ANTI-HLA-B*27 ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for human HLA-B*27, and methods of use thereof.

BACKGROUND

[0002] The major histocompatibility complex (MHC) in humans is known as human leukocyte antigen (HLA). HLA molecules are critically involved in antigen presentation and immune responses. HLA-B*27 is a particular HLA allele associated with a high incidence of ankylosing spondylitis and other seronegative spondyloarthropathies.

[0003] HLA class I molecules such as HLA-B*27 consist of two chains: a membrane-bound heavy chain consisting of three Ig-domains (alpha-1, alpha-2 and alpha-3), and a non-covalently-associated common light chain known as beta-2-microglobulin (β2m). Several allelic subtypes of HLA-B*27 exist, including HLA-B*2701 through HLA-B*2728. Sequence differences between HLA alleles are mostly confined to the peptide-binding cleft between the alpha-1 and alpha-2 domains of the heavy chain. Presentation of peptides of intracellular origin (self- or viral peptides) in the context of HLA-B*27 leads to activation of HLA-restricted T cell clones expressing T cell receptors (TCR) specific for the particular peptide.

[0004] Antibodies against HLA-B*27 are generally known in the art, but are mainly described for use in diagnostic and/or detection applications. (*See, e.g.,* US Patent No. 5,369,010, and Urban *et al.*, *Proc. Natl. Acad. Sci. USA 91*:1534-1538 (1994)). Thus, a need exists in the art for new, high-affinity binding anti-HLA-B*27 antibodies that are suitable for therapeutic applications and other utilities.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides antibodies that specifically bind human HLA-B*27. The antibodies of the invention are useful, *inter alia*, for interfering with T-cell recognition of antigens presented by HLA-B*27 and for treating diseases and disorders caused by or related to HLA-B*27-mediated antigen presentation. For example, the antibodies of the present invention can be administered to a patient for the treatment or alleviation of ankylosing spondylitis and other spondyloarthropathies.

[0006] 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).

[0007] 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, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, and 370, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0008] 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, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, and 378, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0009] 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, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378.

[0010] 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, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, and 376, or a substantially similar sequence 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, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, and 384, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0011] 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, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 120/128, 136/144, 152/160, 168/176, 184/192, 200/208, 216/224, 232/240, 248/256, 264/272, 280/288, 296/304, 312/320, 328/336, 344/352, 360/368, 376/384.

[0012] 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, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, and 372, or a substantially 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, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, and 374, or a substantially 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,

28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, and 380, or a substantially similar sequence 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, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, and 382, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0013] Certain non-limiting, exemplary antibodies and antigen-binding fragments of the invention comprise HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16 (*e.g.* H1M2477N); 20-22-24-28-30-32 (*e.g.* H1M2490N); 36-38-40-44-46-48 (*e.g.* H2M2482N); 52-54-56-60-62-64 (*e.g.* H1M2477N2); 68-70-72-76-78-80 (*e.g.* H1M2490N2); 84-86-88-92-94-96 (*e.g.* H2M2482N2); 100-102-104-108-110-112 (*e.g.* H1M2480N); 116-118-120-124-126-128 (*e.g.* H1M2497N); 132-134-136-140-142-144 (*e.g.* H2M2491N); 148-150-152-156-158-160 (*e.g.* H2M2499N); 164-166-168-172-174-176 (*e.g.* H2M2718N); 180-182-184-188-190-192 (*e.g.* H4H2524P); 196-198-200-204-206-208 (*e.g.* H4H2526P); 212-214-216-220-222-224 (*e.g.* H4H2528P); 228-230-232-236-238-240 (*e.g.* H4H2530S); 244-246-248-252-254-256 (*e.g.* H4H2532P); 260-262-264-268-270-272 (*e.g.* H4H2534S); 276-278-280-284-286-288 (*e.g.* H4H2538P); 292-294-296-300-302-304 (*e.g.* H4H2542P); 308-310-312-316-318-320 (*e.g.* H4H2555P); 324-326-328-332-334-336 (*e.g.* H4H2559P); 340-342-344-348-350-352 (*e.g.* H4H2560P); 356-358-360-364-366-368 (*e.g.* H4H2562S); and 372-374-376-380-382-384 (*e.g.* H4H2564S).

[0014] In a related embodiment, the invention includes an antibody or antigen-binding fragment of an antibody which specifically binds HLA-B*27, 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, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378. 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.

[0015] In a related embodiment, the invention includes an isolated antibody or antigen-binding fragment thereof that exhibits enhanced binding to HLA-B*27 as compared to other HLA-B allelic variants, the antibody or antigen-binding fragment comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains that comprise SEQ ID NOs:52-54-56-60-62-64, respectively, with a histidine substitution in the HCDR1 domain at amino acid position 33, a histidine substitution in the HCDR2 domain at amino acid position 52, or a histidine substitution in the HCDR1 domain at amino acid position 33 and a histidine substitution in the HCDR2 at amino acid position 52.

[0016] In a related embodiment, the invention includes an isolated antibody or antigen-binding fragment thereof that exhibits enhanced binding to HLA-B*27 as compared to other HLA-B allelic variants, the antibody or antigen-binding fragment comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains that comprise SEQ ID NOs:148-150-152-156-158-160, respectively, with one or more histidine substitutions: a histidine substitution in the HCDR1 domain at amino acid position 32; a histidine substitution in the HCDR2 domain at amino acid position 53; a histidine substitution in the HCDR1 domain at amino acid position 32 and a histidine substitution in the HCDR2 domain at amino acid position 53; a histidine substitution in the LCDR1 domain at amino acid positions 27, 30 and 32; a histidine substitution in the LCDR1 domain at amino acid positions 27, 28 and 32; a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 32; a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 31; a histidine substitution in the LCDR1 domain at amino acid positions 27, 28, 30 and 32; a histidine substitution in the LCDR3 domain at amino acid positions 90, 92, 93 and 97; a histidine substitution in the LCDR3 domain at amino acid positions 90, 92 and 97; a histidine substitution in the LCDR3 domain at amino acid positions 92, 93 and 97; a histidine substitution in the LCDR3 domain at amino acid positions 90 and 93; a histidine substitution in the LCDR3 domain at amino acid positions 92 and 93; a histidine substitution in the HCDR3 domain at amino acid positions 98, 100 and 106; a histidine substitution in the HCDR3 domain at amino acid positions 98, 100 and 104; a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106; a histidine substitution in the HCDR3 domain at amino acid positions 98, 100, 104 and 106; a histidine substitution in the HCDR3 domain at amino acid positions 98, 104 and 106; a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 31; a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR3 domain at amino acid positions 90, 92 and 97; or a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR3 domain at amino acid positions 92, 93 and 97.

[0017] In a related embodiment, the invention includes a histidine-substituted isolated antibody or antigen-binding fragment that binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 1.5-

times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

[0018] In another embodiment, the invention includes an isolated antibody or antigen-binding fragment thereof that binds HLA-B*27 at pH 5.75 with a K_D that is at least 5-times, at least 10-times, at least 12-times or at least 25-times greater than the K_D for the antibody binding to HLA-B*27 at pH 7.2. In some embodiments, the antibody or antigen-binding fragment thereof that binds HLA-B*27 at pH 5.75 with a K_D that is greater than the K_D for the antibody binding to HLA-B*27 at pH 7.2 comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 226/234, 258/266, 290/298, 338/346, and 370/378.

[0019] In a related embodiment, the invention includes an isolated antibody or antigen-binding fragment thereof that binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 1.5-times, at least 10-times, at least 12 times or at least 25-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

[0020] In another aspect, the invention provides nucleic acid molecules encoding anti-HLA-B*27 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.

[0021] 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, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, and 369, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0022] 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, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361, and 377, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0023] 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, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, 311, 327, 343, 359, and 375, or a substantially 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, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, and 383, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0024] 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, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, and 371, 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, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341, 357, and 373, or a substantially 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, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, and 379, 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, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, and 381, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0025] 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 NOs: SEQ ID NOs: 1 and 9 (*e.g.* H1M2477N), 17 and 25 (*e.g.* H1M2490N), 33 and 41 (*e.g.* H2M2482N), 49 and 57 (*e.g.* H1M2477N2), 65 and 73 (*e.g.* H1M2490N2), 81 and 89 (*e.g.* H2M2482N2), 97 and 105 (*e.g.* H1M2480N), 113 and 121 (*e.g.* H1M2497N), 129 and 137 (*e.g.* H2M2491N), 145 and 153 (*e.g.* H2M2499N), 161 and 169 (*e.g.* H2M2718N), 177 and 185 (*e.g.* H4H2524P), 193 and 201 (*e.g.* H4H2526P), 209 and 217 (*e.g.* H4H2528P), 225 and 233 (*e.g.* H4H2530S), 241 and 249 (*e.g.* H4H2532P), 257 and 265 (*e.g.* H4H2534S), 273 and 281 (*e.g.* H4H2538P), 289 and 297 (*e.g.* H4H2542P), 305 and 313 (*e.g.* H4H2555P), 321 and 329 (*e.g.* H4H2559P), 337 and 345 (*e.g.* H4H2560P), 353 and 361 (*e.g.* H4H2562S), and 369 and 377 (*e.g.* H4H2564S).

[0026] The present invention includes anti-HLA-B*27 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 human antibody or antigen-binding fragment thereof which specifically binds HLA-B*27 and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition comprising a combination of an anti-HLA-B*27 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-HLA-B*27 antibody. Exemplary agents that may be advantageously combined with an

anti-HLA-B*27 antibody include, without limitation, other agents that inhibit HLA-B*27 activity (including other antibodies or antigen-binding fragments thereof, peptide inhibitors, small molecule antagonists, etc) and/or agents which do not directly bind HLA-B*27 but nonetheless interfere with, block or attenuate HLA-B*27-mediated T-cell activation.

[0028] In yet another aspect, the invention provides methods for inhibiting HLA-B*27-mediated activity using an anti-HLA-B*27 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, prevented or ameliorated can be any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of HLA-B*27 activity (*e.g.*, HLA-B*27-mediated antigen presentation). The anti-HLA-B*27 antibodies or antibody fragments of the invention may function to interfere with the interaction of an HLA-B*27/peptide complex with a T-cell receptor, or to otherwise inhibit HLA-B*27-mediated antigen presentation and/or T-cell activation. Thus, one aspect of the invention are methods for treating, preventing or ameliorating a disease or disorder associated with or mediated by HLA-B*27 activity, the method comprising administering a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention to a patient afflicted with or at risk for a disease or disorder associated with or mediated by HLA-B*27 activity. In some embodiments of the invention, the disease or disorder associated with or mediated by HLA-B*27 activity is spondyloarthropathy, rejection of solid organ transplants, or graft-versus-host disease (GVHD). In some embodiments of the invention, the spondyloarthropathy that is treated, prevented or ameliorated by methods of the invention are ankylosing spondylitis, reactive arthritis (Reiter's Syndrome), acute anterior uveitis, initis, psoriatic arthritis, and/or ulcerative colitis.

[0029] The present invention also includes the use of an anti-HLA-B*27 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 HLA-B*27 activity.

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

DETAILED DESCRIPTION

[0031] 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.

[0032] 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.).

[0033] 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

[0034] The expression "HLA-B*27" (alternatively referred to as "HLA-B27") means a polypeptide complex comprising the alpha-1, alpha-2 and alpha-3 Ig domains of an HLA-B*27 allelic heavy chain, in association with a beta-2-microglobulin polypeptide. The expression "HLA-B*27," as used herein, encompasses all allelic subvariants of the HLA-B*27 family, including HLA-B*2701 through HLA-B*2728. An exemplary HLA-B*27 allelic subvariant is HLA-B*2705 comprising a heavy chain having the amino acid sequence of SEQ ID NO:385. Another exemplary HLA-B*27 allelic subvariant is HLA-B*2709 comprising a heavy chain having the amino acid sequence of SEQ ID NO:386. The amino acid sequences of other HLA-B*27 allelic subvariants are available from public databases and will be well known to persons of ordinary skill in the art. The expression "HLA-B*27" includes cell surface expressed HLA-B*27 complexes (*see*, *e.g.*, Examples 4-6 herein) as well as artificially engineered soluble fusion proteins comprising HLA-B*27 alpha-1, alpha-2 and alpha-3 Ig domains fused to a beta-2-microglobulin protein and optionally an HLA-restricted peptide (*see*, *e.g.*, Example 3 herein). An example of one such soluble HLA-B*27 fusion protein is the construct referred to herein as "scHLA-B27" which comprises the amino acid sequence of SEQ ID NO:387.

[0035] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, HLA-B*27). The term "antibody" includes 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-HLA-B*27 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.

[0036] 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.

[0037] 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) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0038] 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.

[0039] 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)).

[0040] 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.

[0041] 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.*, US Patent Nos. 5,500,362 and 5,821,337, and Clynes *et al.*, *Proc. Natl. Acad. Sci. (USA) 95*:652-656 (1998)). 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.

[0042] 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.

[0043] 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.*, *Nucl. Acids Res. 20*:6287-6295 (1992)) 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 V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0044] 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.

[0045] 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 present nvention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0046] 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, or from a 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. Isolated antibodies are antibodies that have 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.

[0047] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to HLA-B*27 inhibits the interaction between an HLA-B*27/peptide complex and a T-cell receptor. The inhibition caused by an HLA-B*27 neutralizing or blocking antibody need not be complete so long as it is detectable using an appropriate assay. Exemplary assays for determining if an antibody blocks the interaction between HLA-B*27 and a T-cell receptor are known in the art and described elsewhere herein.

[0048] The anti-HLA-B*27 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 from which the antibodies were derived. 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 mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline 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 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 residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original 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. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline 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.

[0049] The present invention also includes anti-HLA-B*27 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-HLA-B*27 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.

[0050] The present invention also includes anti-HLA-B*27 antibodies comprising variants of anti-HLA-B*27 antibodies with pH-dependent binding properties (*i.e.*, reduced binding at low pH as compared to neutral pH), enhanced binding to subvariants of HLA-B*27 (*e.g.*, greater binding affinity for HLA-B*2705 and/or HLA-B*2709 as compared to HLA-B*07), and improved *in vivo* efficacy (*e.g.*, longer antibody serum half-life, prolonged inhibition of T-cell activation, etc.). Such variants include anti-HLA-B*27 antibody variants in which the CDR sequences of a parental anti-HLA-B*27 antibody have been altered to replace one or more non-histidine amino acids by a histidine residue. Examples of "parental" anti-HLA-B*27 antibodies which can be modified, mutated, or otherwise engineered to possess altered binding characteristics (*e.g.*, enhanced pH-dependent binding characteristics) include anti-HLA_B*27 antibodies comprising any of the complementarity determining regions (CDRs) or heavy and light chain variable domains (HCVR/LCVR) as disclosed in Example 2, Table 1, herein.

[0051] 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.

[0052] 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.

[0053] 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, *Methods Mol. Biol. 24*: 307-331 (1994). 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.*, *Science 256*: 1443-1445 (1992). A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0054] 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.*, J. Mol. Biol. *215*:403-410 (1990) and Altschul *et al.*, Nucleic Acids Res. 25:3389-402 (1997).

Biological Characteristics of the Antibodies

[0055] The present invention includes antibodies that specifically bind HLA-B*27. 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 K_D of about $1x10^{-7}$ M or less at 25°C and neutral pH. 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. An isolated antibody that specifically binds a particular HLA-B*27 allelic subvariant may, however, have cross-reactivity to other HLA-B*27 allelic subvariants. Thus, antibodies that "specifically bind HLA-B*27" include antibodies that specifically bind all or a subset of HLA-B*27 allelic subvariants. For example, an antibody that specifically binds HLA-B*2705 and/or HLA-B*2709 is considered an antibody that "specifically binds HLA-B*27."

[0056] The present invention includes anti-HLA-B*27 antibodies that bind a soluble HLA-B*27 fusion protein (*e.g.*, scHLA-B27-mmH [SEQ ID NO:387]) with a K_D of less than about 260 nM at 25°C and neutral pH (*e.g.*, about pH 7.2). For example, the present invention includes anti-HLA-B*27 antibodies that bind scHLA-B27-mmH, as tested by surface plasmon resonance at 25°C and pH 7.2 (*see, e.g.*, Example 3 herein), with a K_D of less than about 250 nM, 200 nM, 150 nM, 100 nM, 50 nM, 1 nM, 900 pM, 850 pM, 800 pM, 750 pM, 700 pM, 650 pM, 600 pM, 550 pM, 500 pM, 450 pm, 400 pM, 350 pM, 300 pM, 250 pM, 200 pM, or less.

[0057] The present invention includes anti-HLA-B*27 antibodies that exhibit pH-dependent binding to HLA-B*27. In some embodiments of the invention, the expression "pH-dependent binding", as used herein, means that an antibody exhibits a K_D value at acidic pH (*e.g.*,pH 5.75) that is at least 5-times greater than its K_D value at neutral pH (*e.g.*, pH 7.2), when tested using surface plasmon resonance, wherein the K_D values at acidic and neutral pH are measured under the same or substantially the same experimental conditions (*e.g.*, temperature, antigen/antibody orientation, reagent concentration, flow rate, etc.). For example, an anti-HLA-B*27 antibody is deemed to have "pH-dependent binding" for purposes of the present disclosure if the ratio of its K_D value for binding to HLA-B*27 at pH 5.75 to its K_D value for binding to HLA-B*27 at pH 7.2 is at least about 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 15.0, 20.0, 25.0, or greater (*see*, *e.g.*, Table 6 [Example 3] herein). In some embodiments of the invention, the expression “pH-dependent binding”, as used herein, means that an antibody exhibits a EC_{50} value (*i.e.*, half maximal effective concentration) in a binding assay (*e.g.*, a chemilumenscence assay) at acidic pH (*e.g.*, pH 6.0) that is at least 3 times greater than the EC_{50} value at neutral pH (*e.g.*, pH 7.2), wherein the EC_{50} values at acidic and neutral pH are measured under the same or substantially the same experimental conditions (*e.g.*, temperature, antigen/antibody orientation, reagent concentration, flow rate, etc.). For example, an anti-HLA-B*27 antibody is deemed to have "pH-dependent binding" for purposes of the present disclosure if the ratio of its EC_{50} value for binding to HLA-B*27 at pH 6.0 to its EC_{50}

value for binding to HLA-B*27 at pH 7.2 is at least about 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 15.0, 20.0, 25.0, or greater (*see*, *e.g.*, Tables 21 and 22 [Example 11] herein). In some embodiments of the invention, pH-dependent binding may be measured in terms of binding associative rate constants (k_a), binding dissociative rate constants (k_d), or dissociative half-lives ($t_{1/2}$).

[0058] The present invention also includes anti-HLA-B*27 antibodies that are capable of blocking or reducing HLA-B*27-mediated T-cell activation *in vitro*. An exemplary assay for determining whether an antibody blocks or reduces HLA-B*27-mediated T-cell activation *in vitro* is shown in Example 4 herein. In this Example, two engineered cell lines are used. The first cell line is one that expresses an HLA-B*27 heavy chain allelic variant along with human β2m and an HLA-restricted peptide on its surface. The second cell line is a reporter cell line that expresses a T cell receptor which, when activated, produces a detectable luciferase signal. Varying amounts of anti-HLA-B*27 antibody are added to the first cell line prior to mixing with the reporter cell line, and IC_{50} values are calculated. The present invention includes antibodies that exhibit an IC_{50} of less than about 12.5 nM when tested in a blocking bioassay as shown in Example 4, or a substantially similar assay. For example, the present invention includes anti-HLA-B*27 antibodies that exhibit an IC_{50} of less than about 12 nM, 10 nM, 8 nM, 6 nM, 4 nM, 2 nM, 1 nM, 900 pM, 850 pM, 800 pM, 750 pM, 700 pM, 650 pM, 600 pM, 550 pM, 500 pM, 450 pm, 400 pM, or less when tested in a blocking bioassay as shown in Example 4, or a substantially similar assay.

[0059] The present invention also includes anti-HLA-B*27 antibodies that exhibit enhanced binding to an HLA-B*27 allelic variant (such as, *e.g.*, HLA-B*2705 and/or HLA-B*2709) as compared to other (non-B*27) HLA-B allelic variants (such as, *e.g.*, HLA-B*07). An exemplary assay format for determining if an antibody exhibits enhanced binding to one or more HLA-B*27 allelic variant(s) is shown in Example 8 herein. In this Example, the relative binding of each antibody to cells expressing either HLA-B*07, HLA-B*2705 or HLA-B*2709, as compared to its binding to parental cells that do not express HLA, was determined. Using this type of assay format, an antibody that exhibits at least 50-fold greater binding to cells expressing an HLA-B*27 allelic variant as compared to binding to the parental cell line (represented by [++] or [+++] in Table 15), but less than 10-fold greater binding to cells expressing the non-HLA-B*27 allelic variant (*e.g.*, HLA-B*07) compared to binding to the parental cells (represented [+] in Table 15), is considered an antibody that exhibits "enhanced binding" to HLA-B*27 as compared to another HLA-B allelic variant(s). In certain instances, an antibody is deemed to exhibit "enhanced binding" to HLA-B*27 as compared to other HLA-B allelic variants if the antibody, when tested in the assay format of Example 8 herein or a substantially similar assay format, shows at least 50-

fold greater binding to cells expressing an HLA-B*27 allelic variant as compared to binding to the parental cells (represented by [++] or [+++] in Table 15), but less than 3-fold greater binding to cells expressing the non-HLA-B*27 allelic variant (*e.g.*, HLA-B*07) compared to binding to the parental cells (represented by [-] in Table 15).

Epitope Mapping and Related Technologies

[0060] The present invention includes anti-HLA-B*27 antibodies which interact with one or more amino acids found within the alpha-1, alpha-2, and/or alpha-3 Ig domains of an HLA-B*27 heavy chain. For HLA-B*2705 (SEQ ID NO:385) and HLA-B*2709 (SEQ ID NO:386), the alpha-1 Ig domain consists of amino acids 25-114, the alpha-2 Ig domain consists of amino acids 115-206, and the alpha-3 Ig domain consists of amino acids 207-298 of the corresponding full heavy chain amino acid sequence. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within one or more Ig domains of an HLA-B*27 heavy chain. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within one or more Ig domains of an HLA-B*27 heavy chain. The antibodies of the present invention may also interact with one or more amino acids within the β2m sequence (SEQ ID NO:388).

[0061] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, *Methods Mol Biol 248*:443-463 (2004)), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, *Protein Science 9*:487-496 (2000)). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring, *Analytical Biochemistry 267(2)*:252-259 (1999); Engen and Smith, *Anal. Chem. 73*:256A-265A (2001).

[0062] The present invention further includes anti-HLA-B*27 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H1M2477N, H1M2490N, H2M2482N, H1M2477N2, H1M2490N2, H2M2482N2, H1M2480N, H1M2497N,

H2M2491N, H2M2499N, H2M2718N, H4H2524P, H4H2526P, H4H2528P, H4H2530S, H4H2532P, H4H2534S, H4H2538P, H4H2542P, H4H2555P, H4H2559P, H4H2560P, H4H2562S, H4H2564S, etc.). Likewise, the present invention also includes anti-HLA-B*27 antibodies that compete for binding to HLA-B*27 with any of the specific exemplary antibodies described herein (*e.g.*, H1M2477N, H1M2490N, H2M2482N, H1M2477N2, H1M2490N2, H2M2482N2, H1M2480N, H1M2497N, H2M2491N, H2M2499N, H2M2718N, H4H2524P, H4H2526P, H4H2528P, H4H2530S, H4H2532P, H4H2534S, H4H2538P, H4H2542P, H4H2555P, H4H2559P, H4H2560P, H4H2562S, H4H2564S, etc.).

[0063] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-HLA-B*27 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-HLA-B*27 antibody of the invention, the reference antibody is allowed to bind to an HLA-B*27 complex (*e.g.*, a soluble HLA-B*27/peptide fusion protein or a cell surface-expressed HLA-B*27 in complex with an HLA-restricted peptide). Next, the ability of a test antibody to bind to the HLA-B*27 complex is assessed. If the test antibody is able to bind to HLA-B*27 following saturation binding with the reference anti-HLA-B*27 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-HLA-B*27 antibody. On the other hand, if the test antibody is not able to bind to the HLA-B*27 complex following saturation binding with the reference anti-HLA-B*27 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-HLA-B*27 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.

[0064] To determine if an antibody competes for binding with a reference anti-HLA-B*27 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to an HLA-B*27 complex under saturating conditions followed by assessment of binding of the test antibody to the HLA-B*27 complex. In

a second orientation, the test antibody is allowed to bind to an HLA-B*27 complex under saturating conditions followed by assessment of binding of the reference antibody to the HLA-B*27 complex. If, in both orientations, only the first (saturating) antibody is capable of binding to the HLA-B*27 complex, then it is concluded that the test antibody and the reference antibody compete for binding to HLA-B*27. 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.

Preparation of Human Antibodies

[0065] 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 HLA-B*27.

[0066] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to HLA-B*27 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

[0067] The anti-HLA-B*27 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 HLA-B*27. 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-HLA-B*27 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-HLA-B*27 antibody or antibody fragment that is essentially bioequivalent to an anti-HLA-B*27 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0068] 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.

[0069] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] Bioequivalent variants of anti-HLA-B*27 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-HLA-B*27 antibody variants comprising amino acid changes which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Immunoconjugates

[0074] The invention encompasses anti-HLA-B*27 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

[0075] 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., J. Immunol. 147*:60-69 (1991); Kufer *et al.*, *Trends Biotechnol. 22*:238-244 (2004). The anti-HLA-B*27 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 HLA-B*27 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.

[0076] 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.

[0077] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab2 bispecific formats (see, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric

complexes with defined composition, valency and geometry. (See, *e.g.*, Kazane et al., J. Am. Chem. Soc. [Epub: Dec. 4, 2012]).

Therapeutic Formulation and Administration

[0078] The invention provides pharmaceutical compositions comprising the anti-HLA-B*27 antibodies or antigen-binding fragments thereof of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that 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.

[0079] The dose of antibody administered to a patient may vary depending upon the age and the size of the patient, 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 HLA-B*27 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 anti-HLA-B*27 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.*, *Pharmaceut. Res. 8*:1351 (1991)).

[0080] 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.*, *J. Biol. Chem. 262*:4429-4432 (1987)). 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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

[0086] The antibodies of the invention (and pharmaceutical compositions comprising the same) are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by HLA-B*27 activity or treatable by blocking or attenuating HLA-B*27-mediated antigen presentation. Exemplary diseases and disorders that can be treated with the anti-HLA-B*27 antibodies of the present invention include spondyloarthropathies, such as, *e.g.*, ankylosing spondylitis, reactive arthritis (Reiter's Syndrome), acute anterior uveitis, initis, psoriatic arthritis, ulcerative colitis, etc. Additional diseases and disorders that can be prevented, treated and/or ameliorated with the anti-HLA-B*27 antibodies of the present invention include rejection of solid organ transplants and graft-versus-host disease (GVHD).

Administration Regimens

[0087] According to certain embodiments of the present invention, multiple doses of anti-HLA-B*27 antibody may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-HLA-B*27 antibody. As used herein, "sequentially administering" means that each dose of anti-HLA-B*27 antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an anti-HLA-B*27 antibody, followed by one or

more secondary doses of the anti-HLA-B*27 antibody, and optionally followed by one or more tertiary doses of the anti-HLA-B*27 antibody.

[0088] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-HLA-B*27 antibody. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-HLA-B*27 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-HLA-B*27 antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0089] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-HLA-B*27 antibody which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0090] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-HLA-B*27 antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0091] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of

administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Combination Therapies

[0092] The present invention includes therapeutic administration regimens which comprise administering an anti-HLA-B*27 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 HLA-B*27 blocking agents such as, *e.g.*, a second anti-HLA-B*27 antibody. Other agents that may be beneficially administered in combination with the anti-HLA-B*27 antibodies of the invention include cytokine inhibitors (including small-molecule cytokine inhibitors and antibodies that bind to cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, IL-18, or to their respective receptors), antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs.

[0093] The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of an anti-HLA-B*27 antibody of the present invention; (for purposes of the present disclosure, such administration regimens are considered the administration of an anti-HLA-B*27 antibody "in combination with" an additional therapeutically active component).

Diagnostic Uses of the Antibodies

[0094] The anti-HLA-B*27 antibodies of the present invention may also be used to detect and/or measure HLA-B*27, or HLA-B*27-expressing cells in a sample, *e.g.*, for diagnostic purposes. For example, an anti-HLA-B*27 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by the presence of an HLA-B*27 allelic subvariant (*e.g.*, HLA-B*2705 or HLA-B*2709). Exemplary diagnostic assays for HLA-B*27 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-HLA-B*27 antibody of the invention, wherein the anti-HLA-B*27 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-HLA-B*27 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, beta-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure HLA-B*27 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0095] Samples that can be used in HLA-B*27 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of HLA-B*27 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of HLA-B*27 in a particular sample obtained from a healthy patient (*e.g.*, a

patient not afflicted with a disease or condition associated with HLA-B*27 or a particular HLA-B*27 allele) will be measured to initially establish a baseline, or standard, level of HLA-B*27. This baseline level of HLA-B*27 can then be compared against the levels of HLA-B*27 measured in samples obtained from individuals suspected of having an HLA-B*27 related disease or condition.

EXAMPLES

[0096] 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 HLA-B*27

[0097] To generate anti-HLA-B*27 antibodies, a VELOCIMMUNE® mouse (*see, e.g.*, US Patent No. 6,596,541) was immunized according to standard methods with either a soluble HLA-B*27 fusion protein (comprising the HLA-B*2705 heavy chain extracellular domain fused to an HLA-restricted peptide and the β2m sequence), or with tissues/cells expressing HLA-B*27, as well as combinations thereof. The antibody immune response was monitored by an HLA-B*27-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 HLA-B*27-specific antibodies. Using this technique several anti-HLA-B*27 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: 2477N, 2490N, 2482N, 2477N2, 2490N2, 2482N2, 2480N, 2497N, 2491N, 2499N, and 2718N.

[0098] Anti-HLA-B*27 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1. Using this method, several fully human anti-HLA-B*27 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: 2524P, 2526P, 2528P, 2530S, 2532P, 2534S, 2538P, 2542P, 2555P, 2559P, 2560P, 2562S, and 2564S.

[0099] Certain biological properties of the exemplary anti-HLA-B*27 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

[00100] Table 1 sets forth the amino acid sequence identifiers (SEQ ID NOs) for the heavy and light chain variable regions (HCVRs and LCVRs) and complementarity determining regions (CDRs) of selected anti-HLA-B*27 antibodies.

Table 1

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
2477N	2	4	6	8	10	12	14	16
2490N	18	20	22	24	26	28	30	32
2482N	34	36	38	40	42	44	46	48
2477N2	50	52	54	56	58	60	62	64
2490N2	66	68	70	72	74	76	78	80
2482N2	82	84	86	88	90	92	94	96
2480N	98	100	102	104	106	108	110	112
2497N	114	116	118	120	122	124	126	128
2491N	130	132	134	136	138	140	142	144
2499N	146	148	150	152	154	156	158	160
2718N	162	164	166	168	170	172	174	176
2524P	178	180	182	184	186	188	190	192
2526P	194	196	198	200	202	204	206	208
2528P	210	212	214	216	218	220	222	224
2530S	226	228	230	232	234	236	238	240
2532P	242	244	246	248	250	252	254	256
2534S	258	260	262	264	266	268	270	272
2538P	274	276	278	280	282	284	286	288
2542P	290	292	294	296	298	300	302	304
2555P	306	308	310	312	314	316	318	320
2559P	322	324	326	328	330	332	334	336
2560P	338	340	342	344	346	348	350	352
2562S	354	356	358	360	362	364	366	368
2564S	370	372	374	376	378	380	382	384

[00101] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H4H", "H1M", "H2M"), followed by a numerical identifier (*e.g.* "2477" or "2524" as shown in Table 1), followed by a "P", "N" or "S" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1M2477N" or "H4H2524P". The H4H, H1M, and H2M prefixes on the antibody designations used herein indicate the particular Fc region of the antibody. For example, an "H1M" antibody has a mouse IgG1 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an H1M or

H2M antibody can be converted to an H4H antibody, and *vice versa*, but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same.

Example 3. Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal Anti-HLA-B*27 Antibodies

[00102] Binding associative and dissociative rate constants (k_a and k_d, respectively) and calculated equilibrium dissociation constants and dissociative half-lives (K_D and $t_{1/2}$, respectively) for antigen binding to purified anti-HLA-B*27 antibodies were determined using a real-time surface plasmon resonance biosensor assay (Biacore T200, 3000, or T100-2). Antibodies were tested for binding to an engineered HLA-B*27 ectodomain in-line fusion protein ("scHLA-B27-mmH", SEQ ID NO:387) consisting of the following components: an N-terminal HLA-restricted peptide sequence (SRYWAIRTR, amino acids 1-9 of SEQ ID NO:387); followed by a first linker sequence (amino acids 10-19 of SEQ ID NO:387); followed by the human beta-2 microglobulin (β2m) sequence (amino acids 20-118 of SEQ ID NO:387); followed by a second linker sequence (amino acids 119-133 of SEQ ID NO:387); followed by the extracellular region of the HLA-B*2705 heavy chain (amino acids 134-409 of SEQ ID NO:387); and terminating with a myc-myc-hexahistidine C-terminal tag (amino acids 410-437 of SEQ ID NO:387).

[00103] Anti-HLA-B*27 antibodies were captured on either a polyclonal goat anti-human Fc-gamma-specific antibody (Jackson Lab, # 109-005-098) or a monoclonal mouse anti-human Fc antibody (GE Healthcare, # BR-1008-39) surface created through direct amine coupling to a Biacore CM5 sensor chip. Different concentrations of scHLA-B27-mmH were injected over the monoclonal antibody captured surface for 4 minutes to determine the association rate, and dissociation was then monitored for 8 minutes. Temperature-dependent binding (Tables 4 and 5) was determined at 25°C and 37°C at a flow rate of 50 µl/min using HBST buffer (0.01M HEPES pH7.4, 0.15M NaCl, 3mM EDTA, 0.05% v/v surfactant P20). pH-dependent binding (Tables 2 and 3) was determined at the same flow rate in PBS buffer containing 0.05% v/v surfactant P20.

[00104] Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0 curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives ($t_{1/2}$) were calculated from the kinetic rate constants as: K_D (M) = k_d / k_a and $t_{½}$ (min) = [ln2/(60*k_d)]. Results are shown in Tables 2-5 (IC=inconclusive; NB = no binding).

Table 2: Kinetics of scHLA-B27-mmH Binding to Different Monoclonal Antibodies at 25ºC in PBS at pH 7.2

mAb Captured	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{½}$ (min)
H4H2524P	1.13E+05	7.85E-03	6.90E-08	1
H4H2526P	4.32E+04	3.32E-05	7.60E-10	348
H4H2528P	2.80E+04	7.64E-05	2.71E-09	151
H4H2530S	3.98E+05	2.66E-04	6.68E-10	43

H4H2532P	4.86E+05	2.22E-04	4.57E-10	52
H4H2534S	3.46E+05	7.35E-04	2.13E-09	16
H4H2538P	4.50E+05	1.66E-04	3.70E-10	69
H4H2542P	9.70E+04	5.03E-03	5.21E-08	2
H4H2555P	4.97E+05	2.44E-04	4.91E-10	47
H4H2559P	4.07E+05	1.77E-04	4.34E-10	65
H4H2560P	5.80E+05	7.25E-04	1.25E-09	16
H4H2562S	9.15E+04	2.79E-03	3.05E-08	4
H4H2564S	6.67E+04	5.90E-04	8.84E-09	20
H4H2477N2	1.30E+04	5.79E-04	4.40E-08	20
H4H2480N	2.01E+04	6.43E-04	3.20E-08	18
H4H2482N2	3.88E+04	5.64E-03	1.45E-07	2
H4H2490N2	2.50E+04	5.98E-03	2.39E-07	2
H4H2491N	5.43E+04	4.38E-04	8.06E-09	26
H4H2497N	2.28E+04	5.07E-04	2.23E-08	23
H4H2499N	IC	IC	IC	IC
H4H2718N	3.14E+04	3.05E-03	9.73E-08	4

Table 3: Kinetics of scHLA-B27-mmH Binding to Different Monoclonal Antibodies at 25ºC in PBS at pH 5.75

mAb Captured	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{½}$ (min)
H4H2524P	3.80E+04	7.68E-03	2.04E-07	2
H4H2526P	5.14E+04	3.84E-05	7.39E-10	301
H4H2528P	3.14E+04	8.57E-05	2.74E-09	135
H4H2530S	7.13E+04	7.62E-04	1.07E-08	15
H4H2532P	4.45E+05	3.20E-04	7.17E-10	36
H4H2534S	4.50E+04	2.40E-03	5.34E-08	5
H4H2538P	4.12E+05	2.64E-04	6.41E-10	44
H4H2542P	3.68E+04	1.80E-02	4.88E-07	1
H4H2555P	4.42E+05	3.78E-04	8.55E-10	31
H4H2559P	3.76E+05	2.71E-04	7.20E-10	43
H4H2560P	2.71E+05	2.09E-03	7.70E-09	6
H4H2562S	6.00E+04	5.71E-03	9.50E-08	2
H4H2564S	5.88E+04	4.40E-03	7.48E-08	3
H4H2477N2	1.32E+04	2.11E-03	1.60E-07	5
H4H2480N	1.92E+04	2.47E-03	1.29E-07	5
H4H2482N2	4.78E+04	6.76E-03	1.42E-07	2
H4H2490N2	3.09E+04	7.33E-03	2.37E-07	2
H4H2491N	9.60E+04	2.76E-03	2.89E-08	4
H4H2497N	2.91E+04	9.88E-04	3.39E-08	12
H4H2499N	IC	IC	IC	IC
H4H2718N	3.28E+04	3.19E-03	9.75E-08	4

Table 4: Kinetics of scHLA-B27-mmH Binding to Different Monoclonal Antibodies at 25ºC in HBST at pH 7.4

mAb Captured	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{½}$ (min)
H4H2524P	2.16E+05	5.17E-03	2.39E-08	2
H4H2526P	5.75E+04	1.20E-04	2.08E-09	96
H4H2528P	2.50E+04	1.51E-04	6.00E-09	77
H4H2530S	5.78E+05	7.05E-04	1.22E-09	16
H4H2532P	1.23E+06	5.55E-04	4.52E-10	21
H4H2534S	3.47E+05	1.80E-03	5.18E-09	6
H4H2538P	9.70E+05	5.86E-04	6.04E-10	20

H4H2542P	9.00E+04	5.20E-03	5.80E-08	2
H4H2555P	1.25E+06	6.49E-04	5.21E-10	18
H4H2559P	6.85E+05	6.13E-04	8.95E-10	19
H4H2560P	1.22E+06	1.79E-03	1.47E-09	6
H4H2562S	2.48E+05	2.47E-03	1.00E-08	5
H4H2564S	1.69E+05	6.03E-04	3.57E-09	19
H4H2477N2	6.50E+04	1.10E-03	1.69E-08	11
H4H2480N	5.50E+04	1.32E-03	2.40E-08	9
H4H2482N2	5.52E+04	6.60E-03	1.20E-07	2
H4H2490N2	3.27E+04	8.46E-03	2.59E-07	1
H4H2491N	1.60E+05	2.87E-04	1.80E-09	40
H4H2497N	2.96E+04	9.95E-04	3.36E-08	12
H4H2499N	7.90E+04	1.68E-03	2.13E-08	7
H4H2718N	6.17E+04	2.77E-03	4.49E-08	4
H4H2477N	NB	NB	NB	NB
H4H2482N	4.71E+04	9.30E-03	1.97E-07	1.2
H4H2490N	3.53E+04	3.52E-04	9.97E-09	33

Table 5: Kinetics of scHLA-B27-mmH Binding to Different Monoclonal Antibodies at 37ºC in HBST at pH 7.4

mAb Captured	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{½}$ (min)
H4H2524P	2.06E+05	5.51E-03	2.68E-08	2
H4H2526P	8.05E+04	3.15E-04	3.91E-09	37
H4H2528P	3.34E+04	6.81E-04	2.04E-08	17
H4H2530S	9.51E+05	1.79E-03	1.88E-09	6
H4H2532P	1.48E+06	1.24E-03	8.37E-10	9
H4H2534S	4.52E+05	3.92E-03	8.70E-09	3
H4H2538P	1.23E+06	1.40E-03	1.13E-09	8
H4H2542P	4.40E+04	7.56E-03	1.70E-07	2
H4H2555P	1.60E+06	1.63E-03	1.02E-09	7
H4H2559P	1.19E+06	1.40E-03	1.18E-09	8
H4H2560P	1.36E+06	2.66E-03	1.96E-09	4
H4H2562S	3.79E+05	2.89E-03	7.63E-09	4
H4H2564S	1.89E+05	1.35E-03	7.17E-09	9
H4H2477N2	8.70E+04	4.06E-03	4.70E-08	3
H4H2480N	NB	NB	NB	NB
H4H2482N2	3.50E+04	1.50E-02	4.30E-07	1
H4H2490N2	3.70E+04	1.31E-02	3.60E-07	1
H4H2491N	2.11E+05	4.78E-04	2.27E-09	24
H4H2497N	1.19E+04	4.01E-03	3.37E-07	3
H4H2499N	1.08E+05	3.00E-03	2.80E-08	4
H4H2718N	1.57E+05	4.58E-03	2.90E-08	3

[00105] At 25°C in PBS at pH 7.2, 20 of 21 anti-HLA-B*27 antibodies bound scHLA-B27-mmH with K_D values ranging from 370 pM to 239 nM as shown in Table 2. One antibody, H4H2499N, produced a low binding signal with kinetic data that could not be fit to standard models (IC).

[00106] At 25°C in PBS at pH 5.75, 20 of 21 anti-HLA-B*27 antibodies bound scHLA-B27-mmH with K_D values ranging from 641 pM to 488 nM as shown in Table 3. One antibody, H4H2499N produced a low binding signal with kinetic data that could not be fit to standard models (IC).

[00107] At 25°C in HBST at pH 7.4, 23 of 24 anti-HLA-B*27 antibodies tested bound scHLA-

B27-mmH with K_D values ranging from 452 pM to 259 nM as shown in Table 4. One antibody, H4H2477N, did not bind scHLA-B27-mmH under these conditions (NB).

[00108] When tested at 37°C in HBST at pH 7.4, 20 of the 21 anti-HLA-B*27 antibodies tested bound scHLA-B27-mmH with K_D values ranging from 837 pM to 430 nM as shown in Table 5. One antibody, H4H2480N, did not bind to scHLA-B27-mmH under these conditions.

[00109] The ratio of K_D values at pH 5.75 to K_D values at pH 7.2 was calculated for each of the tested antibodies to assess the pH-dependent binding properties of the anti-HLA-B*27 antibodies (Table 6). As used herein, an anti-HLA-B*27 antibody exhibits "pH-dependent binding" if the antibody exhibits a higher level of binding (*e.g.*, a lower K_D value) at an increased pH value as compared to binding exhibited at lower pH value; this can be represented as a binding ratio (*e.g.*, wherein the pH-dependent binding is demonstrated by a K_D value at pH 5.75 that is at least 5-times greater than its K_D value at pH 7.2 (as in Table 6 below).

Table 6: pH-Dependent Binding

	K_D (M)		
Antibody	**pH 7.2**	**pH 5.75**	**Ratio K_D @ 5.75 / K_D @ 7.2**
H4H2524P	6.90E-08	2.04E-07	2.96
H4H2526P	7.60E-10	7.39E-10	0.97
H4H2528P	2.71E-09	2.74E-09	1.01
H4H2530S	6.68E-10	1.07E-08	16.02
H4H2532P	4.57E-10	7.17E-10	1.57
H4H2534S	2.13E-09	5.34E-08	25.07
H4H2538P	3.70E-10	6.41E-10	1.73
H4H2542P	5.21E-08	4.88E-07	9.37
H4H2555P	4.91E-10	8.55E-10	1.74
H4H2559P	4.34E-10	7.20E-10	1.66
H4H2560P	1.25E-09	7.70E-09	6.16
H4H2562S	3.05E-08	9.50E-08	3.11
H4H2564S	8.84E-09	7.48E-08	8.46
H4H2477N2	4.40E-08	1.60E-07	3.64
H4H2480N	3.20E-08	1.29E-07	4.03
H4H2482N2	1.45E-07	1.42E-07	0.98
H4H2490N2	2.39E-07	2.37E-07	0.99
H4H2491N	8.06E-09	2.89E-08	3.59
H4H2497N	2.23E-08	3.39E-08	1.52
H4H2499N	IC	IC	N/A
H4H2718N	9.73E-08	9.75E-08	1.00

[00110] As shown in Table 6, the anti-HLA-B*27 antibodies of the present invention that

exhibited pH-dependent binding according to the aforementioned definition are: H4H2530S, H4H2534S, H4H2542P, H4H2560P, and H4H2564S.

Example 4. Anti-HLA-B*27 Antibodies Block HLA-B*27-Induced T Cell Activation In Vitro

[00111] A bioassay was developed to measure T cell activation induced by interaction between HLA-B*27-peptide complex and a corresponding T-cell receptor (TCR) by utilizing a mixed culture derived from two mammalian cell lines: C1R-neo (ATCC, # CRL-2369), a B-lymphoblastoid line previously shown to express low or no endogenous levels of HLA-A and HLA-B molecules; and Jurkat, a human CD4+ T cell line.

[0100] For the first component of the bioassay, the C1R-neo cell line was stably transfected to overexpress the heavy chain of the HLA-B*2705 allelic subvariant (amino acids 25-362 of SEQ ID NO:385) and human beta 2 microglobulin (β2m; amino acids 21-119 of SEQ ID NO:388) along with NP383-391, a nine amino acid HLA-B*27-restricted peptide (SRYWAIRTR [SEQ ID NO:389]) derived from the nucleoprotein of influenza A virus. NP383-391 positive cells were sorted by FACS and a stable cell line was isolated (C1R-neo/HLA-B*2705/β2m/NP383-391), which was maintained in Iscove's medium (Irvine Scientific, Cat. No. # 9032) supplemented with 10% fetal bovine serum and penicillin/streptomycin/L-glutamine.

[0101] For the second component of the bioassay, a reporter cell line, Jurkat/NFAT-Luc (bearing an IL-2 responsive element coupled to luciferase expression [NFAT-luciferase]) was engineered to express: (1) GRb, a TCR consisting of two subunits, alpha (SEQ ID NO:390) and beta (SEQ ID NO:391), which were cloned from an HLA-B*27^{+} individual (the intact receptor, GRbAB, binds specifically the NP383-391 peptide in a complex with HLA-B*2705); and (2) the CD8 co-receptor, consisting of two subunits, alpha (SEQ ID NO:392), and beta (SEQ ID NO:393), which are required for the activation of class I-restricted TCR (normally expressed on CD8+ T cells). The resulting stable cell line (Jurkat/NFAT-Luc/CD8AB/GRbAB) was isolated and maintained in RPMI (Irvine Scientific, # 9160) supplemented with 10% fetal bovine serum and penicillin/streptomycin/L-glutamine.

[0102] For the bioassay C1R-neo/HLA-B*2705/β2m/NP383-391 cells were seeded into 96-well assay plates at 10,000 cells/well. To determine IC_{50} values, 1:3 serial dilutions of anti-HLA-B*27 antibodies starting from 100 nM down to 0.1 nM (plus a no antibody control) were added to cells and incubated for 1 hour at 37°C in 5% CO_2. To stimulate the HLA-TCR interaction, 100,000 Jurkat/NFAT-Luc/CD8AB/GRbAB cells were added to each well and incubated for 5 hours at 37°C in 5% CO_2. Luciferase activity was detected by adding OneGlo substrate (Promega, # E6051) reagent. Results are summarized in Table 7.

Table 7: Anti-HLA-B*27 antibody blocking of C1R-neo/HLA-B*2705/β2m/NP383-391 cell dependent stimulation of Jurkat/NFAT-Luc/CD8AB/GrbAB cells

Antibody	IC_{50} (M)
H4H2524P	1.185E-09
H4H2526P	8.559E-11
H4H2528P	2.639E-10
H4H2530S	3.749E-10
H4H2532P	3.748E-10
H4H2534S	4.432E-10
H4H2538P	3.626E-10
H4H2542P	1.143E-10
H4H2555P	3.465E-10
H4H2559P	1.802E-10
H4H2560P	6.242E-10
H4H2562S	blocks, not sigmoidal
H4H2564S	blocks, not sigmoidal
H4H2477N2	strongly activates, not sigmoidal
H4H2480N	blocks, not sigmoidal
H4H2482N2	blocks, not sigmoidal
H4H2490N2	blocks, not sigmoidal
H4H2491N	no blocking
H4H2497N	1.193E-10
H4H2499N	1.812E-08
H4H2718N	blocks, not sigmoidal
Isotype control	no blocking

[0103] As shown in Table 7, thirteen of the 21 anti-HLA antibodies tested in the mixed culture bioassay blocked C1R-Neo/HLA-B*2705/β2m/NP383-391 cell dependent stimulation of Jurkat/NFAT-Luc/CD8AB/GrbAB cells with IC_{50} values ranging from 85.6pM to 18.1nM. Additionally, 6 of the 21 antibodies also blocked in the assay, but not in a sigmoidal manner. One antibody tested, H4H2477N2, displayed strong activation in the mixed culture bioassay, but not in a sigmoidal manner.

Example 5. Flow Cytometric Analysis to Confirm Binding of Anti-HLA-B*27 Antibodies to Cells Expressing HLA-B*27

[0104] Flow cytometric analysis was utilized to confirm binding of anti-HLA-B*27 antibodies to HLA-B*27$^+$ cells. Tested cells included: (a) the C1R-neo-B*2705 cell line; (b) splenocytes from HLA-B*27 transgenic mice; and (c) primary human peripheral blood mononuclear cells (PBMC) from HLA-B*27$^+$ individuals. The C1R-neo-B*2705 cell line is a derivative of the parental C1R-neo cell line stably transfected to overexpress the heavy chain of the HLA-B*2705 allele and human beta 2 microglobulin (see Example 4). Parental non-transfected C1R-neo cells were

used as a negative control. A transgenic mouse strain was generated by transgenic heterozygous expression of the heavy chain of HLA-B*2705 and human β2microglobulin [C57BL/6J-Tg(HLA-B27) 30-4Trg mouse strain; stock # 003440 from The Jackson Laboratory]. Splenocytes from wild type littermates were used as a negative control for the heterozygous HLA-B*27^{+} splenocytes. Primary human PBMCs were genotyped as positive for HLA-B*27, and expression of the HLA-B*27 allele was confirmed by staining with a commercial anti-HLA-B*27 antibody (HLA ABC Ab; Millipore # MAB1285F). PBMCs from HLA-B*27^{-} donors were used as a negative control.

[0105] For experiments with mouse splenocytes, mice were sacrificed and spleens were removed and mechanically homogenized. Cell suspension was filtered and mononuclear cells were purified on a Histopaque gradient column. Human PBMC were purified from the white blood concentrate (Leukopak; New York Blood Bank, NY, # E3752V00) on a Histopaque gradient column.

[0106] For flow cytometric analysis, 100,000 cells were washed in stain buffer (eBioscience, # 00-4222) and incubated with whole human IgG (10 µg/ml) (Jackson ImmunoResearch, # 009-000-003) for 15 minutes at 4°C to block Fc receptors on the cells. Next, anti-HLA-B*27 antibodies were added at indicated concentrations (0.1 or 1 µg/ml) and incubated for 30 minutes at 4°C. Cells were washed once and then incubated with secondary anti-human Fc antibody (1:2000 dilution) (Jackson ImmunoResearch, # 709-116-149) for 20 minutes at 4°C. Cells were washed twice and fluorescence measurements were acquired using a FACSCalibur flow cytometer (BD Biosciences, MD) and analyzed by FlowJo software. Blocking with whole human IgG alone did not generate binding signal above background when detecting with secondary anti-hFc antibody. Results are summarized in Table 8 (WT = wild-type mouse; TG = HLA-B27/hβ2m transgenic mouse).

Table 8: FACS binding of anti-HLA antibodies to cells with differing HLA expression patterns

	Mean Fluorescence Intensity (MFI)						
	mouse splenocytes			C1R-neo cells		primary PBMCs	
Antibody	WT (1 µg/ml)	TG (0.1 µg/ml)	TG (1 µg/ml)	C1R-neo (1 µg/ml)	C1R-neo-HLA-B27 (1 µg/ml)	B27(neg) (1 µg/ml)	B27(pos) (1 µg/ml)
H4H2477N2	30.0	107	336	13.2	53	91.6	102
H4H2480N	11.9	86.4	337	11	62.9	67.3	72.1
H4H2482N2	13.5	250	360	4.82	141	42.1	76.6
H4H2490N2	14.9	262	348	4.06	137	41.5	77.5
H4H2491N	6.0	27.4	60	2.53	12.8	26.6	35.8
H4H2497N	24.7	285	733	13.4	162	127	194
H4H2499N	15.4	170	378	1.75	89.4	9.13	51.4

H4H2524P	6.5	54.8	436	8.75	51.3	124	169
H4H2526P	8.6	86.7	758	33.7	146	185	252
H4H2528P	7.9	99.7	831	32.4	149	197	255
H4H2530S	8.0	65.8	512	17.9	78.8	158	196
H4H2532P	7.0	70.5	635	7.63	97.3	182	238
H4H2534S	6.8	75.3	555	15.9	64.9	144	186
H4H2538P	7.4	70.4	533	9.01	101	187	245
H4H2542P	7.8	115	884	2.02	117	73.4	170
H4H2555P	6.6	73.7	565	7.49	117	165	229
H4H2559P	8.9	54.6	498	8.47	104	182	230
H4H2560P	6.9	72.2	618	13.1	110	170	205
H4H2562S	6.2	47.6	290	2.24	31.1	50.2	66.6
H4H2564S	8.6	53.7	350	3.11	50.3	64.7	73.7
H4H2718N	9.3	55.6	215	3.84	36.5	43.2	63.8

[0107] As shown in Table 8, all of the tested anti-HLA-B*27 antibodies bound to HLA-B*27$^+$ transgenic splenocytes, while no binding above background level was detected on splenocytes from wild type mice. Similarly, all antibodies bound C1R-neo-B*2705 cells. In parental C1R-neo cells, no binding above background level was detected for H4H2499N, H4H2542P and H4H2562P, while all the other antibodies showed various levels of binding above the background level, but lower than to C1R-neo-B*2705 cells. In human PBMC, all antibodies stained cells isolated from the blood of an individual confirmed to express the HLA-B*27 allele by genotyping ("B27(pos)"). When compared with binding to PBMC from an HLA-B*27 negative donor ("B27(neg)") the binding ratios ranged from 1.1 to 5.6, with H4H2499N exhibiting the highest specificity to the HLA-B*27$^+$ PBMC.

Example 6. Binding of HLA-B*27 Antibodies to Peripheral Blood Mononuclear Cells (PBMC)

[0108] Additional flow cytometry experiments were conducted using PBMCs shown to express alleles other than HLA-B*27. Human blood from 4 donors was collected in K2-EDTA anti-coagulant tubes (BD, #366643). Genomic DNA was purified as per the manufacturer's specifications from whole blood samples using a QIAamp DNA blood mini kit (Qiagen, #51304). Genomic DNA samples were then amplified, and the subsequent HLA locus-specific sequencing reaction was carried out using a SeCore kit (Invitrogen, locus A #5300925, locus B #5311925D, locus C # 5320925). The DNA sequence was determined using an ABI 3730xL DNA Analyzer, and HLA type was determined using uType SBT HLA sequencing software (Invitrogen, #53999-1).

[0109] Human PBMCs were isolated from the blood of the same donors by Ficoll centrifugation. PBMCs were blocked with ChromPure Human IgG (Jackson Immunoresearch, #

009-000-003), stained with 1ug/ml of anti-HLA antibodies for 30 minutes at 4°C followed by washing with staining buffer (BD Biosciences, # 554657) and incubation with a 1:500 dilution of APC-labeled anti-human IgG secondary antibody (Jackson Immunoresearch, #109-136-098) for 20 minutes at 4°C. PBMCs were then washed, resuspended in stabilizing fixative (BD Biosciences, #338036) and sorted on a FACS Canto machine. Data were analyzed using FlowJo software.

[0110] The HLA Class I alleles determined from donors HD1, HD2, HD3, and HD4 are shown in Table 9. None of the 4 tested donors was determined to be HLA-B*27-positive.

Table 9: HLA Class I typing for 4 donor whole blood samples

Donor Sample ID	HLA-A Locus		HLA-B Locus		HLA-C Locus	
	Allele 1	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
D2-1 (HD1)	*11:01:01	*24:03:01	*15:01:01:01	*18:01:01:01	*03:03:01	*12:03:01:01
D4-1 (HD4)	*24:02:01	*33:03:01	*40:06:01:01	*58:01:01	*03:02:01	*15:02:01
D3* (HD3)	*03:01:01:01	*26:01:01	*07:02:01	*38:01:01	*07:02:01:01	*12:03:01:01
D3-5 (HD2)	*02:01:01:01	*36:01	*14:02:01	*53:01:01	*04:01:01:01	*08:02:01

[0111] The binding of anti-HLA antibodies to PBMCs of the HLA-typed donors, as determined by flow cytometry, is shown in Table 10.

Table 10: FACS binding of anti-HLA antibodies to PBMCs from 4 donors

Antibody	**HD1**	**HD2**	**HD3**	**HD4**
Unstained	3.13	3.02	3.02	3.71
anti-human Fc secondary	13.8	28.4	18.2	15
H4H2524P	460	685	703	206
H4H2526P	690	1059	1156	518
H4H2528P	633	959	1143	635
H4H2530S	637	756	995	455
H4H2532P	626	675	955	557
H4H2534S	327	52.1	1010	372
H4H2538P	580	41.7	1032	430
H4H2542P	791	35.4	830	742
H4H2555P	435	35.4	1065	532
H4H2560P	378	771	1125	382
H4H2562S	235	453	980	171
H4H2564S	404	560	876	278
H4H2477N2	704	868	1374	680
H4H2480N	454	513	985	397
H4H2482N2	573	1206	1108	624

H4H2490N2	391	1078	918	640
H4H2491N	438	783	754	388
H4H2497N	281	1097	1073	621
H4H2499N	162	315	424	219
H4H2718N	96.6	210	282	56.6

[0112] As shown in Table 10, all of the anti-HLA antibodies bound to the PBMCs of all donors to some extent, except antibodies H4H2534S, H4H2538P, H4H2542P and H4H2555P, which did not bind to PBMCs from the HD2 donor. Antibodies H4H2499N and H4H2718N (last two rows of Table 10) show the least amount of binding to PBMCs from non-B*27 donors.

Example 7. Binding of HLA-B*27 Antibodies to Surface HLA*B

[0113] Flow cytometric analysis was further utilized to determine specificity of binding of anti-HLA antibodies to cell lines engineered to overexpress different HLA*B alleles. To generate cell lines expressing different HLA alleles, parental K562 (immortalized human myelogenous leukemia) cells were electroporated with DNA plasmids to promote stable integration of 23 different HLA alleles (shown in Table 11) as well as an antibiotic resistant gene to either neomycin or hygromycin. Electroporated cell lines were then placed under antibiotic selection for 2.5 to 5 weeks to select for cells expressing the specific HLA alleles. The resulting K562/HLA cell lines were FACS sorted for populations with the highest levels of surface HLA (top 10% of expressers) using an HLA-A, B, C pan-specific antibody (clone W6/32) conjugated to phycoerythrin (Biolegend, #311406).

Table 11: HLA-B alleles used in the generation of HLA-B expressing K562 cell lines

HLA*B Alleles Expressed	IMGT HLA Accession Number
B*07:02:01	HLA00132
B*08:01:01	HLA00146
B*13:01:01	HLA00152
B*14:02:01	HLA00158
B*15:01:01:01	HLA00162
B*18:01:01:01	HLA00213
B*27:05:02	HLA00225
B*27:09	HLA00230
B*35:01:01:01	HLA00237
B*37:01:01	HLA00265
B*38:02:01	HLA00268
B*39:01:01:01	HLA00271
B*40:01:01	HLA00291
B*42:01:01	HLA00315
B*44:03:01	HLA00319
B*45:01	HLA00329
B*46:01:01	HLA00331

B*48:01:01	HLA00335
B*49:01:01	HLA00340
B*50:01:01	HLA00341
B*52:01:01:01	HLA00362
B*53:01:01	HLA00364
B*54:01:01	HLA00367

[0114] To perform the flow cytometric analysis, ~2 x 10^5 cells/well were washed in 200 uL of Stain Buffer (BSA) (BD Pharmingen, #554657), and blocked with 1 ug/mL of purified human IgG (Jackson ImmunoResearch, # 009-000-003) for 15 minutes at room temperature. Next, anti-HLA antibodies were added at 1 µg/mL and incubated for 30 minutes at 4°C. Cells were washed twice with Stain Buffer and then incubated with a goat polyclonal F(ab')2 secondary reagent conjugated to allophycocyanin that is specific for human IgG Fc-gamma fragment (Jackson ImmunoResearch, # 109-136-098) at 1:500 dilution for 20 minutes at 4°C. Cells were washed twice with 200 uL and fluorescence measurements were acquired using a FACSCanto II instrument (BD Biosciences, MD) and analyzed using FlowJo software (Tree Star).

[0115] Results for the 20 anti-HLA antibodies binding to the K562 cell lines expressing different HLA-B alleles shown as ratio of MFI signal relative to that of the parental K562 cell lines are shown in Tables 12, 13, and 14, below. Of the 20 antibodies tested, H4H2499N exhibited the highest degree of specificity.

Table 12: FACS binding of anti-HLA antibodies to K562 cells expressing seven different HLA-B alleles

		Ratio of MFI relative to parental						
	K652 Parental MFI Value	HLA-B*27:05:02	HLA-B*35:01:01:01	HLA-B*44:03:01	HLA-B*38:02:01	HLA-B*39:01:01:01	HLA-B*40:01:01	HLA-B*42:01:01
Unstained	25	0.7	1.2	1.0	1.4	8.6	1.1	1.2
ahFc secondary	147	0.7	0.6	0.8	1.2	0.8	0.7	1.1
H4H2524P	232	11.1	22.1	12.9	5.7	2.5	5.2	14.0
H4H2526P	328	10.6	23.3	10.7	6.2	3.8	6.1	13.4
H4H2528P	334	11.7	22.1	9.5	6.3	4.1	6.1	14.0
H4H2530S	334	7.8	20.1	12.0	4.6	3.3	4.6	10.6
H4H2532P	309	8.6	15.7	10.2	6.0	4.0	5.1	10.2
H4H2534S	321	8.8	20.3	9.2	4.7	3.1	4.3	11.7
H4H2538P	327	8.9	20.7	9.3	6.7	3.9	5.5	13.5
H4H2542P	234	9.7	4.3	10.0	9.6	1.4	0.9	6.4
H4H2555P	301	10.7	20.3	10.8	6.9	4.3	5.7	13.6
H4H2559P	289	6.7	16.5	7.8	7.2	4.4	5.5	14.6
H4H2560P	295	10.2	21.0	11.7	0.7	3.1	6.3	13.2
H4H2562S	238	7.5	9.7	7.8	3.5	2.1	3.3	9.7
H4H2564S	267	8.7	13.0	8.5	3.9	2.2	3.3	11.6
H4H2477N2	236	10.6	17.0	10.2	5.7	2.8	0.7	1.9
H4H2480N	237	9.6	15.3	8.5	5.9	3.2	1.0	3.3
H4H2482N2	235	12.5	24.8	17.7	4.3	2.4	1.3	4.2
H4H2490N2	237	13.5	23.5	17.1	3.4	2.0	1.1	4.2
H4H2497N	252	13.7	24.2	8.7	6.7	4.0	5.8	17.6
H4H2499N	209	9.8	0.9	13.1	3.7	0.8	0.6	1.0
H4H2718N	225	7.0	11.8	6.3	3.2	1.7	2.2	9.7

Table 13: FACS binding of anti-HLA antibodies to K562 cells expressing six different HLA-B alleles

		Ratio of MFI relative to parental					
	K652 Parental MFI Value	HLA-B* 07:02:01	HLA-B* 37:01:01	HLA-B* 08:01:01	HLA-B* 15:01:01:01	HLA-B* 45:01	HLA-B* 46:01:01
Unstained	33.7	0.8	0.8	0.8	0.8	1.1	1.2
ahFc secondary	83.4	3.4	3.4	1.6	1.3	1.5	1.1
H4H2524P	108	81.2	28.5	31.1	27.5	24.6	18.3
H4H2526P	200	57.9	25.7	28.6	29.7	20.4	16.7
H4H2528P	206	53.4	25.0	26.0	28.7	24.3	16.7
H4H2530S	190	53.0	22.4	23.2	23.0	18.6	16.1
H4H2532P	155	70.8	26.6	33.0	28.4	22.5	20.5
H4H2534S	168	57.6	21.4	25.3	23.5	20.2	16.4
H4H2538P	158	69.3	28.7	31.1	31.0	26.0	21.2
H4H2542P	89.5	22.7	49.2	11.5	9.3	10.1	3.9
H4H2555P	156	61.8	24.4	34.0	30.8	23.2	19.7
H4H2559P	151	63.8	29.5	35.2	31.8	25.0	20.4
H4H2560P	99.9	68.9	34.5	30.6	28.5	27.0	25.8
H4H2562S	109	56.0	25.4	20.7	20.8	18.0	11.8
H4H2564S	95.8	69.2	28.3	27.0	25.5	19.8	16.2
H4H2477N2	110	3.9	21.4	3.1	23.4	13.7	13.3
H4H2480N	110	10.1	26.2	5.6	26.9	15.0	15.9
H4H2482N2	118	10.0	16.4	6.0	25.9	21.3	11.6
H4H2490N2	100	14.2	20.5	8.1	30.9	25.0	14.6
H4H2497N	121	59.5	35.5	28.1	37.9	28.3	20.8
H4H2499N	85.1	1.5	29.1	1.9	1.4	1.4	1.3
H4H2718N	109	45.7	20.7	17.7	18.4	14.1	10.6

Table 14: FACS binding of anti-HLA antibodies to K562 cells expressing ten different HLA-B alleles

		Ratio of MFI relative to parental									
	K652 Parental MFI Value	HLA-B*13:01:01	HLA-B*14:02:01	HLA-B*18:01:01:01	HLA-B*27:09	HLA-B*48:01:01	HLA-B*49:01:01	HLA-B*50:01:01	HLA-B*52:01:01:01	HLA-B*53:01:01	HLA-B*54:01:01
unstained	73.1	0.9	1.1	0.9	1.0	1.4	1.1	0.9	0.9	1.0	1.0
ahFc secondary	80	1.9	1.3	2.1	2.1	1.9	2.2	1.8	1.8	1.5	1.4
H4H2524P	95.7	5.4	3.8	5.3	9.7	5.8	3.2	3.7	4.1	4.6	3.2
H4H2526P	108	6.7	4.7	8.3	14.9	6.9	3.5	4.4	5.1	5.9	4.6
H4H2528P	104	5.4	4.3	7.0	16.5	11.6	3.8	7.2	8.2	6.0	4.9
H4H2530S	84.8	10.2	4.4	12.2	15.4	5.5	3.8	4.6	4.1	4.8	3.5
H4H2532P	88.5	7.7	4.5	6.9	14.8	9.1	3.4	4.8	5.5	6.8	4.6
H4H2534S	89	5.1	3.6	10.8	12.6	8.8	5.4	4.9	5.9	6.7	4.3
H4H2538P	99.4	8.5	5.5	9.5	12.7	7.8	2.8	4.3	6.3	6.5	5.0
H4H2542P	82.7	2.9	1.7	5.3	17.7	3.7	3.9	4.2	7.0	8.7	3.4
H4H2555P	89.1	9.0	5.1	10.4	13.2	9.0	3.5	6.1	6.5	6.9	5.2
H4H2559P	87.4	9.6	5.2	8.9	11.4	9.8	3.2	7.1	7.6	7.9	5.8
H4H2560P	89.8	10.0	5.1	7.4	13.7	9.0	5.0	6.1	6.0	6.4	5.3
H4H2562S	89.3	5.9	3.0	6.5	8.6	8.0	4.4	4.7	3.7	3.2	3.5
H4H2564S	95.7	4.4	2.1	6.0	8.0	2.7	3.2	3.4	3.5	3.1	2.3
H4H2477N2	98.2	5.2	2.6	6.5	8.4	1.8	4.3	3.2	4.0	4.4	2.6
H4H2480N	96.3	4.5	1.8	4.4	7.4	1.7	2.6	2.0	2.6	2.2	1.6
H4H2482N2	88.2	6.5	4.4	6.7	9.6	8.3	4.5	5.7	8.6	7.0	4.9
H4H2490N2	94.9	6.7	2.8	6.3	12.1	4.8	4.4	5.2	6.8	5.9	3.4
H4H2497N	92.4	7.7	3.9	6.9	11.6	7.3	4.0	5.4	6.6	5.2	4.1
H4H2499N	85.9	8.3	1.2	1.9	13.7	1.6	4.1	1.7	6.5	3.5	1.2
H4H2718N	87.3	5.6	1.8	6.9	7.7	4.8	4.4	3.3	4.3	3.0	2.1

Example 8. Preferential Binding of Antibodies to HLA-B*27 Allelic Subvariants

[0116] The ability of anti-HLA-B*27 antibodies to bind to cell-surface human HLA-B*27 allelic subvariants (*i.e.*, HLA-B*2705 and HLA-B*2709), as compared to their ability to bind the HLA-B*07 allelic variant, was measured using electrochemiluminescence detection technology. For these studies C1R-neo cells stably transfected to express either HLA-B*07 heavy chain (CR1-B7, ATCC #CRL-2371), HLA-B*2705 heavy chain (amino acids 25-362 of SEQ ID NO:385), or HLA-B*2709 heavy chain (amino acids 25-362 of SEQ ID NO:386), were used. Each of the HLA-B* expressing cell lines also expressed human beta 2 microglobulin (β2m; amino acids 21-119 of SEQ ID NO:388) which forms a non-covalent complex with HLA heavy chain.

[0117] Cells were harvested and counted using a Cellometer™ Auto T4 cell counter (Nexcelom Bioscience). Approximately 20,000 cells per well were seeded in PBS into carbon

electrode plates (Meso Scale Discovery, # L15-XB-3/L11XB-3) and then incubated for 1 hour at 37°C. After blocking with PBS + 2% (w/v) BSA for 1 hour at 25°C, either 1 nM or 1.1 nM of anti-HLA-B*27 antibodies was transferred to plate-bound cells and incubated for 1 hour at 25°C. Unbound antibodies were washed off using an AquaMax2000 plate washer (MDS Analytical Technologies). Cell-bound anti-HLA-B*27 antibodies were detected with either anti-mFc (Jackson ImmunoResearch, #115-005-164) or anti-hFc (Meso Scale Discovery, #R32AJ-1) antibodies conjugated with SULFO-TAG™. For the cell binding measurement, MSD Read Buffer (Meso Scale Discovery, #R92TD-2) was added to the cell-antibody mixture and allowed to incubate for 15 minutes at room temperature before electrochemical stimulation. Luminescence was detected using a SECTOR Imager 6000 reader (MSD) using a 620 nm wavelength filter. The luminescence intensity correlates with the amount of antibodies bound to cells. Specificity ratios of anti-HLA-B*27 antibodies binding either C1R-B*07, C1R-neo B*2705 or C1R-neo B*2709 cells, relative to C1R-neo parental cells, are shown in Table 15. (-) indicates ratios from 1 to 3 fold above binding to C1R-neo cells; (+) indicates ratios from 3 to 10 fold above binding to C1R-neo cells; (++) indicates ratios from 10 to 50 fold above binding to C1R-neo cells; and (+++) indicates ratios greater than 50 fold above binding to C1R-neo cells.

Table 15: Relative Ratios of Antibody Binding to Cells Expressing HLA-B* Alleles to Antibody Binding to Parental Cells

	Cell Line		
Antibody	**C1R B*7**	**C1R-neo B*2705**	**C1R-neo B*2709**
H1M2477N	+	++	++
H1M2480N	+	++	++
H2aM2482N	++	+++	+++
H1M2490N	-	++	++
H2bM2491N	+	+	+
H1M2497N	+	++	+
H2aM2499N	-	+++	+++
H2bM2718N	+	++	+
H4H2524P	+	++	++
H4H2526P	+	++	++
H4H2528P	+	++	++
H4H2530S	+	++	+
H4H2532P	+	++	++
H4H2534S	+	++	+
H4H2538P	+	++	++
H4H2542P	+	+++	++
H4H2555P	+	++	++
H4H2559P	+	++	++

H4H2560P	+	++	+
H4H2562S	+	++	++
H4H2564S	+	+	+

[0118] As shown in Table 15, only H2aM2482N exhibited significant binding to cells expressing the HLA-B*07 allele (with a specificity ratio of from 10- to 50-fold above binding to C1R-neo cells). Antibodies H1M2477N, H1M2480N, H2aM2482N, H1M2490N, H2aM2499N, H4H2524P, H4H2526P, H4H2528P, H4H2532P, H4H2538P, H4H2555P, H4H2559P, and H4H2562S exhibited approximately equivalent binding to cells expressing the HLA-B*2705 allelic variant as to cells expressing the HLA-B*2709 allelic variant. On the other hand, certain exemplary antibodies exhibited relatively stronger binding to cells expressing the HLA-B*2705 allele as compared to cells expressing the HLA-B*2709 allele (*e.g.*, H1M2497N, H2bM2718N, H4H2530S, H4H2534S, H4H2542P, and H4H2560P).

[0119] All of the antibodies tested, except for H2bM2491N and H4H2564S, exhibited enhanced binding to cells expressing the HLA-B*2705 and/or HLA-B*2709 allelic subvariants compared to cells expressing the HLA-B*07 allelic variant. Antibody H2aM2499N, for example, showed at least 50-fold greater binding to cells expressing HLA-B*2705 and HLA-B*2709 allelic subvariants above binding to C1R-neo cells (represented by [+++] in Table 15), and less than 3-fold greater binding to cells expressing the HLA-B*07 allelic variant above binding to C1R-neo cells (represented by [-] in Table 15). Similar, but less pronounced, enhanced binding characteristics were observed with several other exemplary anti-HLA-B*27 antibodies tested in this Example, as shown in Table 15.

Example 9. Activity of Anti-HLA-B*27 Antibodies Evaluated *In Vivo*

[0120] The activity of anti-HLA-B*27 antibodies *in vivo* was investigated. HLA-B*27 (amino acids 25-362 of UniProt accession number P03989) and human beta 2 microglobulin (β2m; amino acids 21-119 of GenBank accession number NP_004039) were overexpressed in the liver of C57BL/6 wild type (WT) mice obtained from Jackson Laboratory by hydrodynamic delivery of DNA (HDD). For the HDD experiment, WT mice were divided into groups of 2-5 mice per cohort, and each mouse was injected with either 50 μg of plasmid expressing HLA-B*27 plus 50 μg of plasmid expressing β2m or with 50 μg of an empty plasmid control. At days 3 and 10 post-HDD injection, mice in each of four cohorts were intra-peritoneally injected with 30mg/kg of anti-HLA antibodies H4H2542P, H4H2499N or H4H2482P, or an isotype control antibody. Mice were sacrificed on day 14 post-HDD, and cells infiltrating the liver were isolated by collagenase treatment and percoll centrifugation. Liver red blood cells were lysed using ACK lysis buffer (Gibco, #A1492-01). Infiltrating immune cells were stained with a live/dead cell dye (Invitrogen, #L34957) as well as an anti-CD45 antibody (eBioscience, #12-0451-82) to detect infiltrating leukocytes and anti-CD3 (BD Biosciences, #552774), anti-CD4 (Biolegend, #100414) and anti-

CD8 (Biolegend, #100734) antibodies to identify different T cell populations. Flow cytometry was performed on a FACS Canto and data were analyzed using FlowJo software. Levels of inflitration of immune cells generally, and $CD8^+$ and $CD4^+$ cells in particular, into livers of sacrificed animals were measured in terms of frequency, *i.e.*, the percentage of intact/live cells from livers isolated with collagenase treatment and percoll centrifugation that were lymphocytes, $CD8^+$ cells, or $CD4^+$ cells (as opposed to, *e.g.*, hepatocytes).

[0121] The frequency of live cells in the lymphocyte gate (Table 16) as well as frequencies of $CD8^+$ (Table 17) and $CD4^+$ (Table 18) T cells infiltrating the liver were analyzed by flow cytometry. Mice injected with control plasmids showed a frequency of about 10% immune cells and 1% of $CD8^+$ and $CD4^+$ T cells infiltrating the liver, regardless of antibody treatment. In contrast, overexpression of HLA-B*27 and β2m led to an increase in the frequency of immune cell infiltrates (mean = 27%) including an increase in $CD8^+$ and $CD4^+$ T cells (6.1% and 3.3%, respectively) in mice treated with isotype control antibody. Treatment of HLA-B*27/β2m-expressing mice with the anti-HLA antibodies, H4H25422P and H4H2499N, led to a significant reduction in liver-infiltrating immune cells. This was most prominent in mice treated with H4H2499N, which also resulted in a significant decrease in the percentage of $CD8^+$ and $CD4^+$ T cells. In contrast, the anti-HLA antibody H4H2482P and the isotype control had no significant reduction on infiltration of immune cells into the liver. Therefore, the anti-HLA antibody H4H2499N is able to block infiltration of inflammatory cells into the liver of mice overexpressing HLA-B*27 and β2m by HDD.

Table 16: Frequency of live infiltrating immune cells

treatment	H4H2542P		H4H2499N		H4H2482P		Isotype control	
HDD plasmid	B27/ β2m	control	B27/ β2m	control	B27/ β2m	control	B27/ β2m	control
	17.1	14.2	8.68	15.3	19.3	7.55	22.5	5.94
	18.1	9.91	8.8	6.67	22.8	10.8	34	11.5
	12.7		5.63		13.7		28.6	
	10.9		10.7		23.3		23.5	
	23.7		9.35		21.5			
mean	16.5	12.06	8.632	10.99	20.12	9.175	27.15	8.72
SEM	2.242	2.145	0.8317	4.315	1.748	1.625	2.645	2.78
p value	0.0175		0.0002		NS		-	

Table 17: Frequency of total $CD8^+$ T cells in liver

treatment	H4H2542P		H4H2499N		H4H2482P		Isotype control	
HDD plasmid	B27/ β2m	control	B27/ β2m	control	B27/ β2m	control	B27/ β2m	control
	3.55	1.95	2.44	1.6	4.22	0.72	5.06	0.674
	4.12	1.22	2.13	0.61	5.7	1.08	7.43	1.2
	3.14		1.11		3.34		6.84	

	3.45		2.49		6.35		5.19	
	6.75		2.31		5.57			
mean	4.202	1.585	2.096	1.105	5.036	0.9	6.13	0.937
SEM	0.6564	0.365	0.2542	0.495	0.5475	0.18	0.5932	0.263
p value	NS		0.0003		NS		-	

Table 18: Frequency of total $CD4^+$ T cells in liver

treatment	**H4H2542P**		**H4H2499N**		**H4H2482P**		**Isotype control**	
HDD plasmid	**B27/ β2m**	**control**	**B27/ β2m**	**control**	**B27/ β2m**	**control**	**B27/ β2m**	**control**
	1.87	1.34	1.14	1.59	2.7	0.716	2.56	0.612
	2.6	1.18	1.29	0.586	3.25	1.17	4.59	1.12
	1.61		0.762		3.34		2.9	
	1.44		1.57		2.92		3.33	
	3.17		1.58		2.85			
mean	2.138	1.26	1.268	1.088	3.012	0.943	3.345	0.866
SEM	0.3253	0.08	0.1519	0.502	0.1217	0.227	0.4439	0.254
p value	NS		0.0018		NS		-	

Example 10. Construction of Histidine Substitution Variant Anti-HLA-B*27 Antibodies

[0122] In an attempt to generate variants of anti-HLA-B*27 antibodies with pH-dependent binding properties (*i.e.*, reduced binding at low pH as compared to neutral pH) and improved *in vivo* efficacy (*e.g.*, longer antibody serum half-life, prolonged inhibition of T-cell activation, etc.), a series of variant antibodies was constructed. In particular, mutant versions of the parental antibodies H4H2477N2 and H4H2499N were constructed in which each (non-histidine) amino acid within the complementarity determining regions (CDRs) of each antibody was individually mutated to histidine. As shown in Table 1, the heavy chain variable region (HCVR) of the parental H4H2477N2 antibody comprises the amino acid sequence of SEQ ID NO:50 and the light chain variable region (LCVR) of the parental H4H2477N2 antibody comprises the amino acid sequence of SEQ ID NO;58 the heavy chain variable region (HCVR) of the parental H4H2499N antibody comprises the amino acid sequence of SEQ ID NO:146 and the light chain variable region (LCVR) of the parental H4H2499N antibody comprises the amino acid sequence of SEQ ID NO:154; The CDR sequences of the parental H4H2477N2 and H4H2499N antibodies are depicted in Tables 19A and 19B, respectively.

Table 19A: CDR Sequences of mAb H4H2477N2

CDR	Amino Acid Sequence	SEQ ID NO:
HCDR1	GGSFSDYY	52
HCDR2	INHRGNT	54
HCDR3	ARIQLWLRGYDYYGMDV	56
LCDR1	QGIRND	60
LCDR2	AAS	62

LCDR3	LQHNTYPWT	64

Table 19B: CDR Sequences of mAb H4H2499N

CDR	Amino Acid Sequence	SEQ ID NO:
HCDR1	GFTFSDYY	148
HCDR2	ISTSGSTI	150
HCDR3	LLHYYYGMDV	152
LCDR1	QSVSSY	156
LCDR2	DAS	158
LCDR3	QQRSNWPWT	160

[0123] Forty-eight individual histidine substitution variants of H4H2477N2 were made (31 heavy chain CDR mutants and 17 light chain CDR mutants); and 43 individual histidine substitution variants of H4H2499N were made (25 heavy chain CDR mutants and 18 light chain CDR mutants). Variant antibodies obtained from culture medium (supernatant) after transient expression in Chinese hamster ovary (CHO) cells were screened for pH-dependent binding, *i.e.*, reduced binding to cells expressing HLA-B*27 at acidic pH as compared to neutral pH. Based on the initial supernatant screening, several variant antibodies comprising single and multiple histidine substitutions in the CDRs were selected for further study, as summarized in Table 20. The His-substitution designations shown in Table 20 (*e.g.*, "Y33H", "N52H", etc. for H4H2477N2; and "Y32H", "T53H", etc. for H4H2499N) relate to the heavy and light chain variable region (HCVR/LCVR) amino acid sequences of H4H2477N2 (*i.e.*, SEQ ID NOs: 50/58) and H4H2499N (*i.e.*, SEQ ID NOs:146/154). An empty cell in Table 20 denotes the parental sequence.

Table 20: Histidine Substitutions Selected for Further Study

Parental Antibody	His-Substitution Antibody Designation	Substitution					
		HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
H4H2477N2	H4H2477N3	Y33H					
H4H2477N2	H4H2477N4		N52H				
H4H2477N2	H4H2477N5	Y33H	N52H				
H4H2499N	H4H2499N2	Y32H					
H4H2499N	H4H2499N3		T53H				
H4H2499N	H4H2499N4	Y32H	T53H				
H4H2499N	H4H2499N5				Q27H S30H Y32H		
H4H2499N	H4H2499N6				Q27H S28H Y32H		
H4H2499N	H4H2499N7				S28H S30H Y32H		

Parental Antibody	His-Substitution Antibody Designation	Substitution					
		HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
H4H2499N	H4H2499N8				S28H S30H S31H		
H4H2499N	H4H2499N9				Q27H S28H S30H Y32H		
H4H2499N	H4H2499N10						Q90H S92H N93H T97H
H4H2499N	H4H2499N11						Q90H S92H T97H
H4H2499N	H4H2499N12						S92H N93H T97H
H4H2499N	H4H2499N13						Q90H N93H
H4H2499N	H4H2499N14						S92H N93H
H4H2499N	H4H2499N15			L98H Y100H V106H			
H4H2499N	H4H2499N16			L98H Y100H M104H			
H4H2499N	H4H2499N17			Y100H M104H V106H			
H4H2499N	H4H2499N18			L98H Y100H M104H V106H			
H4H2499N	H4H2499N19			L98H M104H V106H			
H4H2499N	H4H2499N20			Y100H M104H V106H	S28H S30H S31H		
H4H2499N	H4H2499N21			Y100H M104H V106H			Q90H S92H T97H
H4H2499N	H4H2499N22			Y100H M104H V106H			S92H N93H T97H

Example 11. pH-selective Binding of Histidine Substitution Variant Anti-HLA-B*27 Antibodies to HLA-B*27 Expressing Cells

[0124] Single-site and multiple-site substitutions of histidine for the parental amino acid(s) in

the CDR regions of two anti-HLA antibodies, H4H2477N2 and H4H2499N, were made to increase pH-selective binding (see Example 10). These histidine variants and their parental antibodies were tested for binding to cell-surface human HLA-B27 expressed on C1R-neo HLA-B*2705 cells at both pH 7.2 and pH 6.0, also using electrochemiluminescence detection technology. Cells were harvested, counted, and seeded into 96 well carbon electrode plates and also blocked as described previously (see Example 8). For the pH 6.0 binding evaluation, plate-bound cells were rinsed with pH 6.0 buffer [50mM MES, 0.0025M KCl, 0.137M NaCl, 0.0005M $MgCl_2x6H_2O$, 0.0009M $CaCl_2$, 0.5% (w/v) BSA; adjusted to pH 6.0 by the addition of 5M NaOH] prior to antibody addition. Anti-HLA antibodies were diluted through 12-point, 3-fold serial dilutions starting at 50nM either in neutral pH buffer [PBS + 0.5% (w/v) BSA; at pH 7.2] for pH 7.2 binding evaluation or in pH 6.0 buffer for pH 6.0 binding evaluation then transferred to the plate-bound cells and incubated for 1 hour at 25°C. Unbound antibodies were washed off using an AquaMax2000 plate washer (MDS Analytical Technologies). Cell-bound anti-HLA antibodies were detected using an anti-human C_H1-specific antibody (generated in-house) conjugated with SULFO-TAG™. Addition of MSD Read Buffer, electrochemical stimulation, and luminescence detection was also performed as described above. The luminescence intensity (expressed as RLU) correlates with the amount of antibodies bound to cells. EC_{50} values for histidine variants of the anti-HLA antibodies H4H2499N and H4H2477N2 binding to C1R-neo B*2705 cells were calculated using Prism Software and are shown in Tables 21 and 22, respectively.

Table 21: Comparison of EC_{50} values and maximum RLU binding signal for H4H2499N and its histidine variants to C1R-neo B*2705 cells at pH 7.2 and pH 6.0

AbPID	EC_{50} (M) of antibodies binding to C1R-neo B*2705 at pH 6.0	EC_{50} (M) of antibodies binding to C1R-neo B*2705 at pH 7.2	Fold increase (weaker binding) in EC_{50} values at pH 6.0 versus pH 7.2 to C1R-neo B*2705 cells	Max RLU binding to C1R-neo B*2705 cells at pH 6.0	Max RLU binding to C1R-neo B*2705 cells at pH 7.2
H4H2499N	1.06E-09	6.65E-10	1.6	18426	16915
H4H2499N*	1.20E-09	4.60E-10	2.6	26317	23734
H4H2499N2*	8.30E-09	2.90E-09	2.9	24141	23728
H4H2499N3*	7.90E-09	2.30E-09	3.5	18921	18387
H4H2499N4*	3.80E-08	6.60E-09	5.9	10315	14195
H4H2499N5	5.10E-09	2.80E-09	1.8	19522	16912
H4H2499N6	4.60E-09	2.50E-09	1.8	20042	17356
H4H2499N7	4.30E-09	2.80E-09	1.5	18058	15677
H4H2499N8	5.20E-09	1.80E-09	2.9	19915	17759
H4H2499N8*	4.80E-09	2.00E-09	2.3	24905	26736
H4H2499N9	5.50E-09	3.70E-09	1.5	15870	15695

H4H2499N10	8.00E-09	3.80E-09	2.1	9502	8599
H4H2499N11	1.20E-08	4.60E-09	2.6	9549	9605
H4H2499N11*	1.70E-08	4.80E-09	3.6	11970	14364
H4H2499N12	2.20E-09	1.30E-09	1.7	24334	23014
H4H2499N12*	3.20E-09	1.20E-09	2.7	31917	32762
H4H2499N13	2.22E-08	2.11E-08	1.1	8124	9563
H4H2499N14	1.50E-09	7.70E-10	1.9	19137	17315
H4H2499N15	1.92E-08	9.20E-09	2.1	9681	8001
H4H2499N16	IC	IC	IC	10010	11377
H4H2499N17	1.00E-08	3.20E-09	3.2	16905	18931
H4H2499N17*	1.60E-08	3.10E-09	5.3	21621	23850
H4H2499N18	8.10E-09	3.90E-09	2.1	14600	13055
H4H2499N19	IC	2.53E-08	IC	7870	9986
H4H2499N20*	8.70E-09	2.50E-09	3.5	22709	24372
H4H2499N21*	5.60E-08	8.00E-09	7.1	9680	13192
H4H2499N22*	6.10E-09	1.90E-09	3.3	32823	31612

*Performed on a separate day from a first experiment
IC=inconclusive

Table 22: Comparison of EC_{50} values and maximum RLU binding signal for H4H2477N2 and its histidine variants binding to C1R-neo B*2705 cells at pH 7.2 and pH 6.0

AbPID	EC_{50} (M) of antibodies binding to C1R-neo B*2705 at pH 6.0	EC_{50} (M) of antibodies binding to C1R-neo B*2705 at pH 7.2	Fold increase (weaker binding) in EC_{50} values at pH 6.0 versus pH 7.2 to C1R-neo B*2705 cells	Max RLU binding to C1R-neo B*2705 cells at pH 6.0	Max RLU binding to C1R-neo B*2705 cells at pH 7.2
H4H2477N2	7.90E-10	5.60E-10	1.4	46624	42761
H4H2477N3	3.60E-08	4.70E-09	7.7	22164	30529
H4H2477N4	6.40E-08	2.90E-09	21.8	25658	37452
H4H2477N5	IC	IC	IC	108897	137977

[0125] Binding experiments for parental antibody H4H2499N and its histidine variants were performed on two separate days. Some of the antibodies were included in both experiments, and the data from both experiments are shown in Table 21. Several of the histidine variants (H4H2499N3, H4H2499N4, H4H2499N11, H4H2499N17, H4H2499N20, H4H2499N21, and H4H2499N22) displayed greater than a 3-fold decrease in EC_{50} value at pH 6.0 compared with their EC_{50} value at pH 7.2, while the parental antibody, in two experiments, exhibited a 1.6-fold and a 2.6-fold decrease in EC_{50} value at pH 6.0 compared with its EC_{50} value at pH 7.2. EC_{50} values for two of the histidine variants, H4H2499N16 and H4H2499N19, could not be assigned at both pH 6.0 and pH 7.2 due to the lack of a complete sigmoidal curve at tested antibody concentrations.

[0126] As shown in Table 22, two of the histidine variants of parental antibody H4H2477N2 (H4H2477N3 and H4H2477N4) tested for binding to C1R-neo B*2705 cells displayed 7.7-fold and 21.8-fold decreases, respectively, in EC_{50} values at pH 6.0 compared with their EC_{50} values at pH 7.2, while the parental H4H2477N2 antibody exhibited no measurable decrease in EC_{50} value at pH 6.0 compared with its EC_{50} value at pH 7.2. For histidine variant H4H2477N5, EC_{50} values could not be assigned at both pH 7.2 and pH 6.0 due to the lack of a complete sigmoidal curve at tested antibody concentrations.

[0127] 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 antibody or antigen-binding fragment thereof that exhibits enhanced binding to HLA-B*27 as compared to other HLA-B allelic variants.

2. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof exhibits enhanced binding to HLA-B*2705 or HLA-B*2709 as compared to HLA-B*07.

3. The isolated antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, and 370; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, and 378.

4. The isolated antibody or antigen-binding fragment of claim 3, wherein the antibody or antigen-binding fragment comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378.

5. The isolated antibody or antigen-binding fragment of claim 4, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; 308-310-312-316-318-320; 324-326-328-332-334-336; 340-342-344-348-350-352; 356-358-360-364-366-368; and 372-374-376-380-382-384.

6. An isolated antibody, or antigen-binding fragment thereof, that specifically binds HLA-B*27, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274,

290, 306, 322, 338, 354, and 370; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, and 378.

7. The isolated antibody or antigen-binding fragment of claim 6, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378.

8. An isolated antibody or antigen-binding fragment thereof that binds to the same epitope on HLA-B*27 as a reference anti-HLA-B*27 antibody, wherein the reference antibody comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378.

9. An isolated antibody or antigen-binding fragment thereof that competes with a reference anti-HLA-B*27 antibody for binding to HLA-B*27, wherein the reference antibody comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, and 370/378.

10. An isolated antibody or antigen-binding fragment thereof that exhibits enhanced binding to HLA-B*27 as compared to other HLA-B allelic variants, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains comprising SEQ ID NOs:52-54-56-60-62-64, respectively, with a histidine substitution in the HCDR1 domain at amino acid position 33, a histidine substitution in the HCDR2 domain at amino acid position 52, or a histidine substitution in the HCDR1 domain at amino acid position 33 and a histidine substitution in the HCDR2 at amino acid position 52.

11. An isolated antibody or antigen-binding fragment thereof that exhibits enhanced binding to HLA-B*27 as compared to other HLA-B allelic variants, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains comprising SEQ ID NOs:148-150-152-156-158-160, respectively, with one or more substitutions selected from group consisting of:

(a) a histidine substitution in the HCDR1 domain at amino acid position 32;

(b) a histidine substitution in the HCDR2 domain at amino acid position 53;

(c) a histidine substitution in the HCDR1 domain at amino acid position 32 and a histidine

substitution in the HCDR2 domain at amino acid position 53;

(d) a histidine substitution in the LCDR1 domain at amino acid positions 27, 30 and 32;

(e) a histidine substitution in the LCDR1 domain at amino acid positions 27, 28 and 32;

(f) a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 32;

(g) a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 31;

(h) a histidine substitution in the LCDR1 domain at amino acid positions 27, 28, 30 and 32;

(i) a histidine substitution in the LCDR3 domain at amino acid positions 90, 92, 93 and 97;

(j) a histidine substitution in the LCDR3 domain at amino acid positions 90, 92 and 97;

(k) a histidine substitution in the LCDR3 domain at amino acid positions 92, 93 and 97;

(l) a histidine substitution in the LCDR3 domain at amino acid positions 90 and 93;

(m) a histidine substitution in the LCDR3 domain at amino acid positions 92 and 93;

(n) a histidine substitution in the HCDR3 domain at amino acid positions 98, 100 and 106;

(o) a histidine substitution in the HCDR3 domain at amino acid positions 98, 100 and 104;

(p) a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106;

(q) a histidine substitution in the HCDR3 domain at amino acid positions 98, 100, 104 and 106;

(r) a histidine substitution in the HCDR3 domain at amino acid positions 98, 104 and 106;

(s) a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR1 domain at amino acid positions 28, 30 and 31;

(t) a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR3 domain at amino acid positions 90, 92 and 97; and

(u) a histidine substitution in the HCDR3 domain at amino acid positions 100, 104 and 106 and a histidine substitution in the LCDR3 domain at amino acid positions 92, 93 and 97.

12. An isolated antibody or antigen-binding fragment thereof that binds HLA-B*27 at pH 5.75 with a K_D that is at least 5-times greater than the K_D for the antibody binding to HLA-B*27 at pH 7.2.

13. The isolated antibody or antigen-binding fragment of claim 12, wherein the antibody or antigen-binding fragment thereof binds HLA-B*27 at pH 5.75 with a K_D that is at least 10-times greater than the K_D for the antibody binding to HLA-B*27 at pH 7.2.

14. The isolated antibody or antigen-binding fragment of claim 13, wherein the antibody or antigen-binding fragment thereof binds HLA-B*27 at pH 5.75 with a K_D that is at least 25-times greater than the K_D for the antibody binding to HLA-B*27 at pH 7.2.

15. The isolated antibody or antigen-binding fragment of claim 14, wherein the antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 226/234, 258/266, 290/298, 338/346, and 370/378.

16. An isolated antibody or antigen-binding fragment thereof that binds HLA-B*2705 at pH 6.0 with an EC_{50} that is at least 1.5-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

17. The isolated antibody or antigen-binding fragment of claim 16, wherein the antibody or antigen-binding fragment thereof binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 10-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

18. The isolated antibody or antigen-binding fragment of claim 17, wherein the antibody or antigen-binding fragment thereof binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 25-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2..

19. The isolated antibody or antigen-binding fragment of claim 10, wherein the antibody or antigen-binding fragment thereof binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 1.5-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

20. The isolated antibody or antigen-binding fragment of claim 11, wherein the antibody or antigen-binding fragment thereof binds HLA-B*2705 at pH 6.0 with a EC_{50} that is at least 1.5-times greater than the EC_{50} for the antibody binding to HLA-B*2705 at pH 7.2.

21. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1, 10, 11, 12 or 16 and a pharmaceutically acceptable carrier or diluent.

22. A method for treating, preventing or ameliorating a disease or disorder associated with or mediated by HLA-B*27 activity, the method comprising administering the pharmaceutical composition of claim 21 to a patient afflicted with or at risk for a disease or disorder associated with or mediated by HLA-B*27 activity.

23. The method of claim 22, wherein the disease or disorder associated with or mediated by HLA-B*27 activity is selected from the group consisting of a spondyloarthropathy, rejection of solid organ transplants, and graft-versus-host disease (GVHD).

24. The method of claim 23, wherein the spondyloarthropathy is selected from the group consisting of ankylosing spondylitis, reactive arthritis (Reiter's Syndrome), acute anterior uveitis, initis, psoriatic arthritis, and ulcerative colitis.