HUMAN ANTIBODIES TO HUMAN TNF-LIKE LIGAND 1A (TL1A)

Field of the invention

[0001] The present invention relates to human antibodies and antigen-binding fragments of human antibodies that specifically bind human TNF-like ligand 1A (hTL1A), and therapeutic methods of using those antibodies.

Statement of Related Art

[0002] TL1A is a type II cell membrane protein of the tumor necrosis factor superfamily (TNFSF) and also designated as TNFSF15. It is expressed on the surface of endothelial cells, and activated cells of the hematopoietic lineage, including monocytes, macrophages, lymphocytes, lamina propria mononuclear cells, dendritic cells and plasma cells (Tan, K.B. *et al.*, 1997, *Gene* 204:35-46; Prehn, J.L. *et al.*, 2007, *J Immunol* 178:4033-4038). It is also expressed in kidney, lung, prostate and thymus (Tan *et al.*, 1997, *supra*). In endothelial cells, expression of TL1A is upregulated by IL-1α and TNFα (Migone, T.S. *et al.*, 2002, *Immunity* 16:479-492). In human fresh blood monocytes and monocyte-derived dendritic cells, TL1A expression is upregulated by FcγR-mediated or Toll-like receptor (TLR) signaling (Prehn *et al.*, 2007, *supra*; Meylan, F. *et al.*, 2008, *Immunity* 29:79-89). TL1A can be cleaved from the cell membrane via a mechanism analogous to TNFα and a soluble ectodomain form of TL1A has been reported (Migone *et al.*, 2002, *supra*; Kim, S. *et al.*, 2005, *J Immunol Methods* 298:1-8; Yang, C.R. *et al.*, 2004, *Cancer Res* 64:1122-1129). Protein sequencing has confirmed that this form of TL1A is liberated following cleavage of the membrane-anchored precursor between residues Ala-71 and Leu-72 (Migone *et al.*, 2002, *supra*). Two variant cDNAs that potentially encode N-terminally truncated versions of TL1A have been identified: VEGI-174 (or TL1) (Zhai, Y. *et al.*, 1999, *FASEB J 13*:181-189) and VEGI-192 (Chew, L.J. *et al.*, 2002, *FASEB J* 16:742-744). The published data suggest the biologically active products of the TL1A gene are the full-length type II transmembrane protein (residues 1-251) and its proteolytically cleaved ectodomain (residues 72-251) (Migone *et al.*, 2002, *supra*; Jin *et al.*, 2007, *Biochem Biophys Res Commun* 364:1-6). A variant of hTL1A, designated as "Fhm", containing a single amino acid substitution of Gln-167 with Arg, is disclosed in US 6,521,422.

[0003] TL1A mediates signals via its cognate receptor Death Receptor 3 (DR3; also known as TNFRSF25; the nucleic acid and amino acid sequences of SEQ ID NO:251 and 252, respectively), resulting in promoting cell survival and secretion of pro-inflammatory cytokines, or promoting apoptosis, in a context-dependent manner. TL1A is one of three known ligands (in addition to FasL and LIGHT) that are bound by the endogenous soluble decoy receptor, DcR3 (also known as TR6, NTR3 or TNFRSF21; the nucleic acid and amino acid sequences of SEQ ID NO:253 and 254, respectively) (Migone *et al.*, 2002, *supra*; Yang C.R. *et al.*, 2004, *Cancer Res* 64:1122-1129).

[0004] DR3 is a TNF receptor-related death-domain receptor expressed on the majority of activated T lymphocytes and NK cells (Migone *et al.*, 2002, *supra*; Screaton G.R. *et al.*, 1997, *Proc Natl Acad Sci (USA)* 94:4615-4619). TL1A engages DR3 on T cells, enhancing their responsiveness to IL-2 (Migone *et al.*, 2002, *supra*), potentiating T cell proliferation and release of IFNγ and GM-CSF under conditions of suboptimal costimulation (Migone *et al.*, 2002, *supra*; Meylan *et al.*, 2008, *supra*). TL1A has also been shown to synergize with suboptimal levels of IL-12/IL-18 to induce IFNγ production by $CD4^+$ T cells (Papadakis, K.A. *et al.*, 2004, *J Immunol* 172:7002-7007; Prehn, J.L. *et al.*, 2004, *Clin Immunol* 112:66-77; Papadakis, K.A. *et al.*, 2005, *J Immunol* 174:4985-4990; Cassatella, M.A. *et al.*, 2007, *J Immunol* 178:7325-7333).

[0005] TL1A has been implicated in various inflammatory diseases and/or auto immune diseases, including inflammatory bowel diseases [*e.g.*, ulcerative colitis (UC) and Crohn's disease (CD)], rheumatoid arthritis, multiple sclerosis (MS), atherosclerosis, and the like (*see* Bayry, J., 2010, *Nature Reviews/Rheumatology* 6:67-68; Takedatsu, H. *et al.*, 2008, *Gastroenterology* 135:552-567; Prehn *et al.*, 2004, *supra*; Bamias, G. *et al.*, 2008, *Clin Immunol* 129:249-255; Bull, M.J. *et al.*, 2008, *J Exp Med* 205:2457-2464; Pappu, B.P. *et al.*, 2008, *J Exp Med* 205:1049-1062; Bamias, G. *et al.*, 2003, *J Immunol* 171:4868-4874; Kang, Y. *et al.*, 2005, *Cytokine* 29:229-235). Although the majority of the published data are consistent with a pivotal role for TL1A in driving differentiation of T_H1 and T_H17 effector function, a recent study has proposed a role for the TL1A/DR3 interaction in development of T_H2 T cell responses in asthma models (Fang, L. *et al.*, 2008, *J Exp Med* 205:1037-1048). Thus, the use of TL1A inhibitors, such as fully human antibodies against TL1A with high affinities and neutralizing activity, alone or in combination with currently available anti-inflammatory agents, immunosuppresants (*e.g.*, TNF-α antagonists, cortisone or steroids, and the like), and/or anti-allergy agents, provides effective treatment for these diseases and disorders.

[0006] The nucleic acid and the amino acid sequences of human TL1A are shown in SEQ ID NOS: 243 and 244, respectively, and those of Fhm are shown in SEQ ID NOS:245 and 246, respectively. Antibodies to TL1A are disclosed in, for example, US 7,597,886, US 7,820,798 and US 2009/0280116.

BRIEF SUMMARY

[0007] Provided herein are fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that specifically bind and neutralize human TL1A (hTL1A) activity.

[0007a] In one aspect, there is provided an isolated human antibody or antigen-binding fragment thereof that specifically binds human TNF-like ligand 1A (hTL1A), comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 sequence combination of SEQ ID NO: 20/22/24; and/or a light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 sequence combination of SEQ ID NO: 28/30/32.

[0007b] In one aspect, there is provided an antibody or antigen-binding fragment thereof which binds the same epitope on hTL1A as an antibody or antigen-binding fragment comprising heavy chain and light chain complementarity determining regions (CDRs) of SEQ ID NO:20/22/24/28/30/32.

[0007c] In one aspect, there is provided an isolated human antibody or antigen-binding fragment thereof that specifically binds human TNF-like ligand 1A (hTL1A), comprising a heavy chain variable region (HCVR) comprising an amino acid sequence of SEQ ID NO: 18 and/or a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.

[0008] The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, *e.g.*, to eliminate residual effector functions (Reddy *et al.*, 2000, *J. Immunol.* 164:1925-1933).

[0009] In one embodiment, the disclosure features an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:2, 18, 34, 50, 66, 82, 98, 114, 118, 134, 138, 154, 158, 174, 178, 194, 198, 214, 218 and 234, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In another embodiment, the antibody or an antigen-binding fragment thereof comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:2, 18, 34, 50, 66, 134, 174 and 234. In yet another embodiment, the antibody or fragment thereof comprises a HCVR comprising SEQ ID NO:2, 18, 174 or 234.

[0010] In one embodiment, the antibody or fragment thereof further comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 116, 126, 136, 146, 156, 166, 176, 186, 196, 206, 216, 226 and 236, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In another embodiment, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:10, 26, 42, 58, 74, 136, 176 and 236. In yet another embodiment, the antibody or fragment thereof comprises a LCVR comprising SEQ ID NO:10, 26, 176 or 236.

[0011] In further embodiments, the antibody or fragment thereof comprises 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, 114/116, 118/126, 134/136, 138/146, 154/156, 158/166, 174/176, 178/186, 194/196, 198/206, 214/216, 218/226 and 234/236. In one embodiment, the antibody or fragment thereof comprises a HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 134/136, 174/176 and 234/236. In another embodiment, the antibody or fragment thereof comprises a HCVR/LCVR pair comprising SEQ ID NO:2/10, 18/26, 174/176 or 234/236.

[0012] In one embodiment, the disclosure features an antibody or antigen-binding fragment of an antibody comprising a heavy chain complementarity determining region 3 (HCDR3) amino acid sequence selected from the group consisting of SEQ ID NO:8, 24, 40, 56, 72, 88, 104, 124, 144, 164, 184, 204 and 224, 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) amino acid sequence selected from the group consisting of SEQ ID NO:16, 32, 48, 64, 80, 96, 112, 132, 152, 172, 192, 212 and 232, or substantially similar sequences thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In one embodiment, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:8/16, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 124/132, 144/152, 164/172, 184/192, 204/212 or 224/232. In another embodiment, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO: 8/16, 24/32,

40/48, 56/64, 72/80, 124/132, 164/172 or 224/232. In yet another embodiment, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:8/16, 24/32, 164/172 or 224/232.

[0013] In a further embodiment, the disclosure features an antibody or fragment thereof further comprising a heavy chain CDR1 (HCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:4, 20, 36, 52, 68, 84, 100, 120, 140, 160, 180, 200 and 220, 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) amino acid sequence selected from the group consisting of SEQ ID NO:6, 22, 38, 54, 70, 86, 102, 122, 142, 162, 182, 202 and 222, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or a light chain CDR1 (LCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:12, 28, 44, 60, 76, 92, 108, 128, 148, 168, 188, 208 and 228, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or a light chain CDR2 (LCDR2) amino acid sequence selected from the group consisting of SEQ ID NO:14, 30, 46, 62, 78, 94, 110, 130, 150, 170, 190, 210 and 230, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In one embodiment, the antibody or fragment thereof comprises a combination of HCDR1/HCDR2/HCDR3 selected from the group consisting of SEQ ID NO:4/6/8, 20/22/24, 36/38/40, 52/54/56, 68/70/72, 84/86/88, 100/102/104, 120/122/124, 140/142/144, 160/162/164, 180/182/184, 200/202/204 and 220/222/224; and/or a combination of LCDR1/LCDR2/LCDR3 selected from the group consisting of SEQ ID NO:12/14/16, 28/30/32, 44/46/48, 60/62/64, 76/78/80, 92/94/96, 108/110/112, 128/130/132, 148/150/152, 168/170/172, 188/190/192, 208/210/212 and 228/230/232. In another embodiment, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination selected from the group consisting of SEQ ID NO: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, 120/122/124/128/130/132, 140/142/144/148/150/152, 160/162/164/168/170/172, 180/182/184/188/190/192, 200/202/204/208/210/212 and 220/222/224/228/230/232. In another embodiment, the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR sequences of SEQ ID NO: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, 120/122/124/128/130/132, 160/162/164/168/170/172 or 220/222/224/228/230/232. In yet another embodiment, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination of SEQ ID NO:4/6/8/12/14/16, 20/22/24/28/30/32, 160/162/164/168/170/172 or 220/222/224/228/230/232.

[0014] In a related embodiment, the disclosure comprises an antibody or antigen-binding fragment of an antibody which specifically binds hTL1A, wherein the antibody or fragment

thereof comprises heavy and light chain CDR domains contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/116, 118/126, 134/136, 138/146, 154/156, 158/166, 174/176, 178/186, 194/196, 198/206, 214/216, 218/226 and 234/236. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are known in the art and can be applied to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Conventional definitions that can be applied to identify the boundaries of CDRs include 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. In one embodiment, the antibody or fragment thereof comprises CDR sequences contained within a HCVR and LCVR pair selected from the group consisting of the amino acid sequence pairs of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 134/136, 174/176 and 234/236. In another embodiment, the antibody or fragment thereof comprises CDR sequences contained within the HCVR and LCVR sequence pair of SEQ ID NO: 2/10, 18/26, 174/176 or 234/236.

[0015] In another related embodiment, the disclosure provides an antibody or antigen-binding fragment thereof that competes for specific binding to hTL1A with an antibody or antigen-binding fragment comprising heavy and light chain CDR sequences of SEQ ID NO: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, 120/122/124/128/130/132, 160/162/164/168/170/172 or 220/222/224/228/230/232. In one embodiment, the antibody or antigen-binding fragment thereof competes for specific binding to hTL1A with an antibody or antigen-binding fragment comprising heavy and light chain CDR sequences of SEQ ID NO:4/6/8/12/14/16, 20/22/24/28/30/32, 160/162/164/168/170/172 or 220/222/224/228/230/232. In another embodiment, the antibody or antigen-binding fragment of the disclosure competes for specific binding to hTL1A with an antibody or antigen-binding fragment comprising a HCVR/LCVR sequence pair of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 134/136, 174/176 or 234/236. In yet another embodiment, the antibody or antigen-binding fragment thereof competes for specific binding to hTL1A with an antibody or antigen-binding fragment comprising a HCVR/LCVR sequence pair of SEQ ID NO:2/10, 18/26, 174/176 or 234/236.

[0016] In another related embodiment, the disclosure provides an antibody or antigen-binding fragment thereof that binds the same epitope on hTL1A that is recognized by an antibody or fragment thereof comprising heavy and light chain CDR sequences of SEQ ID

NO: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, 120/122/124/128/130/132, 160/162/164/168/170/172 or 220/222/224/228/230/232. In one embodiment, the antibody or antigen-binding fragment thereof binds the same epitope on hTL1A that is recognized by an antibody or antigen-binding fragment thereof comprising heavy and light chain CDR sequences of SEQ ID NO:4/6/8/12/14/16, 20/22/24/28/30/32, 160/162/164/168/170/172 or 220/222/224/228/230/232. In another embodiment, the antibody or antigen-binding fragment of the disclosure recognizes the same epitope on hTL1A that is recognized by an antibody or antigen-binding fragment thereof comprising a HCVR/LCVR sequence pair of SEQ ID NO: SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 134/136, 174/176 or 234/236. In yet another embodiment, the antibody or antigen-binding fragment thereof recognizes the same epitope on hTL1A that is recognized by an antibody or antigen-binding fragment thereof comprising a HCVR/LCVR sequence pair of SEQ ID NO:2/10, 18/26, 174/176 or 234/236.

[0016b] In one aspect, there is provided an isolated nucleic acid molecule encoding the antibody or antigen-binding fragment as defined above.

[0017] In one embodiment, the disclosure provides nucleic acid molecules encoding anti-TL1A antibodies or fragments thereof described above. Recombinant expression vectors carrying the nucleic acids of the disclosure, and isolated host cells, *e.g.*, bacterial cells, such as *E. coli*, or mammalian cells, such as CHO cells, into which such vectors have been introduced, are also encompassed by the disclosure, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0017a] In one aspect, there is provided an expression vector comprising the nucleic acid molecule as defined above.

[0017b] In one aspect, there is provided an isolated host cell comprising the expression vector as defined above.

[0017c] In one aspect, there is provided a method of producing an anti-hTL1A antibody or antigen-binding fragment thereof, comprising growing the host cell as defined above under conditions permitting production of the antibody or fragment thereof, and recovering the antibody or fragment thereof so produced.

[0018] In one embodiment, the disclosure 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, 117, 133, 137, 153, 157, 173, 177, 193, 197, 213, 217 and 233, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, 17, 33, 49, 65, 133, 173 and 233. In yet another embodiment, the antibody or

fragment thereof comprises a HCVR encoded by the nucleic acid sequence of SEQ ID NO:1, 17, 173 or 233.

[0019] In one embodiment, the antibody or fragment thereof further comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, 25, 41, 57, 73, 89, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225 and 235, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, 25, 41, 57, 73, 135, 175 and 235. In yet another embodiment, the antibody or fragment thereof comprises a LCVR encoded by the nucleic acid sequence of SEQ ID NO:9, 25, 175 or 235.

[0020] In further embodiments, the antibody or fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) sequence pair encoded by a nucleic acid sequence pair selected from the group consisting of SEQ ID NO:1/9, 17/25, 33/41, 49/57, 65/73, 81/89, 97/105, 113/115, 117/125, 133/135, 137/145, 153/155, 157/165, 173/175, 177/185, 193/195, 197/205, 213/215, 217/225 and 233/235. In one embodiment, the antibody or fragment thereof comprises a HCVR/LCVR sequence pair encoded by a nucleic acid sequence pair selected from the group consisting of SEQ ID NO:1/9, 17/25, 33/41, 49/57, 65/73, 133/135, 173/175 and 233/235. In yet another embodiment, the antibody or fragment thereof comprises a HCVR/LCVR pair encoded by a nucleic acid sequence pair of SEQ ID NO:1/9, 17/25, 173/175 or 233/235.

[0021] In one embodiment, the disclosure features 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, 123, 143, 163, 183, 203 and 223, 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, 131, 151, 171, 191, 211 and 231, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO:7/15, 23/31, 39/47, 55/63, 71/79, 87/95, 103/111, 123/131, 143/151, 163/171, 183/191, 203/211 or 223/231. In another embodiment, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO:7/15, 23/31, 39/47, 55/63, 71/79, 123/131, 163/171 or 223/231. In yet another embodiment, the HCDR3/LCDR3 sequence pair is encoded by the nucleic acid sequence pair of SEQ ID NO:7/15, 23/31, 163/171 or 223/231.

[0022] In a further embodiment, the antibody or fragment thereof 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, 119, 139, 159, 179, 199 and 219, 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, 121, 141, 161, 181, 201 and 221, 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, 127, 147, 167, 187, 207 and 227, 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, 129, 149, 169, 189, 209 and 229, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology

thereof. In one embodiment, the antibody or fragment thereof comprises a combination of HCDR1/HCDR2/HCDR3 encoded by SEQ ID NO:3/5/7, 19/21/23, 35/37/39, 51/53/55, 67/69/71, 83/85/87, 99/101/103, 119/121/123, 139/141/143, 159/161/163, 179/181/183, 199/201/203 or 219/221/223; and a combination of LCDR1/LCDR2/LCDR3 encoded by SEQ ID NO:11/13/15, 27/29/31, 43/45/47, 59/61/63, 75/77/79, 91/93/95, 107/109/111, 127/129/131, 147/149/151, 167/169/171, 187/189/191, 207/209/211 or 227/229/231. In one embodiment, the antibody or fragment thereof comprises heavy and light chain CDR sequences encoded by a nucleic acid sequence combination selected from the group consisting of SEQ ID NO:3/5/7/11/13/15, 19/21/23/27/29/31, 35/37/39/43/45/47, 51/53/55/59/61/63, 67/69/71/75/77/79, 83/85/87/91/93/95, 99/101/103/107/109/111, 119/121/123/127/129/131, 139/141/143/147/149/151, 159/161/163/167/169/171, 179/181/183/187/189/191, 199/201/203/207/209/211 and 219/221/223/227/229/231. In another embodiment, the antibody or antigen-binding portion thereof comprises heavy and light chain CDR sequences encoded by a nucleic acid sequence combination of SEQ ID NO:3/5/7/11/13/15, 19/21/23/27/29/31, 35/37/39/43/45/47, 51/53/55/59/61/63, 67/69/71/75/77/79, 119/121/123/127/129/131, 159/161/163/167/169/171 or 219/221/223/227/229/231. In yet another embodiment, the antibody or antigen-binding portion thereof comprises heavy and light chain CDR sequences encoded by a nucleic acid sequence combination of SEQ ID NO: 3/5/7/11/13/15, 19/21/23/27/29/31, 159/161/163/167/169/171 or 219/221/223/227/229/231.

[0023] In one embodiment, the disclosure features an isolated antibody or antigen-binding fragment of an antibody that specifically binds hTL1A, comprising a HCDR3 and a LCDR3, wherein the HCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} (SEQ ID NO:239), wherein X^1 is Thr or Ala, X^2 is Lys, Arg or absent, X^3 is Glu, Gly or absent, X^4 is Asp, Pro or absent, X^5 is Leu or absent, X^6 is Arg, Tyr, Glu or absent, X^7 is Gly, Asp, Ala or absent, X^8 is Asp, Ser or Tyr, X^9 is Tyr or Trp, X^{10} is Tyr or Asp, X^{11} is Tyr, Lys or Ile, X^{12} is Gly, Tyr, Asn, or Ser, X^{13} is Val, Gly or Ser, X^{14} is Phe or Met, X^{15} is Asp, and X^{16} is Tyr or Val; and the LCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 (SEQ ID NO:242), wherein X^1 is Gln, X^2 is Gln, X^3 is Tyr, Leu or Phe, X^4 is His, Tyr or Asn, X^5 is Arg or Ser, X^6 is Ser, Thr or Tyr, X^7 is Trp or Pro, X^8 is Phe, Leu or absent, and X^9 is Thr.

[0024] In a further embodiment, the antibody or fragment thereof further comprises a HCDR1 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:237), wherein X^1 is Gly, X^2 is Phe, X^3 is Thr, X^4 is Phe, X^5 is Ser, X^6 is Thr, Ser or Asn, X^7 is Tyr, and X^8 is Gly, Trp, Val or Ala; a HCDR2 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:238), wherein X^1 is Ile or Val, X^2 is Ser or Lys, X^3 is Gly or Glu, X^4 is Thr, Asp, Ser or Arg, X^5 is Gly, X^6 is Arg, Ser or Gly, X^7 is Thr, Glu or Ser, and X^8 is Thr or Lys; a LCDR1 sequence comprising an amino acid

sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} (SEQ ID NO:240), wherein X^1 is Gln, X^2 is Thr, Ser, Ala or Gly, X^3 is Ile, X^4 is Ser or Leu, X^5 is Tyr or absent, X^6 is Ser or absent, X^7 is Ser or absent, X^8 is Asn or absent, X^9 is Asn or absent, X^{10} is Lys or absent, X^{11} is Ser, Asn or Thr, and X^{12} is Trp or Tyr; and a LCDR2 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:241) wherein X^1 is Ala, Trp or Ser, X^2 is Ala or Thr, and X^3 is Ser.

[0025] In one embodiment, the disclosure features a human anti-TL1A antibody or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR) encoded by nucleotide sequence segments derived from V_H, D_H and J_H germline sequences, and a light chain variable region (LCVR) encoded by nucleotide sequence segments derived from V_K and J_K germline sequences. In certain embodiments, the antibody or antigen-binding fragment thereof comprises the HCVR and the LCVR encoded by nucleotide sequence segments derived from a germline gene combination selected from the group consisting of: (i) V_H3-23, D_H2-21, J_H4, V_K1-5 and J_K1; (ii) V_H3-7, D_H1-7, J_H6, V_K4-1 and J_K3; (iii) V_H3-23, D_H2-2, J_H6, V_K1-9 and J_K2; (iv) V_H3-23, D_H6-6, J_H4, V_K1-9 and J_K4; (v) V_H1-2, D_H2-15, J_H3, V_K1-12 and J_K4; (vi) V_H4-34, D_H3-9, J_H4, V_K3-20 and J_K4; (vii) V_H4-34, D_H1-1, J_H4, V_K3-20 and J_K4; and (viii) V_H4-34, D_H3-3, J_H4, V_K2-24 and J_K4.

[0026] In one embodiment, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to hTL1A or Fhm with an equilibrium dissociation constant (K_D) of about 1 nM or less, as measured by surface plasmon resonance assay (for example, BIACORE™). In certain embodiments, the antibody of the disclosure exhibits a K_D of about 800 pM or less; about 700 pM or less; about 600 pM or less; about 500 pM or less; about 400 pM or less; about 300 pM or less; about 200 pM or less; about 150 pM or less; about 100 pM or less; about 90 pM or less; about 80 pM or less; about 50 pM or less; or 30 pM or less.

[0027] In one embodiment, the present disclosure provides an anti-hTL1A antibody or antigen-binding fragment thereof that binds hTL1A protein of SEQ ID NO:244, but does not cross-react with a variant thereof, such as Fhm of SEQ ID NO:246, as determined by, for example, ELISA, surface plasmon resonance assay, or Luminex® xMAP® Technology, as described herein. Fhm contains a single amino acid substitution at position 167, corresponding to Gln in hTL1A, with Arg (*see* U.S. Patent No. 6,521,422). In related embodiments, the disclosure also provides an anti-hTL1A antibody or antigen-binding fragment thereof that binds a hTL1A protein and cross-reacts with an Fhm. In another related embodiment, the disclosure provides an anti-hTL1A antibody or antigen binding fragment thereof that does not cross-react with mouse TL1A (mTL1A: SEQ ID NO:250, encoded by the nucleotide sequence of SEQ ID NO:249) but does cross-react with TL1A of cynomolgus monkey (*Macaca fascicularis*, or MfTL1A: SEQ ID NO:248, encoded by the nucleotide sequence of SEQ ID NO:247) or rhesus monkey (*Macaca mulatta*: *the same amino acid sequence as* MfTL1A). *In further related embodiments, the*

disclosure provides an anti-hTL1A antibody or antigen-binding fragment thereof that cross-reacts with both mTL1A and MfTL1A.

[0028] *The disclosure encompasses anti-hTL1A antibodies having a modified glycosylation* pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or *e.g.*, removal of a fucose moiety to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) JBC 277:26733). In other applications, removal of N-glycosylation site may reduce undesirable immune reactions against the therapeutic antibodies, or increase affinities of the antibodies. In yet other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0028a] In one aspect, there is provided a pharmaceutical composition comprising the antibody or antigen-binding fragment as defined above, wherein the antibody or antigen-binding fragment thereof further neutralizes hTL1A, and a pharmaceutically acceptable carrier.

[0029] In one embodiment, the disclosure features a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds hTL1A and a pharmaceutically acceptable carrier. In one embodiment, the disclosure features a composition which is a combination of an antibody or antigen-binding fragment thereof of the disclosure, and a second therapeutic agent. The second therapeutic agent may be one or more of any agent such as immunosuppressants, anti-inflammatory agents, analgesic agents, anti-allergy agents, and the like, many of which may have overlapping therapeutic effects of one another. Suitable immunosuppressants to be used in combination with the anti-hTL1A antibodies of the disclosure include, but are not limited to, glucocorticoids, cyclosporin, methotrexate, interferon β (IFN-β), tacrolimus, sirolimus, azathioprine, mercaptopurine, opioids, mycophenolate, TNF-binding proteins, such as infliximab, eternacept, adalimumab, and the like, cytotoxic antibiotics, such as dactinomycin, anthracyclines, mitomycin C, bleomycin, mithramycin, and the like, antibodies targeting immune cells, such as anti-CD20 antibodies, anti-CD3 antibodies, and the like. Suitable anti-inflammatory agents and/or analgesics for combination therapies with anti-hTL1A antibodies include, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, naproxen, Cox-2 inhibitors, and the like, TNF-α antagonists, IL-1 antagonists, IL-6 antagonists, acetaminophen, morphinomimetics, and the like. Suitable anti-allergy agents include antihistamines, glucocorticoids, epinephrine (adrenaline), theophylline, cromolyn sodium and anti-leukotrienes, as well as anti-cholinergics, decongestants, mast cell stabilizers, and the like.

[0030] In one embodiment, the disclosure features methods for inhibiting hTL1A activity using the anti-hTL1A antibody or antigen-binding portion of the antibody of the disclosure, 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 disclosure and, optionally, one or more additional therapeutic agents described above.

The disease or disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented, or its occurrence rate reduced compared to that without anti-hTL1A antibody treatment, by removal, inhibition or reduction of TL1A activity. Examples of diseases

or disorders treatable by the methods of the disclosure include, but are not limited to, inflammatory diseases and/or autoimmune diseases, such as inflammatory bowel diseases (IBD) including UC and CD, RA, MS, type 1 and type 2 diabetes, psoriasis, psoriatic arthritis, ankylosing spondylitis, atopic dermatitis, and the like; allergic reactions or conditions, including asthma, allergic lung inflammation, and the like; cancers atherosclerosis, infections, neurodegenerative diseases, graft rejection, graft vs. host diseases (GVHD), cardiovascular disorders/diseases, and the like.

[0031] Other embodiments will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0032] Before the present invention is described in detail, 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.

[0033] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[0034] The term "human TNF-like ligand 1A" or "hTL1A", as used herein, refers to hTL1A having the nucleic acid sequence shown in SEQ ID NO:243 and the amino acid sequence of SEQ ID NO:244, or a biologically active fragment thereof, as well as hTL1A variants, including Fhm having the nucleic acid sequence shown in SEQ ID NO:245 and the amino acid sequence of SEQ ID NO:246, or a biologically active fragment thereof, unless specifically indicated otherwise.

[0035] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (HCVR) and a heavy chain constant region (C_H; comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (LCVR) and a light chain constant region (C_L). The HCVR and LCVR can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more

conserved, termed framework regions (FR). Each HCVR and LCVR 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, and FR4.

[0036] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 *J Mol Biol* 320:415-428).

[0037] CDR residues not contacting antigen can be identified based on previous studies (for example, residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[0038] 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 mAbs 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 mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human FR sequences.

[0039] The fully-human anti-TL1A antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are 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 residues(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 back-mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the 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 residues 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.

[0040] The present invention also includes anti-TL1A 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-TL1A 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, 2 or 1, conservative amino acid substitution(s) relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0041] Unless specifically indicated otherwise, the term "antibody" (Ab), as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. 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-display 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.

[0042] 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.

[0043] 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.

[0044] 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)).

[0045] 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.

[0046] In certain embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope.

[0047] 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 physiological conditions. Specific binding can be characterized by an equilibrium dissociation constant (K_D) of about 3000 nM or less (*i.e.*, a smaller K_D denotes a tighter binding), about 2000 nM or less, about 1000 nM or less; about 500 nM or less; about 300 nM or less; about 200 nM or less; about 100 nM or less; about 50 nM or less; about 1 nM or less; or about 0.5 nM or less. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds hTL1A may, however, exhibit cross-reactivity to other antigens, such as TL1A molecules from other species, for example, cynomolgus monkey TL1A (SEQ ID NO:248), and/or mouse TL1A (SEQ ID NO:250), and/or a TL1A variant, such as Fhm (SEQ ID NO:246). Moreover, multi-specific antibodies (*e.g.*, bispecifics) that bind to hTL1A and one or more additional antigens are nonetheless considered antibodies that "specifically bind' hTL1A, as used herein.

[0048] The term "high affinity" antibody refers to those antibodies having a binding affinity to hTL1A, expressed as K_D, of about 1×10^{-9} M or less, about 0.5×10^{-9} M or less, about 0.25×10^{-9} M or less, about 1×10^{-10} M or less, or about 0.5×10^{-10} M or less, as measured by surface plasmon resonance, *e.g.*, BIACORE™ or solution-affinity ELISA.

[0049] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0050] By the term "slow off rate", "Koff" or "k_d" is meant an antibody that dissociates from hTL1A with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, e.g., BIACORE™.

[0051] By the term "intrinsic affinity constant" or "k_a" is meant an antibody that associates with hTL1A at a rate constant of about 1×10^{3} $M^{-1}s^{-1}$ or higher, as determined by surface plasmon resonance, e.g., BIACORE™.

[0052] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other mAbs having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hTL1A is substantially free of Abs that specifically bind antigens other than hTL1A). An isolated antibody that specifically binds hTL1A may, however, have cross-reactivity to other antigens, such as TL1A molecules from other species, such as cynomolgus monkey and mouse, and/or hTL1A variants, such as Fhm.

[0053] A "neutralizing antibody", as used herein (or an "antibody that neutralizes TL1A activity"), is intended to refer to an antibody whose binding to TL1A results in inhibition of at least one biological activity of TL1A. This inhibition of the biological activity of TL1A can be assessed by measuring one or more indicators of TL1A biological activity by one or more of several standard *in vitro* or *in vivo* assays known in the art (also see examples below).

[0054] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[0055] The term "epitope" is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0056] 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 90%, and more preferably at least about 95%, 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.

[0057] 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 90% sequence identity, even more preferably at least 95%, 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 or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) *Methods Mol. Biol.* 24: 307-331, which is herein incorporated by reference. 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: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) *Science* 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0058] Sequence similarity for polypeptides 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 with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 2000, *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.*, 1990, *J. Mol. Biol.* 215: 403 410 and, 1997, *Nucleic Acids Res.* 25:3389 402, each of which is herein incorporated by reference.

[0059] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, the age and the size of a subject treated, the route of administration, and the like, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

Preparation of Human Antibodies

[0060] Methods for generating human antibodies in transgenic mice 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 TL1A.

[0061] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to TL1A 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, and the like.

[0062] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} M through about 10^{-9} M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions, for example, wild-type IgG1 (SEQ ID NO:255) or IgG4 (SEQ ID NO:256), or modified IgG1 or IgG4 (for example, IgG4 with Ser-108 substituted with Pro as shown in SEQ ID NO:257), to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics of the antibodies reside in the variable region.

Epitope Mapping and Related Technologies

[0063] To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke, 2004, *Methods Mol Biol 248*:443-63) (herein specifically incorporated by reference in its entirety), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, *Protein Science* 9: 487-496) (herein specifically incorporated by reference in its entirety).

[0064] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0065] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of mAbs directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly

different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical mAbs, such that characterization can be focused on genetically distinct mAbs. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the anti-TL1A mAbs of the invention into groups of mAbs binding different epitopes.

[0066] The present invention includes hTL1A antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein. Likewise, the present invention also includes anti-hTL1A antibodies that compete for binding to hTL1A or a hTL1A fragment with any of the specific exemplary antibodies described herein.

[0067] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-hTL1A 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-hTL1A antibody of the invention, the reference antibody is allowed to bind to a hTL1A protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the hTL1A molecule is assessed. If the test antibody is able to bind to hTL1A following saturation binding with the reference anti-hTL1A antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-hTL1A antibody. On the other hand, if the test antibody is not able to bind to the hTL1A molecule following saturation binding with the reference anti-hTL1A antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-hTL1A antibody of the invention.

[0068] To determine if an antibody competes for binding with a reference anti-hTL1A antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a hTL1A molecule under saturating conditions followed by assessment of binding of the test antibody to the hTL1A molecule. In a second orientation, the test antibody is allowed to bind to a hTL1A molecule under saturating conditions followed by assessment of binding of the reference antibody to the TL1A molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the TL1A molecule, then it is concluded that the test antibody and the reference antibody compete for binding to hTL1A. 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 identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0069] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, 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 have 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 have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0070] 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, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0071] The invention encompasses a human anti-TL1A monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxin agents include any agent that is detrimental to cells. Examples of suitable cytotoxin agents and chemotherapeutic agents for forming immunoconjugates are known in the art, see for example, WO 05/103081, herein specifically incorporated by reference).

Bispecifics

[0072] The antibodies of the present invention may be monospecific, bispecific, or multispecific. Multispecific mAbs may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, *J. Immunol.* 147:60-69. The human anti-hTL1A mAbs can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment, to produce a bispecific or a multispecific antibody with a second binding specificity.

[0073] 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.

Bioequivalents

[0074] The anti-hTL1A antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described mAbs, but that retain the ability to bind human TL1A. Such variant mAbs 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 mAbs. Likewise, the hTL1A mAb-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-hTL1A antibody or antibody fragment that is essentially bioequivalent to an anti-hTL1A antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0075] 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. In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] Bioequivalent variants of anti-hTL1A 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.

Therapeutic Administration and Formulations

[0080] The invention provides therapeutic compositions comprising the anti-hTL1A antibodies or antigen-binding fragments thereof of the present invention and the therapeutic methods using the same. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA, 1998, *J Pharm Sci Technol* 52:238-311.

[0081] The dose may vary depending upon the age and the size of a subject to be administered, target disease, the purpose of the treatment, conditions, route of administration, and the like. When the antibody of the present invention is used for treating various conditions and diseases directly or indirectly associated with TL1A, including inflammatory diseases/disorders, autoimmune diseases/disorders, allergic reactions, and the like, in an adult patient, it is advantageous to intravenously or subcutaneously administer the antibody of the present invention 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. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0082] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.*, 1987, *J. Biol. Chem.* 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0083] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat *et al.*, 1989, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

[0084] 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.), CRC Pres., Boca Raton, Florida (1974). 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, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984).

[0085] 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. 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.

[0086] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, 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 certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly).

[0087] 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 0.1 to about 800 mg per dosage form in a unit dose; especially in the form of injection, the aforesaid antibody is contained in about 1 to about 500 mg, in about 5 to 300 mg, in about 8 to 200 mg, and in about 10 to about 100 mg for the other dosage forms.

Combination Therapies

[0088] The invention further provides therapeutic methods for treating diseases or disorders, which is directly or indirectly associated with hTL1A, by administering the hTL1A mAb or fragment thereof of the invention in combination with one or more additional therapeutic agents. The additional therapeutic agent may be one or more of any agent that is advantageously combined with the antibody or fragment thereof of the invention, including immunosuppressants, anti-inflammatory agents, analgesic agents, anti-allergy agents, and the like. Suitable immunosuppressants include, but are not limited to, glucocorticoids, cyclosporin, methotrexate, interferon β (IFN-β), tacrolimus, sirolimus, azathioprine, mercaptopurine, opioids, mycophenolate, TNF-binding proteins, such as infliximab, eternacept, adalimumab, and the like, cytotoxic antibiotics, such as dactinomycin, anthracyclines, mitomycin C, bleomycin, mithramycin, and the like, antibodies targeting immune cells, such as anti-CD20 antibodies, anti-CD3 antibodies, and the like. Suitable anti-inflammatory agents and/or analgesics for *combination therapies with the anti-hTL1A antibodies include, corticosteroids, non-steroidal* anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, naproxen, Cox-2 inhibitors, and the like, TNF-α antagonists (*e.g.*, Infliximab or REMICADE® by Centocor Inc.; golimumab by Centocor Inc.; etanercept or ENBREL® by Amgen/Wyeth; adalimumab or HUMIRA® by Abbott Laboratories, and the like), IL-1 antagonists (*e.g.*, IL-1-binding fusion proteins, for example, ARCALYST® by Regeneron Pharmaceuticals, Inc., *see* US Patent No. 6,927,044; KINERET® by Amgen, and the like), IL-6 antagonists (*e.g.*, anti-IL-6 receptor antibodies as disclosed in US Patent No. 7,582,298, and ACTEMRA® by Roche), acetaminophen, morphinomimetics, and the like. Suitable anti-allergy agents, which can block the action of allergic mediators, or to prevent activation of cells and degranulation processes, include antihistamines, glucocorticoids, epinephrine (adrenaline), theophylline, cromolyn sodium and anti-leukotrienes, such as montelukast (SINGULAIR® by Merck) or zafirlukast (ACCOLATE® by AstraZeneca), as well as anti-cholinergics, decongestants, mast cell stabilizers, and other compounds that can impair eosinophil chemotaxis.

[0089] The hTL1A mAb or fragment thereof of the invention and the additional therapeutic agent(s) can be co-administered together or separately. Where separate dosage formulations are used, the antibody or fragment thereof of the invention and the additional agents can be administered concurrently, or separately at staggered times, *i.e.*, sequentially, in appropriate orders.

EXAMPLES

[0090] 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 but some experimental errors and deviations should be accounted for. Unless indicated otherwise, 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 TL1A

[0091] VELOCIMMUNE™ mice were immunized with human TL1A, and the antibody immune response monitored by antigen-specific immunoassay using serum obtained from these mice. Anti-hTL1A antibody-expressing B cells were harvested from the spleens of immunized mice shown to have elevated anti-hTL1A antibody titers and were fused with mouse myeloma cells to form hybridomas. The hybridomas were screened and selected to identify cell lines expressing hTL1A-specific antibodies using assays as described below. The assays identified several cell lines that produced chimeric anti-hTL1A antibodies designated as H2M1681N, H2M1704N, H2M1804N, H2M1805N, H2M1817N and H2M1818N. These antibodies were later converted to hIgG4 isotype by replacing the respective mouse constant regions with the hIgG4 amino acid sequence of SEQ ID NO:257, which contains a S108P mutation in the hinge region, and designated as H4H1681N, H4H1704N, H4H1804N, H4H1805N, H4H1817N and H4H1818N, respectively.

[0092] Human TL1A-specific antibodies were also isolated directly from antigen-immunized B cells without fusion to myeloma cells, as described in U.S. Patent No. 7,582,298, which is hereby incorporated by reference in its entirety. Heavy and light chain variable regions were cloned to generate fully human anti-hTL1A antibodies designated as H4H1719P, H4H1725P, H4H1738P, H4H1742P, H4H1745P, H4H1750P and H4H1752P. Stable recombinant antibody-expressing CHO cell lines were established.

Example 2. Variable Gene Utilization Analysis

[0093] To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 1 shows the gene usage for selected antibodies in accordance with the invention.

Table 1

Antibody	HCVR			LCVR	
	V_H	D_H	J_H	V_K	J_K
H2M1704	3-7	1-7	6	4-1	3
H2M1681	3-23	2-21	4	1-5	1
H2M1817	4-34	3-9	4	3-20	4
H2M1804	4-34	1-1	4	3-20	4
H2M1818	3-11	4-17	6	4-1	1
H2M1805	4-34	3-3	4	2-24	4
H4H1719	3-9	3-3	6	2-28	2
H4H1725	1-2	2-15	3	1-12	4
H4H1738	3-15	4-4	6	2-28	2
H4H1742	3-23	2-2	6	1-9	2
H4H1745	3-23	6-6	4	1-9	4
H4H1750	3-30	4-17	6	1-17	1
H4H1752	3-23	1-7	4	1-5	1

[0094] Table 2 shows the heavy and light chain variable region amino acid sequence pairs of selected anti-hTL1A antibodies and their corresponding antibody identifiers. The N and P designations refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (i.e., in the framework regions). Thus, N and P variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but contain modifications within the framework regions.

Table 2

mAb Name (H2M- or H4H-)	HCVR/LCVR SEQ ID NOS	mAb Name (H2M- or H4H-)	HCVR/LCVR SEQ ID NOS
1704N	2/10	1738N	138/146
1681N	18/26	1738P	154/156
1804N	34/42	1745N	158/166
1805N	50/58	1745P	174/176
1817N	66/74	1750N	178/186
1818N	82/90	1750P	194/196
1719N	98/106	1752N	198/206
1719P	114/116	1752P	214/216
1725N	118/126	1742N	218/226
1725P	134/136	1742P	234/236

Example 3. TL1A Binding Affinity Determination.

[0095] Binding affinities and kinetic constants were determined by surface plasmon resonance at 25°C and 37°C as indicated in Tables 3-5 for human monoclonal anti-TL1A antibodies binding to the following TL1A species variants: human (h) (CHO-expressed, residues 72-251 of SEQ ID NO:244, with N-terminal His_6-tag), cynomolgus monkey (Mf) (*E. coli*-expressed, residues 72-251 of SEQ ID NO:248, with or without N-terminal Met), cynomolgus monkey (CHO-expressed, residues 72-251 of SEQ ID NO:248, with N-terminal His_6-tag), mouse (m) (*E. coli*-expressed, residues 76-252 of SEQ ID NO:250, with or without N-terminal Met), mouse (CHO-expressed, residues 76-252 of SEQ ID NO:250, with N-terminal His_6-tag), and rat (CHO cell-expressed; residues 76-252 of SEQ ID NO:258, with N-terminal His_6-tag). Binding constants were also determined for the hTL1A variant, Fhm (*E. coli*-expressed, residues 72-251 of SEQ ID NO:246 containing Q167R substitution, with or without N-terminal Met). Measurements were conducted on a T100 BIACORE™ instrument. Antibodies, expressed with either mouse Fc (designated with prefix "H2M") or human IgG4(S108P) Fc (designated with prefix "H4H"), were captured onto an anti-Fc sensor surface, and at least three different concentrations of the soluble TL1A proteins ranging from 1.25 nM to 100 nM were injected over the sensor surface. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the data to a 1:1 binding model using BIAevaluation 4.1 curve fitting software (BIAcore Life Sciences). Molar concentrations of TL1A/Fhm used in the data fitting assumed a monomeric state for TL1A in solution. 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 $t_{1/2}$ (min) = [ln2/(60*k_d)]. NB: No binding under the conditions tested; NT: Not tested in this experiment; *: Fitted k_d values below $1x10^{-6}$ (1/s) are slower than the detection limit under these experimental conditions; therefore, k_d values were set at $1x10^{-6}$ (1/s) for the purpose of approximating K_D and $t_{1/2}$; **: Equilibrium dissociation constants for antibodies were determined under steady state conditions.

[0096] As shown in Tables 3 and 4, antibodies bound with high affinity to CHO-expressed forms of both human and monkey TL1A proteins at 25°C (13 and 12 antibodies with K_D < 1 nM, respectively) and at 37°C (13 and 12 antibodies with K_D < 1 nM, respectively). H4H1750P bound significantly weaker to the monkey compared to the human TL1A protein. H4H1704N bound to CHO-expressed mTL1A with K_D < 2nM at both 25°C and 37°C. H4H1818N bound CHO-expressed mTL1A at 25°C (K_D ~7nM) but not at 37°C. Five antibodies, H4H1681N, H4H1738P, H4H1750P, H4H1752P and H4H1805N, did not bind to CHO-expressed rat TL1A at either 25°C or 37°C; the other eight antibodies bound to rat TL1A at both temperatures with K_D ranging from ~0.6 pM to ~16 nM.

[0097] As shown in Table 5, three antibodies (H2M1681N, H4H1752P and H2M1805N) did not demonstrate binding to the *E. coli*-expressed Fhm variant [hTL1A(Q167R)] under the conditions

tested. Three antibodies (H2M1704N, H4H1725P, and H2M1818N) demonstrated weak binding (K_D ranging from ~60 nM to ~170 nM) to mouse TL1A expressed in *E. coli* as assessed under steady-state conditions, while all other tested antibodies did not bind to the mouse TL1A protein under the tested conditions.

Table 3

mAb	CHO-expressed TL1A at 25°C							
	hTL1A K_D (pM)	hTL1A $t_{1/2}$ (min)	MfTL1A K_D (pM)	MfTL1A $t_{1/2}$ (min)	mTL1A K_D (pM)	mTL1A $t_{1/2}$ (min)	rTL1A K_D (pM)	rTL1A $t_{1/2}$ (min)
H4H1681N	263	36	404	25	NB	NB	NB	NB
H4H1704N	39.2	453	59.9	276	194	46	404	25
H4H1719P	481	44	417	45	NB	NB	364	38
H4H1725P	63.6	185	346	64	NB	NB	317	26
H4H1738P	608	64	361	93	NB	NB	NB	NB
H4H1742P	60.4	755	115	577	NB	NB	78	144
H4H1745P	164	172	115	231	NB	NB	2.7 (nM)	4
H4H1750P	15.8*	11550*	8.6 (nM)	21	NB	NB	NB	NB
H4H1752P	156	197	213	139	NB	NB	NB	NB
H4H1804N	291	51	264	49	NB	NB	321	43
H4H1805N	365	73	342	74	NB	NB	NB	NB
H4H1817N	321	103	356	92	NB	NB	2.5 (nM)	25
H4H1818N	124	120	119	122	7.1 (nM)	12	88	92

Table 4

mAb	CHO-expressed TL1A at 37°C							
	hTL1A K_D (pM)	hTL1A $t_{1/2}$ (min)	MfTL1A K_D (pM)	MfTL1A $t_{1/2}$ (min)	mTL1A K_D (pM)	mTL1A $t_{1/2}$ (min)	rTL1A K_D (pM)	rTL1A $t_{1/2}$ (min)
H4H1681N	254	31	226	32	NB	NB	NB	NB
H4H1704N	1.00*	11550*	0.93*	11550*	1.3 (nM)	27	46	133
H4H1719P	7.61	1912	2.01	5784	NB	NB	78	86
H4H1725P	23.9	758	17.7	888	NB	NB	411	15
H4H1738P	571	51	465	60	NB	NB	NB	NB
H4H1742P	4.37*	11550*	4.45*	11550*	NB	NB	653	27
H4H1745P	177	129	173	124	NB	NB	16.5 (nM)	11
H4H1750P	13.8*	11550*	17.1 (nM)	11	NB	NB	NB	NB
H4H1752P	225	96	223	91	NB	NB	NB	NB
H4H1804N	45.8	286	78	167	NB	NB	127	88
H4H1805N	299	80	352	68	NB	NB	NB	NB
H4H1817N	27.8	1098	25.6	1150	NB	NB	1.6 (nM)	26
H4H1818N	0.925*	11550*	0.804*	11550*	NB	NB	0.61*	11550*

Table 5

mAb	*E. coli*-expressed Fhm/TL1A								
	25°C		37°C		25°C		37°C		25°C
	Fhm K_D (pM)	Fhm $t_{1/2}$ (min)	Fhm K_D (pM)	Fhm $t_{1/2}$ (min)	MfTL1A K_D (pM)	MfTL1A $t_{1/2}$ (min)	MfTL1A K_D (pM)	MfTL1A $t_{1/2}$ (min)	mTL1A K_D (pM)**
H2M1681N	NB	NB	NT	NT	546	54	1.5 (nM)	14	NB
H2M1704N	282	52	NT	NT	285	57	751	16	127 (nM)
H4H1719P	109	96	174	42	242	79	289	40	NB
H4H1725P	28.9	447	43.6	194	63.7	292	62.2	174	62 (nM)
H4H1738P	1020	19	3100	4	1360	18	4.2 (nM)	4	NB
H4H1742P	437	129	696	45	593	97	945	35	NB
H4H1745P	115	226	207	71	141	221	281	74	NB
H4H1750P	204	623	244	335	52.7 (nM)	4	456 (nM)	1	NB
H4H1752P	NB	NB	NB	NB	274	122	1320	29	NB
H2M1804N	192	108	NT	NT	176	172	218	118	NB
H2M1805N	NB	NB	NT	NT	345	67	439	36	NB
H2M1817N	451	57	NT	NT	1250	37	1.0 (nM)	30	NB
H2M1818N	1080	17	NT	NT	1840	10	4.1 (nM)	3	171 (nM)

Experiment 4: Inhibition of TL1A by Anti-hTL1A Antibodies

[0098] HEK293 cell lines (CRK01573, ATCC) were generated to stably express human DR3 (full-length; SEQ ID NO:252) or mouse DR3 (full-length; SEQ ID NO:259) along with a luciferase reporter [NFκB response element (5x)-luciferase-IRES-GFP]. NFκB activation by TL1A has been shown previously (Migone *et al.*, 2002, *Immunity* 16:479-492). In order to test the membrane-bound form of TL1A and TL1A variants, HEK293 cell lines were generated that stably express full length human TL1A (SEQ ID NO:244), full-length human TL1A with Gln-167 substituted by Arg [Fhm; TL1A(Q167R); SEQ ID NO:246], full-length TL1A from cynomolgus monkey, *Macaca fascicularis* (MfTL1A; SEQ ID NO:248), full length mouse TL1A (SEQ ID NO:250), and full length rat TL1A (SEQ ID NO:258). The stable cell lines were isolated and maintained in 10% fetal bovine serum (FBS; Irvine Scientific), Dulbecco's Modified Eagle Medium(DMEM; Irvine Scientific), non-essential amino acids (NEAA; Irvine Scientific), Penicillin/Streptomycin (Invitrogen), and G418 (Invitrogen).

[0099] For the bioassay, human or mouse DR3 reporter cells were seeded into 96-well assay plates at 1 x 10^4 cells/well in low serum media, *i.e.*, 0.1% FBS and OPTIMEM® (Invitrogen), and incubated at 37°C and 5% CO_2 overnight. The next day, soluble TL1A or Fhm (sTL1A or sFhm) was serially diluted at 1:3 and added to cells at concentrations ranging from 0.002 nM to 100 nM (plus a buffer control containing no TL1A). For inhibition, antibodies were serially diluted at 1:3 and added to cells at concentrations ranging from 0.002 nM to 100 nM (plus a buffer control containing no antibody) in the presence of constant concentrations of TL1A or Fhm: 800 pM hTL1A (CHO cell-expressed; residues 72-251 of SEQ ID NO:244, with N-terminal His_6-tag), 100 pM hTL1A (*E. coli*-expressed; residues 72-251 of SEQ ID NO:244, with or without N-terminal Met), 500 pM Fhm (CHO cell-expressed; residues 72-251 of SEQ ID NO:246), 400 pM MfTL1A (CHO cell-expressed; residues 72-251 of SEQ ID NO:248, with N-terminal His_6-tag), 400 pM MfTL1A (*E. coli*-expressed; residues 72-251 of SEQ ID NO:248, with or without N-terminal Met), 50 pM mouse TL1A (CHO cell-expressed; residues 76-252 of SEQ ID NO:250, with N-terminal His_6-tag), 20 pM mouse TL1A (*E. coli*-expressed; residues 76-252 of SEQ ID NO:250, with or without N-terminal Met), and 50 pM rat TL1A (CHO cell-expressed; residues 76-252 of SEQ ID NO:258, with N-terminal His_6-tag). Luciferase activity was detected after 5.5 hours of incubation at 37°C and 5% CO_2. The results are shown in Table 6. Control mAb1: Positive control (an anti-hTL1A antibody with heavy and light chain variable domains having the amino acid sequences corresponding to SEQ ID NOS:21 and 27 of US 2009/0280116); control mAb2: Negative control (irrelevant antibody); NB: No binding under the conditions tested; NT: Not tested in this assay; *: Inhibition is not to baseline at the highest antibody concentration of 100 nM.

Table 6

sTL1A or sFhm		hTL1A (CHO)	hTL1A (*E. Coli*)	Fhm (CHO)	MfTL1A (CHO)	MfTL1A (*E. Coli*)	mTL1A (CHO)	mTL1A (*E. Coli*)	rTL1A (CHO)
EC50 (nM)		0.63	0.12	0.32	0.86	2.02	0.08	0.01	0.06
Constant TL1A or Fhm (pM)		800	100	500	400	400	50	20	50
IC50 [nM]	H4H1681N	0.17	0.02	NB	0.03	0.02	NB	NB	NB
	H4H1704N	0.13	0.06	0.17	0.01	0.03	NB	NB	0.40
	H4H1804N	0.07	0.03	0.10	0.03	0.02	NB	NB	3.64
	H4H1805N	0.10	0.04	185.50*	0.03	0.02	NB	NB	NB
	H4H1817N	0.11	0.04	0.12	0.04	0.02	NB	NB	23.87
	H4H1818N	0.37	0.29	0.62	0.13	0.06	NB	NB	1.10
	H4H1719P	0.06	0.02	0.08	0.01	0.02	NT	NB	NT
	H4H1725P	0.05	0.02	0.07	0.01	0.02	NB	NB	63.43
	H4H1738P	0.39	0.16	0.33	0.39	0.07	NT	NB	NT
	H4H1742P	0.31	0.19	0.53	0.26	0.07	NB	NB	6.12
	H4H1745P	0.09	0.06	0.15	0.05	0.03	NB	NB	NB
	H4H1750P	0.90	2.17	3.10	154.70	32.47*	NT	NB	NT
	H4H1752P	0.36	0.21	NB	0.12	0.05	NT	NB	NT
	Control mAb1	NB	0.74	NB	NB	3.25	NT	NB	NT
	Control mAb2	NB	NB	NB	NB	NB	NB	NT	NB

[0100] As shown in Table 6, thirteen anti-TL1A antibodies were shown to inhibit soluble human TL1A (CHO and *E. coli*-expressed) stimulation of the human DR3 receptor expressed on HEK293 cells as determined using a luciferase reporter for NFκB activation. A positive control antibody (control mAb1) inhibited *E. coli*-expressed, but not CHO-expressed, hTL1A. Ten antibodies also inhibited stimulation of hDR3-expressing cells by Fhm (hTL1A with Q167R). H4H1681N, H4H1805N and H4H1752P did not fully inhibit Fhm at the highest antibody concentration of 100 nM. All thirteen antibodies also blocked MfTL1A (Table 6). Mouse TL1A (produced from both CHO and E. coli) stimulated NFκB activation in the hDR3 reporter cells; however, none of the 13 anti-human TL1A antibodies inhibited *E. coli*-expressed mouse TL1A in this assay (Table 6). Nine selected antibodies were further tested and did not demonstrate blocking of CHO-expressed mouse TL1A in this assay (Table 6).

[0101] To test the ability of TL1A expressed on cells to stimulate signaling in the hDR3 reporter system, bioassays were performed as described above for soluble TL1A with the following changes: Adherent HEK293/TL1A cells were dissociated using Enzyme-Free Dissociation Solution (Chemicon) and added to adherent hDR3 reporter cells after serially diluting the TL1A cells at 1:2 starting from 2 x 10^5 cells to 195 cells (plus a no-cell control). For inhibition by antibodies, 1 x 10^4 cells were added together with serially diluted antibodies from 100 nM to 0.002 nM (plus a control containing no antibody). The results are shown in Table 7. Control mAb1 and mAb2: Same as the assays above. NB: No binding under the conditions tested; NT: Not tested in this assay; *: Inhibition is not to baseline at the highest antibody concentration of 100 nM.

Table 7

Cell-Bound TL1A or Fhm		HEK293/ hTL1A	HEK293/ Fhm	HEK293/ MfTL1A	HEK293/ mTL1A	HEK293/ rTL1A
EC50 (cells)		23474	47921	8465	12366	9773
Constant TL1A/Fhm		10,000 (# of cells)				
IC50 [nM]	H4H1681N	0.66	NB	3.07	NT	NT
	H4H1704N	1.11	1.58	3.76	NB	NT
	H4H1804N	0.56	1.23	2.23	NB	3.99
	H4H1805N	0.82	54.10*	1.93	NB	NB
	H4H1817N	1.11	0.62	5.04	NB	94.96*
	H4H1818N	1.54	1.42	4.29	NT	NT
	H4H1719P	0.82	0.84	3.00	NT	NT
	H4H1725P	0.66	0.94	2.82	NB	190.30*
	H4H1738P	7.47	7.75	20.25	NT	NT
	H4H1742P	6.55	8.39	18.45	NB	NT
	H4H1745P	0.76	2.12	4.28	NT	NT
	H4H1750P	12.82	29.52*	107.40*	NT	NT
	H4H1752P	1.99	138.10*	11.67	NT	NT
	Control mAb1	NB	NB	NB	NB	NT
	Control mAb2	NB	NB	NB	NB	NB

[0102] As shown in Table 7, all thirteen antibodies blocked the stimulation of hDR3-expressing cells by hTL1A expressed on cells. With cell-bound Fhm, all antibodies inhibited significantly except H4H1681N, H4H1805N, H4H1750P and H4H1752P, which did not inhibit fully at the highest tested antibody concentration of 100 nM. With cell-bound MfTL1A, all antibodies inhibited, except H4H1750 that did not inhibit completely at the highest tested antibody concentration of 100 nM. Six of the antibodies H4H1704N, H4H1804N, H4H1805N, H4H1817N, H4H1725P, and H4H1742P were tested for blocking cell-surface mTL1A stimulation of mDR3 cells; and none showed inhibition. Reporter cells expressing mouse DR3 could also be stimulated by rTL1A-expressing 293 cells, with an observed EC_{50} of 9773 cells (Table 7). Four antibodies were tested in the rTL1A/mDR3 assay: three antibodies H4H1804N, H4H1817N, H4H1725P blocked while H4H1805N did not block stimulation of mDR3 cells by cell-surface rTL1A (Table 7). Control mAb1 blocked E. coli-expressed soluble hTL1A and MfTL1A stimulation of hDR3 cells, but failed to block the CHO-expressed forms of hTL1A and MfTL1A under all tested conditions (Table 6). Control mAb1 also did not inhibit stimulation of hDR3-expressing cells by any of the cell-surface expressed TL1A and Fhm under all tested conditions (Table 7).

Experiment 5: Blocking of TL1A to hDR3 and DcR3 by Anti-TL1A Antibodies

[0103] The ability of antibodies to block human TL1A binding to its cognate receptors, the DR3 and DcR3 receptors, was measured using a competition sandwich ELISA. In addition, blocking of a human TL1A variant Fhm (human TL1A Q167R) and the cynomolgus monkey (*Macaca fascicularis*) TL1A (MfTL1A) protein binding to the human DR3 or DcR3 receptors was measured in the same manner. Constant amounts of biotinylated human TL1A or Fhm (both expressed with a 6-His tag in CHO cells) or biotinylated MfTL1A (expressed in CHO cells) were separately titrated with varying amounts of antibodies. The antibody-protein complexes were incubated in solution (1hr, 25°C) and then transferred to microtiter plates coated with human DR3 (hDR3) or human DcR3 (hDcR3) expressed as human IgG1 Fc fusion proteins. After one hour at 25°C the wells were washed, and bound human or monkey TL1A was detected with streptavidin conjugated with horseradish peroxidase (HRP). Wells were developed with a TMB solution to produce a colorimetric reaction and quenched with aqueous sulfuric acid before reading absorbance at 450nm on a Perkin-Elmer Victor X5 plate reader. A sigmoidal dose-response curve was fit to the data using the Prism™ data analysis package. The calculated IC50 value, defined as the concentration of antibody required to block 50% of TL1A binding to hDR3 or hDcR3, was used as an indicator of blocking potency. Both fully human anti-hTL1A mAbs and comparator antibodies, *i.e.*, control mAb1 (an anti-hTL1A antibody with heavy and light chain variable domains having the amino acid sequences corresponding to SEQ ID NOS:21 and 27, respectively, of US 2009/0280116) and control mAb3 (an anti-hTL1A antibody with heavy and light chain variable domains having the amino acid sequences corresponding to

SEQ ID NOS:57 and 48, respectively, of US 2009/0280116), were included in the study. The results are shown in Table 8. NB: No binding under the conditions tested; NT: Not tested. Concentrations of biotinylated soluble ligands: (1) 150 pM; (2) 500 pM; (3) 10 pM; and (4) 50 pM.

[0104] As shown in Table 8, most of the fully human mAbs show effective blocking of the TL1A/hDR3 and TL1A/hDcR3 binding interaction, with several showing IC50 values below 50 pM. Two of the antibodies, H4H1752P and H4H1805N, strongly blocked binding of both human and monkey TL1A binding to both hDR3 and hDcR3 but failed to block binding of Fhm to either hDR3 or hDcR3, suggesting that the binding epitope for these two antibodies may involve the region near the Fhm mutation site (hTL1A with Q167R). The crystal structure of hTL1A shows that residue Q167 occurs within a surface-exposed loop (Zhan et al., 2009, Biochemistry 48: 7636-7645).

Table 8

mAb ID	hDR3 hTL1A (CHO)[1] IC_{50} (pM)	hDR3 Fhm (CHO)[1] IC_{50} (pM)	hDR3 MfTL1A (CHO)[2] IC_{50} (pM)	DcR3 hTL1A (CHO)[3] IC_{50} (pM)	DcR3 Fhm (CHO)[3] IC_{50} (pM)	DcR3 MfTL1A (CHO)[4] IC_{50} (pM)
H4H1681N	60	>10000	141	17	90	93
H2M1681N	37	>10000	61	13	234	149
H4H1704N	30	44	42	77	170	110
H2M1704N	NT	NT	NT	NT	NT	NT
H4H1719P	22	23	46	44	45	61
H4H1725P	15	18	16	68	85	145
H4H1738P	69	152	117	122	150	68
H4H1742P	64	214	240	181	231	127
H4H1745P	18	44	50	58	85	118
H4H1750P	341	589	5209	656	626	NB
H4H1752P	104	NB	110	31	NB	56
H4H1804N	40	69	71	175	>10000	34
H2M1804N	46	102	81	120	>10000	9
H4H1805N	14	NB	26	33	436	313
H2M1805N	6	NB	13	12	2241	1138
H4H1817N	114	235	101	270	NT	322
H2M1817N	154	249	137	890	666	776
H4H1818N	119	202	123	232	NT	1102
H2M1818N	154	317	239	396	NB	55
Control mAb1	>10000	NB	5300	>1000	NT	21000
Control mAb3	>10000	NB	17000	8600	NT	NT

Example 6: Cell Surface Binding Competition of TL1A Antibodies with Soluble hTL1A

[0105] Human embryonic kidney 293 cells stably transfected to over-express cell-surface hTL1A were first stained in a flow cytometric experiment with eight anti-hTL1A antibodies at four concentrations (1, 0.1, 0.01, and 0.003 µg/ml). Bound human antibodies were detected using an allophycocyanin-labeled goat $F(ab')_2$ specific for human Fcγ [or anti-hFcγ-APC $F(ab')_2$, Jackson ImmunoResearch, # 109-136-170]. The lowest antibody concentration providing significant staining levels was then used in a competition binding experiment. A negative isotype control antibody (human IgG4) was used at 1 µg/ml to define the background signal. For the competition experiment, eight antibody samples, at the minimal concentrations identified above, were first treated with soluble hTL1A expressed from CHO cells at concentrations ranging from 0.03 µg/ml to 10 µg/ml. After pre-incubation for 30 min on ice, the antibody/hTL1A mixture was added to 293/HEK-hTL1A cells that had been isolated by centrifugation in a 96-well conical plate. After incubation for an additional 10 minutes on ice, the cells were washed. The secondary reagent, anti-hFcγ-APC $F(ab')_2$, was added to all wells at a 200-fold dilution to detect bound antibodies. Samples were incubated for 15 minutes on ice, away from light, and then washed. Cells were processed on an BD™ LSR II Flow Cytometer (BD Biosciences) to detect anti-hTL1A antibodies bound to the cell surface, and data were analyzed using FlowJo software (version 8.8.6; Tree Star Inc.). The results are shown in Table 9. Maximum signal: Anti-hTL1A antibody binding in the absence of soluble hTL1A; Minimum signal: Signal recorded when 1 µg /ml of isotype control antibody was added in place of the anti-hTL1A antibody. NT: Not tested.

Table 9

	Mean Fluorescence Intensity for anti-hTL1A antibodies (H4H) binding to cell-surface hTL1A							
Soluble hTL1A (µg/ml)	**1704N**	**1725P**	**1742P**	**1804N**	**1805N**	**1817N**	**1681N**	**1745P**
	0.1 µg/ml	0.1 µg/ml	1 µg/ml	0.1 µg/ml	0.1 µg/ml	1 µg/ml	1 µg/ml	1 µg/ml
10	NT	NT	NT	NT	NT	NT	16.9	15
3	22.6	34.9	19.9	28.9	23.6	25.2	28.5	19.5
1	26	29.2	32.7	33.7	25.1	29.1	80.9	26.9
0.3	31.5	40.7	44.7	33.5	36.8	23.6	236	115
0.1	132	84.5	79.4	51.1	60.2	97.2	327	93.1
0.03	163	207	126	126	156	85.3	318	80
Maximum signal	116	211	126	127	158	110	320	87.2
Minimum signal	27.5	27.5	27.5	27.5	27.5	27.5	17.9	17.9

[0106] As shown in Table 9, the signals from the eight tested antibodies could be competed down to baseline levels by the addition of excess soluble hTL1A, demonstrating the specificity of binding of the antibodies to cell-surface hTL1A.

Example 7: Blocking of hTL1A-Dependent CD4$^+$ T-cell Stimulation by Anti-TL1A Antibodies

[0107] To determine the ability of anti-hTL1A antibodies to block hTL1A-dependent stimulation of human CD4$^+$ T-cells, an *in vitro* assay was developed in which hTL1A/anti-CD3/anti-CD28-stimulated release of IFN-gamma (IFN-γ) was measured in the presence or absence of antibodies. Human CD4$^+$ T-cells were isolated from fresh buffy coats prepared from human blood samples obtained from the New York Blood Center. Cells from a single donor were kept separate from other donor cells for each assay. The CD4$^+$ T-cells were added to the wells of a 96-well plate at 3.5 x 10^5 cells per well. To each well was then added soluble hTL1A (residues 72-251 of NP_005109.2 with an N-terminal hexa-histidine tag, expressed from CHO cells) to a final concentration of 1 µg/ml (16 nM, assuming hTL1A forming trimers in solution) in RPMI + 10% FBS, L-glutamine and penicillin/streptomycin. To each well was also added the anti-hTL1A antibodies or an isotype control antibody to final concentrations of 1.0 µg/ml or 3.0 µg/ml (6.7 nM or 20 nM, respectively). The samples were incubated for 15 minutes at 4°C in the dark, followed by the additions of anti-hCD3 (BD Pharmingen, cat # 555336) and anti-hCD28 (BD Pharmingen, cat # 555725) to each well to final concentrations of 1.0 µg/ml. Samples were incubated for 24 hours at 37°C, the supernatants harvested, and IFN-γ levels determined by ELISA. The blocking effect (average from two separate wells for each condition) of each antibody on each human CD4$^+$ T-cell donor sample is represented as the reduction from maximal signal divided by maximal response window; *i.e.*, % Blocking = [(Max - Inhib)/(Max - Min)] x 100, where "Max", "Inhib", and "Min" are concentrations of IFN-γ measured for CD4$^+$ human T-cells treated as follows: "Max" – treated with [hTL1A + anti-hCD3 + anti-hCD28 + isotype control mAb]; "Min: " – treated with [anti-hCD3 + anti-hCD28 + isotype control mAb]; and "Inhib" – treated with [hTL1A + anti-hCD3 + anti-hCD28 + anti-hTL1A test mAb]. Antibodies for which IFN-γ blockade surpassed the "Min" baseline level are represented as 100% blockade. Ratio (Max/Min) is the ratio of the IFN-γ concentration produced from human CD4$^+$ T-cells treated under Max and Min conditions as defined above.

[0108] As shown in Table 10, the antibodies H4H1725P, H4H1805N, H4H1817N, and H4H1804N significantly blocked hTL1A-stimulated IFN-γ release at both 1 µg/ml and 3 µg/ml concentrations, with nearly complete blockade (>80%) observed for most donors at the higher antibody concentration. The results for blockade of IFN-γ secretion by thirteen different anti-hTL1A antibodies against CD4$^+$ T-cells from 10 different human donors are further summarized in Table 11. SD: Standard deviation.

Table 10

mAb ID		% Blocking of IFN-γ production in human T-cells from 10 donors									
	Donor #	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
	Ratio (Max/Min)	5	4	10	10	4	3	4	3	8	2
H4H1725P	mAb 1 µg/ml (6.7 nM)	90	100	70	85	45	80	60	85	50	95
H4H1805N		100	100	90	100	100	100	100	100	90	100
H4H1817N		100	100	90	90	100	45	55	100	55	80
H4H1804N		95	100	80	90	100	50	10	100	50	0
H4H1725P	mAb 3 µg/ml (20 nM)	95	100	95	100	90	80	90	100	90	100
H4H1805N		100	100	95	100	80	100	100	100	70	100
H4H1817N		100	100	100	100	100	100	100	100	95	85
H4H1804N		100	100	95	100	100	100	100	100	100	80

Table 11

mAb ID	Average %Blocking (SD) 0.1 µg/ml mAb	Average %Blocking (SD) 1 µg/ml mAb	Average %Blocking (SD) 3 µg/ml mAb
H4H1681N	20% (24)	45% (30)	95% (7)
H4H1704N	22% (29)	53% (27)	98% (5)
H4H1719P	10% (22)	37% (23)	78% (30)
H4H1725P	13% (14)	80% (20)	94% (7)
H4H1738P	25% (30)	41% (35)	81% (18)
H4H1742P	10% (22)	58% (33)	83% (16)
H4H1745P	22% (27)	36% (36)	91% (7)
H4H1750P	11% (14)	42% (35)	89% (15)
H4H1752P	18% (25)	52% (35)	71% (30)
H4H1804N	26% (30)	68% (38)	98% (6)
H4H1805N	21% (28)	98% (4)	94% (10)
H4H1817N	25% (33)	81% (22)	98% (5)
H4H1818N	25% (33)	42% (33)	71% (35)
Isotype Control	16% (21)	26% (33)	28% (27)

[0109] IFN-γ levels were also measured at six different antibody concentrations (ranging from 0.03 µg/ml to 10 µg/ml) for each of six different antibodies added to $CD4^+$ T-cells from twelve human donors. Curve fitting to the data allowed estimation of the antibody concentration at which half-maximal inhibition was achieved for each antibody for each donor cell sample. The average (± SD) concentrations for achieving half-maximal inhibition are provided in Table 12.

Table 12

Donor #	mAb IC_{50} (nM)					
	H4H1725P	H4H1742P	H4H1805N	H4H1817N	H4H1804N	H4H1704N
D1	3.4	24	2.4	6.6	6.8	-
D2	3.2	4.6	5.5	3.1	8.6	-
D3	5.4	10	3.4	4.7	8.6	-
D4	4.7	13	2.5	2.4	7.7	-
D5	7.0	12	2.9	8.5	6.7	17
D6	5.4	10	3.4	4.7	8.6	11
D7	13	31	8.0	7.0	12	12
D8	12	27	5.1	7.0	11	19
D9	6.4	56	5.9	9.5	8.1	7.5
D10	4.1	12	2.8	7.3	12	10
D11	6.1	27	3.0	7.3	7.6	11
D12	6.4	14	3.1	9.4	7.5	8.0
Average (± SD)	6.4 (3.1)	20 (14)	4.0 (1.7)	6.5 (2.3)	8.8 (1.9)	12 (4.1)

[0110] Four of the antibodies, H4H1725P, H4H1804N, H4H1805N, H4H1817N exhibited average half-maximal inhibition concentrations below 10 nM (ranging approximately 4-9 nM).

What is claimed:

1. An isolated human antibody or antigen-binding fragment thereof that specifically binds human TNF-like ligand 1A (hTL1A), comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 sequence combination of SEQ ID NO: 20/22/24; and/or a light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 sequence combination of SEQ ID NO: 28/30/32.

2. The antibody or antigen-binding fragment according to claim 1, wherein heavy and light chain CDR sequences, HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3, comprise a CDR sequence combination of SEQ ID NO: 20/22/24/28/30/32.

3. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 18.

4. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 26.

5. The antibody or antigen-binding fragment of claim 2, wherein the antibody or fragment does not cross-react with Fhm comprising the amino acid sequence of SEQ ID NO:246.

6. An antibody or antigen-binding fragment thereof which binds the same epitope on hTL1A as an antibody or antigen-binding fragment comprising heavy chain and light chain complementarity determining regions (CDRs) of SEQ ID NO:20/22/24/28/30/32.

7. An isolated human antibody or antigen-binding fragment thereof that specifically binds human TNF-like ligand 1A (hTL1A), comprising a heavy chain variable region (HCVR) comprising an amino acid sequence of SEQ ID NO: 18 and/or a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.

8. The antibody or antigen-binding fragment of claim 7, comprising a HCVR/LCVR sequence pair of SEQ ID NO: 18/26.

9. An isolated nucleic acid molecule encoding the antibody or antigen-binding fragment of claim 1.

10. An expression vector comprising the nucleic acid molecule of claim 9.

11. An isolated host cell comprising the expression vector of claim 10.

12. A method of producing an anti-hTL1A antibody or antigen-binding fragment thereof, comprising growing the host cell of claim 11 under conditions permitting production of the antibody or fragment thereof, and recovering the antibody or fragment thereof so produced.

13. A pharmaceutical composition comprising the antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof further neutralizes hTL1A, and a pharmaceutically acceptable carrier.