residue (Val-42 of SEQ ID NO:851).

[0156] Significantly, this experiment indicates that antibodies H4H581P and H4H588N both interact with the first V and D residues located C-terminal to the activating PAR-2 protease cleavage site (*i.e.*, Val-42 and Asp-43 of SEQ ID NO:851), whereas the Comparator 1 and 3 antibodies do not interact with either of these residues, and the Comparator 2 antibody interacts with Asp-43 but not Val-42. The shifted binding of H4H581P on PAR-2 as compared to the comparator antibodies may explain the functional superiority of H4H581P over the comparator as demonstrated in the following *in vivo* examples.

Example 10. Dose Response of Anti-PAR-2 Antibody in Pruritus Models

[0157] In this Example, the ability of the anti-PAR-2 antibody H4H581P to attenuate itch in two different protease-induced pruritus models was assessed. Transgenic mice expressing human PAR-2 ($hPAR2^{+/+}$) were used for all cohorts in these experiments. Separate cohorts of mice received 150 mg/kg (s.c.) of an isotype control mAb or 10, 25, 50, 75, 100, and 150 mg/kg (s.c.) of H4H581P. Twenty-four hours after antibody dosing all cohorts received 150 µg of porcine trypsin, or 10 µg recombinant human beta tryptase, (s.c., interscapular), which produced bouts of scratching behavior for 30 to 60 minutes. A dose-response relationship was observed in mice receiving H4H581P prior to trypsin injection, with an estimated ED_{50} of 25 mg/kg. The results of these experiments, expressed in terms of the percent change in the total number of scratching bouts recorded over a 30 minute period following trypsin administration, or over a 60 minute period following tryptase administration, are shown in Table 29 (all data are represented as mean ± SEM; ND = not determined; * = $p<0.05$ compared to the isotype control group).

Table 29

Dose of mAb H4H581P (mg/kg)	Percent Change in Scratching Bouts Relative to Control	
	Trypsin	Tryptase
10	9.9 ± 23.1	-40.7 ± 23.4

25	-26.6 ± 11.3	-38.5 ± 11.1
50	-31.3 ± 13.5	-47.7 ± 8.0*
75	-34.9 ± 4.7*	ND
100	-55.2 ± 8.0*	-39.6 ± 6.9*
150	-42.9 ± 9.7*	ND

[0158] As shown in this Example, mAb H4H581P was able to block protease-induced pruritus behaviors in a dose dependent manner using two different protease-induced itch models.

Example 11. Reduction of Pruritus Behaviors by Administration of an Anti-PAR-2 Antibody in a Hapten-Induced Chronic Dermatitis Model

[0159] To further assess the ability of the anti-PAR-2 antibody H4H581P to reduce pruritus behaviors in a physiologically relevant disease state, a mouse model of chronic dermatitis was used. In this model, mice received repeated cutaneous applications of the haptenizing agent, oxazolone. This chronic oxazolone-induced dermatitis model has been shown to recapitulate many of the clinical, histological, and immunological hallmarks of atopic dermatitis in humans (Man *et al.*, 2008, *J. Invest. Dermatol. 128(1)*:79-86).

[0160] Mice were sensitized with a single cutaneous application of 1% oxazolone on the left ear or vehicle (100 mg/kg, s.c.). The mice then received nine total cutaneous applications (challenges) of 0.6% oxazolone between the scapulae beginning seven days after the sensitization application. Weekly dosing (3 total) of the H4H581P anti-PAR2 antibody was initiated 24 hours prior to the first oxazolone challenge (100 mg/kg, s.c.). This dosing paradigm significantly reduced pruritus behaviors as measured by reduced numbers of scratching bouts elicited by the final oxazolone challenge. All data are represented as mean number of scratching bouts ± SEM for n=6 mice/group; * = $p<0.05$ by Tukey post-hoc test as compared to the Oxazolone + IgG control group; # = $p<0.05$ by Tukey post-hoc test compared to the Vehicle + IgG control group).

Table 30

Treatment	Time (minutes)						Total
	0-10	10-20	20-30	30-40	40-50	50-60	
Vehicle + IgG control	0.0±0.0	0.8±0.6	2.2±1.4	3.4±1.3	4.8±2.0	2.4±1.0	13.6±4.2
Oxazolone + IgG control	0.5±0.5	11.3±3.9	24.8±9.7	28.0±7.3	21.0±7.9	21.3±7.3	#106.8±33.2
Oxazolone + H4H581P	0.2±0.2	1.6±1.2	*6.0±2.4	16.6±5.1	18.2±4.8	18.0±5.5	60.6±11.6

[0161] Histological analysis showed a significant increase in epidermal hyperplasia and immune cell infiltrate in the oxazolone-challenged animals. (Data not shown). No significant differences were observed in any of these parameters between the H4H581P anti-PAR2 antibody and the isotype control.

Example 12. Comparison of Pruritus Inhibiting Activities of mAb H4H581P

[0162] In this Example, the ability of mAb H4H581P to attenuate itching bouts in a mouse pruritus model was compared to that of a comparator anti-PAR-2 mAb (Comparator "1A1" described in WO 2009/005726.

[0163] Transgenic mice expressing human PAR-2 ($hPAR2^{+/+}$) were divided into 3 cohorts. Cohort A received 50 mg/kg (s.c.) of an isotype control mAb, cohort B received 50 mg/kg (s.c.) of H4H581P, and Cohort C received 50 mg/kg (s.c.) of the comparator anti-PAR-2 antibody. Twenty-four hours after antibody dosing all cohorts received 150 µg of trypsin (s.c., interscapular), which produced bouts of scratching behavior for 30 minutes. The percent change in the number of scratching bouts observed for the treated mice as compared to control-treated mice is shown in Table 29 (all data are represented as mean ± SEM).

Table 31

Antibody Treatment (50 mg/kg)	**(% change in scratching bouts from control)**
mAb H4H581P	- 41.1 ± 13.5
Comparator mAb	- 13.9 ± 9.9

[0164] As shown in this Example, mAb H4H581P was substantially more effective than the comparator mAb in reducing pruritus behaviors in the trypsin-induced itch model used herein.

[0165] The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated human antibody or antigen-binding fragment thereof that specifically binds to human PAR-2 (SEQ ID NO:851) and interacts with Val-42 and Asp-43 of human PAR-2.

2. The isolated human antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment also interacts with one or more residues selected from the group consisting of Ser-37, Leu-38, Ile-39, Gly-40, and Gly-44 of human PAR-2.

3. The isolated human antibody or antigen-binding fragment of claim 1 or 2, wherein the antibody or antigen-binding fragment does not interact with Lys-41 of human PAR-2.

4. The isolated human antibody or antigen-binding fragment of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment interacts with Ser-37, Leu-38, Ile-39, Gly-40, Val-42 and Asp-43 of human PAR-2, but does not interact with Lys-41 of human PAR-2.

5. The isolated human antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment comprises the complementarity determining regions (CDRs) of a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs:98/106 and SEQ ID NOs:714/692.

6. The isolated human antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 amino acid sequences selected from the group consisting of: (a) SEQ ID NOs:100-102-104 / 108-110-112; and (b) SEQ ID NOs:700-702-704 / 708-710-712.

7. The isolated human antibody or antigen binding fragment of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment blocks trypsin cleavage of human PAR-2 at the activating cleavage site located at the junction of residues Arg-36 and Ser-37 of human PAR-2.

8. The isolated human antibody or antigen-binding fragment of claim 7, wherein the antibody or antigen-binding fragment does not block trypsin cleavage of human PAR-2 at one or more non-activating cleavage sites selected from the non-activating cleavage site located at the junction of residues Arg-31 and Ser-32 of human PAR-2 and the non-activating cleavage site located at the junction of residues Lys-34 and Gly-35 of human PAR-2.

9. An isolated human antibody or antigen-binding fragment thereof that specifically binds to human PAR-2 (SEQ ID NO:851), wherein the antibody or antigen-binding fragment interacts with Ser-37, Leu-38, Ile-39, Gly-40, Val-42 and Asp-43 of human PAR-2, but does not interact with Lys-41 of human PAR-2, and wherein the antibody or antigen-binding fragment

blocks trypsin cleavage of human PAR-2 at the activating cleavage site located at the junction of residues Arg-36 and Ser-37 of human PAR-2, but does not block trypsin cleavage of human PAR-2 at the non-activating cleavage site located at the junction of residues Arg-31 and Ser-32 of human PAR-2.

10. An isolated human antibody or antigen-binding fragment thereof that binds to the same epitope on human PAR-2 as a reference antibody selected from the group consisting of: (a) an antibody comprising a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:714, and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:692; and (b) an antibody comprising a HCVR having the amino acid sequence of SEQ ID NO:98, and a LCVR having the amino acid sequence of SEQ ID NO:106.

11. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1 to 10, and a pharmaceutically acceptable carrier or diluent.

12. A therapeutic method comprising administering the pharmaceutical composition of claim 11 to a patient, wherein the patient exhibits one or more symptoms or indicia of a disease or disorder caused by PAR-2 activity.

13. The therapeutic method of claim 12, wherein the disease or disorder caused by PAR-2 activity is a disease or disorder selected from the group consisting of pain, pruritus, asthma, rheumatoid arthritis, fibrosis, atopic dermatitis, inflammatory bowel disease, ulcerative colitis, pancreatitis, ulcer, Crohn's disease, cancer, and Netherton's disease.

14. The therapeutic method of claim 12 or 13, further comprising administering to the patient at least one additional therapeutically active component selected from the group consisting of an IL-1 inhibitor, an IL-18 inhibitor, an IL-4 inhibitor, an IL-4 receptor inhibitor, an IL-6 inhibitor, an IL-6 receptor inhibitor, a nerve growth factor (NGF) inhibitor, a tumor necrosis factor (TNF) inhibitor, a TNF receptor inhibitor, a uric acid synthesis inhibitor and a corticosteroid.

15. The isolated human antibody or antigen-binding fragment of any one of claims 1 to 10, or the pharmaceutical composition of claim 11, for use in treating a patient who exhibits one or more symptoms or indicia of a disease or disorder caused by PAR-2 activity, wherein the disease or disorder caused by PAR-2 activity is a disease or disorder selected from the group consisting of pain, pruritus, asthma, rheumatoid arthritis, fibrosis, atopic dermatitis, inflammatory bowel disease, ulcerative colitis, pancreatitis, ulcer, Crohn's disease, cancer, and Netherton's disease.

16. Use of the isolated antibody or antigen-binding fragment of any one of claims 1 to 10, or the pharmaceutical composition of claim 11, in the manufacture of a medicament for use

in treating a patient who exhibits one or more symptoms or indicia of a disease or disorder caused by PAR-2 activity, wherein the disease or disorder caused by PAR-2 activity is a disease or disorder selected from the group consisting of pain, pruritus, asthma, rheumatoid arthritis, fibrosis, atopic dermatitis, inflammatory bowel disease, ulcerative colitis, pancreatitis, ulcer, Crohn's disease, cancer, and Netherton's disease.