

ANTI-TREM-1 ANTIBODIES AND USES THEREOF

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0001] The content of the electronically submitted sequence listing in ASCII text file (Name: 3338_092PC01_SeqListing.txt; Size: 106,162 bytes; and Date of Creation: March 27, 2019) filed with the application is herein incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] TREM-1 is an activating receptor expressed on monocytes, macrophages, and neutrophils. These cells play a central role in chronic inflammatory diseases by releasing cytokines and other mediators that drive inflammation. TREM-1 mRNA and protein expression is up-regulated in patients with rheumatic arthritis (RA) and inflammatory bowel disease (IBD), and TREM-1-positive cells accumulate at sites of inflammation, correlating with disease severity. *See* Bouchon *et al.*, *Nature* 410:1103-1107 (2001); Schenk *et al.*, *Clin Invest* 117:3097-3106 (2007); and Kuai *et al.*, *Rheumatology* 48:1352-1358 (2009). Peptidoglycan-recognition-protein 1 (PGLYRP1) expressed primarily by activated neutrophils is a ligand for TREM-1 and mediate TREM-1 signaling upon binding..

[0003] *In vitro*, engagement of TREM-1 triggers secretion of pro-inflammatory cytokines including TNF, IL-8, and monocyte chemotactic protein-1. In addition, TREM-1 signaling synergizes with multiple Toll-like Receptors (TLRs) to further boost pro-inflammatory signals. In turn, this up-regulates expression of TREM-1, leading to a vicious cycle amplifying the inflammation. *See* Bouchon *et al.*, *J Immunol* 164:4991-4995 (2000). Increasing evidence indicates that TLRs contribute to the development and progression of chronic inflammatory diseases such as RA and IBD.

[0004] Humanized anti-TREM-1 mAbs that inhibit both human and cynomolgus TREM-1 function have been disclosed elsewhere. *See* WO 2013/120553 A1 and WO 2016/009086 A1. However, such antibodies either have viscosity profile that can hamper manufacturing process or have other issues that can limit their therapeutic potential (e.g., cytokine storm and ADCC). *See* Shire *et al.*, *J. Pharm. Sci.* 93:1390-1402 (2004); and Warncke *et al.*, *J Immunol.* 188:4405-11 (2012). Accordingly, there is a need for an anti-TREM-1 antibody that can specifically bind to and inhibit TREM-1 function but without the issues of the earlier anti-TREM-1 antibodies.

SUMMARY ~~OF THE DISCLOSURE~~

[0004a] In one aspect, provided herein is a heavy chain comprising SEQ ID NO: 50, SEQ ID NO: 51, or SEQ ID NO: 85.

[0004b] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85, and wherein the light chain comprises SEQ ID NO: 54.

[0004c] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.

[0004d] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 51 and wherein the light chain comprises SEQ ID NO: 54.

[0004e] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 52 and wherein the light chain comprises SEQ ID NO: 54.

[0004f] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRFQAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; wherein the IgG1 heavy chain constant region comprises three or more amino acid substitutions comprising L234A, L235E, and G237A, per EU numbering and wherein the heavy chain lacks a C-terminal lysine.

[0004g] In one aspect, provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRFQAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; wherein the IgG1 heavy chain constant region comprises three or more amino acid substitutions, wherein the substitutions comprise L234A, L235E, and G237A, per EU numbering, or wherein the IgG1 heavy chain constant region comprises three or more amino acid substitutions and is lacking one or more amino acids at the C-terminus, wherein the substitutions comprise L234A, L235E, and G237A, per EU numbering.

[0005] Provided herein are isolated antibodies, such as monoclonal antibodies, in particular human (e.g., monoclonal) antibodies, that specifically bind triggering receptor expressed on myeloid cells-1 (TREM-1) and have desirable functional properties. In some ~~embodiments~~examples, the antibody comprises a heavy chain variable region (VH), a light chain variable region (VL), and an IgG1 heavy

chain constant region, wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions compared to a wild-type IgG1 heavy chain constant region (SEQ ID NO: 9). In some [embodiments](#)[examples](#), the antibody cross-competes with mAb 0318 for binding to blocking TREM-1 and comprises a heavy chain variable region (VH), a light chain variable region (VL), and an IgG1 heavy chain constant region, wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions compared to a wild-type IgG1 heavy chain constant region (SEQ ID NO: 9).

[0006] In some [embodiments](#)[examples](#), the antibody binds to the same TREM-1 epitope as mAb 0318. In some [embodiments](#)[examples](#), the antibody specifically binds to a TREM-1 epitope comprising one or more amino acid residues selected from the group consisting of the D38, V39, K40, C41, D42, Y43, T44, L45, E46, K47, F48, A49, S50, S51, Q52, K53, A54, W55, Q56, Y90, H91, D92, H93, G94, L95, and L96 of SEQ ID NO: 1. In some [embodiments](#)[examples](#), the antibody specifically binds to a TREM-1 epitope comprising amino acids D38 to L45, E46 to Q56, and/or Y90 to L96 of SEQ ID NO: 1.

[0007] In some [embodiments](#)[examples](#), the IgG1 heavy chain constant region of the antibody disclosed herein comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, D356E, and L358M, per EU numbering. In some [embodiments](#)[examples](#), the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, A330S, P331S, D356E, and L358M, per EU numbering. In some [embodiments](#)[examples](#), the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, C226S, C229S, and P238S, per EU numbering. In some [embodiments](#)[examples](#), the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of S131C, K133R, G137E, G138S, Q196K, I199T, N203D, K214R, C226S, C229S, and P238S, per EU numbering.

[0008] In some [embodiments](#)[examples](#), the antibody disclosed herein comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR3 comprises DMGIRRQFAY (SEQ ID NO: 26) or DMGIRRQFAY (SEQ ID NO: 26) except one or two substitutions. In some [embodiments](#)[examples](#), the heavy chain CDR3 comprises DQGIRRQFAY (SEQ ID NO: 72).

[0009] In some [embodiments](#)[examples](#), the antibody disclosed herein comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR2 comprises RIRTKSSNYATYYAASVKG (SEQ ID NO: 25) or RIRTKSSNYATYYAASVKG (SEQ ID NO: 25) except one or two substitutions.

[0010] In some [embodiments](#)[examples](#), the antibody disclosed herein comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR1 comprises TYAMH (SEQ ID NO: 24) or TYAMH (SEQ ID NO: 24) except one or two substitutions.

[0011] In some [embodiments](#)[examples](#), the antibody disclosed herein comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the light chain CDR1 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27) or RASQSVDTFDYSFLH (SEQ ID NO: 27)

except one or two substitutions. In some [embodiments](#)[examples](#), the light chain CDR2 comprises RASNLES (SEQ ID NO: 28) or RASNLES (SEQ ID NO: 28) except one or two substitutions. In some [embodiments](#)[examples](#), the light chain CDR3 comprises QQSNQDPYT (SEQ ID NO: 29) or QQSNQDPYT (SEQ ID NO: 29) except one or two substitutions.

[0012] In some [embodiments](#)[examples](#), the VH of the antibody disclosed herein comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth as SEQ ID NO: 14. In some [embodiments](#)[examples](#), the VL of the antibody disclosed herein comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth as SEQ ID NO: 15. In some [embodiments](#)[examples](#), the VH and VL comprises SEQ ID NOs: 14 and 15, respectively.

[0013] In some [embodiments](#)[examples](#), the antibody of the present disclosure comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53. In some [embodiments](#)[examples](#), the light chain comprises SEQ ID NO: 54.

[0014] Provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; and wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of L234A, L235E, and G237A, per EU numbering.

[0015] Provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; and wherein the IgG1 heavy chain constant region comprises amino acid substitutions selected from the group consisting of L234A, L235E, G237A, A330S, and P331S, per EU numbering.

[0016] Provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively;

and wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, C226S, C229S, and P238S, per EU numbering.

[0017] Provided herein is an isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region, wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively; wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; and wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of S131C, K133R, G137E, G138S, Q196K, I199T, N203D, K214R, C226S, C229S, and P238S, per EU numbering.

[0018] In some [embodiments](#)[examples](#), TREM-1 comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 7.

[0019] In some [embodiments](#)[examples](#), the antibody of the present disclosure has a decreased binding affinity to FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIA (CD16a), FcγRIIB (CD16b), or any combination thereof compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54. In some [embodiments](#)[examples](#), the antibody has a decreased binding affinity to FcγRI (CD64) by at least two fold, at least three fold, at least four fold, at least five fold, at least six fold, at least seven fold, at least eight fold, at least nine fold, or at least 10 fold compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

[0020] In some [embodiments](#)[examples](#), the antibody disclosed herein is less immunogenic compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54. In some [embodiments](#)[examples](#), the antibody does not agonize TREM-1 signaling upon binding to TREM-1 and in the absence of a stimulator. In some [embodiments](#)[examples](#), the antibody does not induce expression of an inflammatory cytokine in immature dendritic cells (iDCs) when the cells are incubated in the presence of the antibody and in the absence of a stimulator compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

[0021] In some [embodiments](#)[examples](#), the antibody disclosed herein blocks the production of an inflammatory cytokine in a cell when the cells are activated in the presence of both the antibody and a stimulator. In some [embodiments](#)[examples](#), the stimulator is a TREM-1 ligand. In some [embodiments](#)[examples](#), the inflammatory cytokine is selected from a group consisting of IL-6, TNF-α, IL-8, IL-1β, IL-12, chitinase-3-like protein 1 (CHI3L1), and combinations thereof.

[0022] In some [embodimentsexamples](#), the antibody of the present disclosure binds human FcRn, cynomolgus FcRn, and/or mouse FcRn in a pH dependent manner. In some [embodimentsexamples](#), the antibody disclosed herein is more thermally stable compared to a reference antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54, as measured by a Capillary Differential scanning calorimeter (CAP-DSC). In some [embodimentsexamples](#), about 10% to 20%, about 20% to 30% (e.g., 24%), or about 30% to 40% of the antibody is reversible when it is heated to 77 °C. In some [embodimentsexamples](#), the antibody has a higher melting temperature (T_m) compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

[0023] In some [embodimentsexamples](#), the antibody disclosed herein has a viscosity of less than 5 cP, less than 4 cP, less than 3 cP, less than 2.5 cP, less than 2.4 cP, less than 2.3 cP, less than 2.2 cP, less than 2.1 cP, less than 2 cP, less than 1.9 cP, less than 1.8 cP, less than 1.7 cP, less than 1.6 cP, less than 1.5 cP, less than 1.4 cP, less than 1.3 cP, less than 1.2 cP, less than 1.1 cP, less than 1.0 cP, less than 0.9 cP, less than 0.8 cP, less than 0.7 cP, less than 0.6 cP, less than 0.5 cP, less than 0.4 cP, less than 0.3 cP, less than 0.2 cP, or less than 0.1 cP at a concentration of 80 mg/mL. In some [embodimentsexamples](#), the antibody has a viscosity of less than 10 cP (e.g., 9 cP) at a concentration of 130 mg/mL.

[0024] In some [embodimentsexamples](#), the antibody binds to human TREM-1 with of a K_D of less than 4 nM (e.g., 3.4 nM) as measured by Biacore. In some [embodimentsexamples](#), the antibody binds to cynomolgus TREM-1 with a K_D of less than 1 nM (e.g., 0.91 nM), as measured by Biacore.

[0025] In some [embodimentsexamples](#), the antibody is monomeric as observed by size-exclusion high-performance liquid chromatography (SE-HPLC). In some [embodimentsexamples](#), the antibody exhibits minimal risk for fragmentation as observed by two-dimensional liquid chromatography-tandem mass spectrometry (2D-LC/MS) or intact mass analysis using liquid chromatography-tandem mass spectrometry (LC/MS). In some [embodimentsexamples](#), the antibody has an isoelectric point of 8 to 9 (e.g., 8.75).

[0026] In some [embodimentsexamples](#), the antibody is stable in a formulation comprising histidine, sucrose, arginine, and NaCl. In some [embodimentsexamples](#), the antibody is stable for at least 2 months in a formulation comprising 20 mM histidine, 150 mM sucrose, 25 mM arginine, and 50 mM NaCl. In some [embodimentsexamples](#), the formulation is at a pH of 6.0 and/or wherein the formulation is stored at 4°C, 25°C, or 40°C.

[0027] [In one aspect, A](#)also provided herein are bispecific molecules comprising an anti-TREM-1 antibody of the present disclosure, linked to a molecule having a second binding specificity.

[0028] Provided herein are nucleic acids encoding the antibody disclosed herein, vectors comprising the nucleic acids, and cells transformed with the vectors.

[0029] [In one aspect, P](#)provided herein are immunoconjugates comprising the anti-TREM-1 antibodies disclosed herein, linked to an agent.

[0030] In one aspect, ~~P~~provided herein are compositions comprising anti-TREM-1 antibodies, or antigen binding portions thereof, bispecific molecules, or immunoconjugates described herein, and a carrier. In another aspect, ~~A~~also provided herein are kits comprising the anti-TREM-1 antibodies, or antigen binding portions thereof, bispecific molecules, or immunoconjugates described herein, and instructions for use.

[0030a] In one aspect, provided herein is a use of the antibody as described herein in the manufacture of a medicament for inhibiting TREM-1 activity in a human subject in need thereof.

[0030b] In one aspect, provided herein is a use of the antibody as described herein in the manufacture of a medicament for the treatment of an inflammatory disease or an autoimmune disease in a human subject in need thereof.

[0030c] In one aspect, provided herein is a use of an isolated antibody in the manufacture of a medicament for the treatment of Crohn's disease in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85 and wherein the light chain comprises SEQ ID NO: 54.

[0030d] In one aspect, provided herein is a use of an isolated antibody in the manufacture of a medicament for the treatment of ulcerative colitis in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85 and wherein the light chain comprises SEQ ID NO: 54.

[0030e] In one aspect, provided herein is a use of an isolated antibody in the manufacture of a medicament for the treatment of Crohn's disease in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.

[0030f] In one aspect, provided herein is a use of an isolated antibody in the manufacture of a medicament for the treatment of ulcerative colitis in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.

[0030g] In one aspect, provided herein is the antibody of as described herein for use in therapy.

[0031] Provided herein is a method of inhibiting TREM-1 activity in a subject in need thereof, comprising administering the anti-TREM-1 antibody, the bispecific molecule, the nucleic acid, the vector, the cell, or the immunoconjugate of the present disclosure.

[0032] Provided herein is a method of treating an inflammatory disease or an autoimmune disease in a subject in need thereof, comprising administering the anti-TREM-1 antibody, the bispecific molecule, the nucleic acid, the vector, the cell, or the immunoconjugate of the present disclosure. In some ~~embodiments~~examples, the inflammatory disease or the autoimmune disease is selected from the group consisting of an inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome, rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, systemic lupus erythematosus (SLE), lupus nephritis, vasculitis, sepsis, systemic

inflammatory response syndrome (SIRS), type I diabetes, Grave's disease, multiple sclerosis (MS), autoimmune myocarditis, Kawasaki disease, coronary artery disease, chronic obstructive pulmonary disease, interstitial lung disease, autoimmune thyroiditis, scleroderma, systemic sclerosis, osteoarthritis, atopic dermatitis, vitiligo, graft versus host disease, Sjogrens's syndrome, autoimmune nephritis, Goodpasture's syndrome, chronic inflammatory demyelinating polyneuropathy, allergy, asthma, other autoimmune diseases that are a result of either acute or chronic inflammation, and any combinations thereof. In some [embodiments](#)[examples](#), the method further comprises administering one or more additional therapeutics. In some [embodiments](#)[examples](#), the additional therapeutics is an anti-IP-10 antibody or an anti-TNF- α antibody.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0033] FIGs. 1A and 1B shows that all the mAb 0318 variants bind to human and cynomolgus TREM-1 with similar affinity as the original mAb 0318 antibody (IgG4). FIG. 1A shows the binding affinity data for the 318-IgG1.3f variant to both human (top row) and cynomolgus (bottom row) TREM-1. FIG. 1B shows the binding affinity data for several different mAb 0318 variants to human TREM-1. The different variants shown include: (i) 318-IgG1.1f, (ii) 318-IgG1.3f, (iii) 318-IgG4-Aba, (iv) 318-IgG1-Aba, and (v) 318-IgG1.1f (M to Q mutant). The binding affinity data for the mAb 0318 antibody (IgG4 A and B) were provided for comparison purposes in FIG. 1B.

[0034] FIGs. 2A and 2B show the internalization of mAb 0318-IgG1.3f upon binding to TREM-1 expressed on CD14+ monocytes at various time points post-TREM-1 binding. In FIG. 2A, TREM-1 receptor expression was analyzed at 0, 6, and 24 hours post-binding. In FIG. 2B, TREM-1 receptor expression was analyzed at 0, 4, and 20 hours post-binding. FIG. 2B also provides data using the TREM26 antibody, which does not compete with the 0318 antibody variants for binding to TREM-1.

What is claimed:

1. A heavy chain comprising SEQ ID NO: 50, SEQ ID NO: 51, or SEQ ID NO: 85.
2. The heavy chain of claim 1 comprising SEQ ID NO: 50.
3. The heavy chain of claim 1 comprising SEQ ID NO: 85.
4. An isolated antibody which specifically binds to ~~a triggering receptor expressed on myeloid cells 1 (TREM-1),~~ comprising a heavy chain ~~variable region (VH), and~~ a light chain ~~variable region (VL), and an IgG1 heavy chain constant region,~~ wherein the IgG1 heavy chain ~~constant region comprises one or more amino acid substitutions compared to a wild-type IgG1 heavy chain constant region (SEQ ID NO: 9).~~ SEQ ID NO: 85, and wherein the light chain comprises SEQ ID NO: 54.
- 2.
5. An isolated antibody which ~~cross-competes with mAb-0318 for binding to blocking~~ specifically binds to TREM-1, comprising a heavy chain and a light chain, wherein the heavy chain ~~comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.~~
6. An isolated antibody which specifically binds to TREM-1, comprising a heavy chain ~~variable region (VH), and~~ a light chain ~~variable region (VL), and an IgG1 heavy chain constant region,~~ wherein the ~~IgG1 heavy chain constant region comprises one or more amino acid substitutions compared to a wild-type IgG1 heavy chain constant region (SEQ ID NO: 9).~~ 51 and wherein the light chain comprises SEQ ID NO: 54.
3. ~~The antibody of claim 1 or 2, wherein the antibody binds to the same TREM-1 epitope as mAb-0318.~~
4. ~~The antibody of claim 1 or 2, wherein the antibody specifically binds to a TREM-1 epitope comprising one or more amino acid residues selected from the group consisting of the D38, V39, K40, C41, D42, Y43, T44, L45, E46, K47, F48, A49, S50, S51, Q52, K53, A54, W55, Q56, Y90, H91, D92, H93, G94, L95, and L96 of SEQ ID NO: 1.~~
5. ~~The antibody of claim 1 or 2, wherein the antibody~~
7. An isolated antibody which ~~specifically binds to a TREM-1 epitope,~~ comprising amino acids D38 to L45, E46 to Q56, and/or Y90 to L96 of SEQ ID NO: 1.

6. ~~The antibody of any one of claims 1 to 5, wherein the IgG1 a heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, D356E, and L358M, per EU numbering.~~

7. ~~The antibody of any one of claims 1 to 5, wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, A330S, P331S, D356E, and L358M, per EU numbering.~~

8. ~~The antibody of any one of claims 1 to 5, wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, C226S, C229S, and P238S, per EU numbering.~~

9. ~~The isolated antibody of any one of claims 1 to 5, wherein the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of S131C, K133R, G137E, G138S, Q196K, I199T, N203D, K214R, C226S, C229S, and P238S, per EU numbering.~~
a light chain

~~10. The antibody of any one of claims 1 to 9, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR3 comprises DMGIRRQFAY (SEQ ID NO: 26) or DMGIRRQFAY (SEQ ID NO: 26) except one or two substitutions.~~

~~11. The antibody of claim 10, wherein the heavy chain CDR3 comprises SEQ ID NO: 52 and wherein the light chain comprises DQGIRRQFAY (SEQ ID NO: 72).~~

~~12. The antibody of any one of claims 1 to 11, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR2 comprises RIRTKSSNYATYYAASVKG (SEQ ID NO: 25) or RIRTKSSNYATYYAASVKG (SEQ ID NO: 25) except one or two substitutions.~~

~~13. The antibody of any one of claims 1 to 12, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the heavy chain CDR1 comprises TYAMH (SEQ ID NO: 24) or TYAMH (SEQ ID NO: 24) except one or two substitutions.~~

14. ~~The antibody of any one of claims 1 to 13, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the light chain CDR1 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27) or RASQSVDTFDYSFLH (SEQ ID NO: 27) except one or two substitutions.~~ 54.

~~15. — The antibody of any one of claims 1 to 14, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the light chain CDR2 comprises RASNLES (SEQ ID NO: 28) or RASNLES (SEQ ID NO: 28) except one or two substitutions.~~

8.

~~16. — The antibody of any one of claims 1 to 15, comprising a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, and CDR3, wherein the light chain CDR3 comprises QQSNQDPYT (SEQ ID NO: 29) or QQSNQDPYT (SEQ ID NO: 29) except one or two substitutions.~~

~~17. — The antibody of any one of claims 1 to 16, wherein the VH comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth as SEQ ID NO: 14.~~

~~18. — The antibody of any one of claims 1 to 17, wherein the VL comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth as SEQ ID NO: 15.~~

~~19. — The antibody of any one of claims 1 to 18, comprising a heavy chain variable region and a light chain variable region, wherein the VH comprises SEQ ID NO: 14 and the VL comprises SEQ ID NO: 15.~~

~~20. — The antibody of claim 19, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.~~

~~21. — The antibody of claim 20, comprising a heavy chain and a light chain, wherein the light chain comprises SEQ ID NO: 54.~~

22. An isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region,

wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively;

wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; and wherein the IgG1 heavy chain constant region comprises three or more amino acid substitutions comprising L234A, L235E, and G237A, per EU numbering and wherein the heavy chain lacks a C-terminal lysine.

~~wherein the IgG1~~

9. The antibody of claim 8 comprising a heavy chain ~~constant~~variable region (VH) and a light chain variable region (VL), wherein the VH comprises ~~one or more amino acid substitutions selected from~~SEQ ID NO: 14 and the ~~group consisting of L234A, L235E, and G237A, per EU numbering.~~ VL comprises the VL of SEQ ID NO: 15.

10. The antibody of claim 9, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85, and wherein the light chain comprises SEQ ID NO: 54.

11, 23. An isolated antibody which specifically binds to TREM-1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region,

wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively;

wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; ~~and~~

wherein the IgG1 heavy chain constant region comprises ~~amino acid substitutions selected from the group consisting of L234A, L235E, G237A, A330S, and P331S, per EU numbering.~~

~~24. An isolated antibody which specifically binds to TREM 1, comprising a heavy chain CDR1, CDR2, CDR3; a light chain CDR1, CDR2, CDR3; and an IgG1 heavy chain constant region;~~

~~wherein the heavy chain CDR1, CDR2, and CDR3 comprises TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (SEQ ID NO: 25), and DMGIRRQFAY (SEQ ID NO: 26), respectively;~~

~~wherein the light chain CDR1, CDR2, and CDR3 comprises RASQSVDTFDYSFLH (SEQ ID NO: 27), RASNLES (SEQ ID NO: 28), and QQSNQDPYT (SEQ ID NO: 29), respectively; and~~

~~wherein the IgG1 heavy chain constant region comprises one~~ three ~~or more amino acid substitutions, wherein the substitutions selected from the group consisting of K214R, C226S, C229S~~ comprise L234A, L235E, and P238SG237A, per EU numbering. , or

wherein the IgG1 heavy chain constant region comprises three or more amino acid substitutions and is lacking one or more amino acids at the C-terminus, wherein the substitutions comprise L234A, L235E, and G237A, per EU numbering.

~~12.25. An isolated~~ The antibody ~~which specifically binds to TREM-1 of claim 11,~~ comprising a heavy chain ~~CDR1, CDR2, CDR3;~~ variable region (VH) and a light chain ~~CDR1, CDR2, CDR3; and an IgG1 heavy chain constant~~ variable region;

(VL), wherein the ~~heavy chain CDR1, CDR2, and CDR3~~ VH comprises ~~TYAMH (SEQ ID NO: 24), RIRTKSSNYATYYAASVKG (14 and the VL comprises the VL of~~ SEQ ID NO: 25), and ~~DMGIRRQFAY (SEQ ID NO: 26), respectively;~~ 15.

~~13. wherein the~~ The antibody of claim 12, comprising a heavy chain and a light chain ~~CDR1, CDR2, and CDR3, wherein the heavy chain~~ comprises ~~RASQSVDTFDYSFLH (SEQ ID NO: 50, SEQ ID NO: 27), RASNLES (51, or~~ SEQ ID NO: 28), and ~~QQSNQDPYT (SEQ ID NO: 29), respectively~~ 85; and wherein the light chain comprises SEQ ID NO: 54.

14. The antibody of claim 12, wherein the IgG1 heavy chain constant region ~~comprises one or more amino acid substitutions selected from the group consisting of S131C, K133R, G137E, G138S, Q196K, H199T, N203D, K214R, C226S, C229S~~ has three substitutions, wherein the substitutions are L234A, L235E, and ~~P238S~~ G237A, per EU numbering.

~~26.~~

15. The antibody of ~~any one of claims 1 to 25~~ claim 12, wherein the IgG1 heavy chain constant region has three substitutions, wherein the substitutions are L234A, L235E, and G237A, per EU numbering, and a C-terminal lysine residue of the IgG1 heavy chain constant region is present.

16. The antibody of claim 12, wherein the IgG1 heavy chain constant region has three substitutions, wherein the substitutions are L234A, L235E, and G237A, per EU numbering, and wherein the heavy chain lacks a C-terminal lysine residue.

~~17. TREM-1~~ The antibody of claim 11, comprising a heavy chain and a light chain, wherein the heavy chain comprises ~~an amino acid sequence of~~ SEQ ID NO: 1, 85, and wherein the light chain comprises SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 7. 54.

18. The antibody of claim 11, comprising a heavy chain, wherein the heavy chain lacks a C-terminal lysine residue.

19. The antibody of claim 11, wherein the IgG1 heavy chain constant region has three substitutions, wherein the substitutions are L234A, L235E, and G237A, per EU numbering.

20. The antibody of claim 11, wherein the IgG1 heavy chain constant region has three substitutions, wherein the substitutions are L234A, L235E, and G237A, per EU numbering, and a C-terminal lysine residue of the IgG1 heavy chain constant region is present.

~~21.27.~~ The antibody of any one of claims 1 to 26, which has a decreased binding affinity to FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIA (CD16a), FcγRIIB (CD16b), or any combination thereof compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

~~28.~~ The antibody of any one of claims 1 to 26, which has a decreased binding affinity to FcγRI (CD64) by at least two fold, at least three fold, at least four fold, at least five fold, at least six fold, at least seven fold, at least eight fold, at least nine fold, or at least 10 fold compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

~~29.~~ The antibody of any one of claims 1 to 28, which is less immunogenic compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

~~30.~~ The antibody of any one of claims 1 to 29, which does not agonize TREM-1 signaling upon binding to TREM-1 and in the absence of a stimulator.

~~31.~~ The antibody of any one of claims 1 to 30, which does not induce expression of an inflammatory cytokine in immature dendritic cells (iDCs) when the cells are incubated in the presence of the antibody and in the absence of a stimulator compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54.

~~32.~~ The antibody of ~~any one of claims 1 to 31, which blocks the production of an inflammatory cytokine in a cell when~~claim 11, wherein the cells are activated in IgG1 heavy chain constant region has three substitutions, wherein ~~the presence of both the antibody and a stimulator.~~
substitutions are L234A, L235E,

~~33.~~ The antibody of any one of claims 30 to 31, wherein the stimulator is a TREM-1 ligand.

~~34.~~ The antibody of claim 31 or 32, wherein the inflammatory cytokine is selected from a group consisting of IL-6, TNF- α , IL-8, IL-1 β , IL-12, chitinase 3-like protein 1 (CHI3L1), and combinations thereof.

~~35.~~ The antibody of any one of claims 1 to 34, which binds human FeRn, cynomolgus FeRn, and/or mouse FeRn in a pH dependent manner.

~~36.~~ The antibody of any one of claims 1 to 35, which is more thermally stable compared to a reference antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76 and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54, as measured by a Capillary Differential scanning calorimeter (CAP-DSC).

~~37.~~ The antibody of any one of claims 1 to 36, wherein about 10% to 20%, about 20% to 30% (e.g., 24%), or about 30% to 40% of the antibody is reversible when it is heated to 77 °C.

~~38.~~ The antibody of claim 36 or 37, which has a higher melting temperature (T_m) compared to an antibody comprising a heavy chain consisting of the amino acid sequence as set forth in SEQ ID NO: 76G237A, per EU numbering, and a light chain consisting of the amino acid sequence as set forth in SEQ ID NO: 54,wherein the heavy chain lacks a C-terminal lysine residue.

~~39.~~ The antibody of any one of claims 1 to 38, which has a viscosity of less than 5 cP, less than 4 cP, less than 3 cP, less than 2.5 cP, less than 2.4 cP, less than 2.3 cP, less than 2.2 cP, less than 2.1 cP, less than 2 cP, less than 1.9 cP, less than 1.8 cP, less than 1.7 cP, less than 1.6 cP, less than 1.5 cP, less than 1.4 cP, less than 1.3 cP, less than 1.2 cP, less than 1.1 cP, less than 1.0 cP, less than 0.9 cP, less than 0.8 cP, less than 0.7 cP, less than 0.6 cP, less than 0.5 cP, less than 0.4 cP, less than 0.3 cP, less than 0.2 cP, or less than 0.1 cP at a concentration of 80 mg/mL.

~~40.~~ The antibody of any one of claims 1 to 38, which has a viscosity of less than 10 cP (e.g., 9 cP) at a concentration of 130 mg/mL.

~~41.~~ The antibody of any one of claims 1 to 40, which binds to human TREM-1 with of a K_D of less than 4 nM (e.g., 3.4 nM) as measured by Biacore.

~~42. — The antibody of any one of claims 1 to 41, which binds to cynomolgus TREM-1 with a K_D of less than 1 nM (e.g., 0.91 nM), as measured by Biacore.~~

~~43. — The antibody of any one of claims 1 to 42, which is monomeric as observed by size exclusion high performance liquid chromatography (SE-HPLC).~~

~~44. — The antibody of any one of claims 1 to 43, which exhibits minimal risk for fragmentation as observed by two dimensional liquid chromatography tandem mass spectrometry (2D-LC/MS) or intact mass analysis using liquid chromatography tandem mass spectrometry (LC/MS).~~

~~45. — The antibody of any one of claims 1 to 44, which has an isoelectric point of 8 to 9 (e.g., 8.75).~~

~~46. — The antibody of any one of claims 1 to 45, which is stable in a formulation comprising histidine, sucrose, arginine, and NaCl.~~

~~47. — The antibody of claim 46, which is stable for at least 2 months in a formulation comprising 20 mM histidine, 150 mM sucrose, 25 mM arginine, and 50 mM NaCl.~~

~~48. — The antibody of claim 46 or 47, wherein the formulation is at a pH of 6.0 and/or wherein the formulation is stored at 4°C, 25°C, or 40°C.~~

~~49~~

22. A bispecific molecule comprising the antibody of any one of claims ~~14~~ to ~~48~~21, linked to a molecule having a second binding specificity.

~~50. — A nucleic acid encoding the antibody of any one of claims 1 to 48.~~

~~51. — A vector comprising the nucleic acid of claim 50.~~

~~52. — A cell comprising the vector of claim 51.~~

~~53.~~

23. An immunoconjugate comprising the antibody of any one of claims ~~14~~ to ~~48~~21, linked to an agent.

~~54.~~

24. A composition comprising the antibody of any one of claims ~~1 to 48~~, ~~the bispecific molecule of claim 49~~, ~~the nucleic acid of claim 50~~, ~~the vector of claim 51~~, ~~the cell of claim 52~~, ~~or the immunoconjugate of claim 53~~4 to 21, and a carrier.

~~55.~~

25. A kit comprising the antibody of any one of claims ~~1 to 48, the bispecific molecule of claim 49, the nucleic acid of claim 50, the vector of claim 51, the cell of claim 52, or the immunoconjugate of claim 53~~ 4 to 21, and an instruction for use.

~~56. — A method of~~

26. Use of the antibody of any one of claims 4 to 21 in the manufacture of a medicament for inhibiting TREM-1 activity in a human subject in need thereof, ~~comprising administering the antibody of any one of claims 1 to 48, the bispecific molecule of claim 49, the nucleic acid of claim 50, the vector of claim 51, the cell of claim 52, or the immunoconjugate of claim 53 to the subject.~~

~~57. — A method~~

27. The use of ~~treating~~ claim 26, wherein the medicament is to be administered in combination with one or more additional therapeutics.

28. Use of the antibody of any one of claims 4 to 21 in the manufacture of a medicament for the treatment of an inflammatory disease or an autoimmune disease in a human subject in need thereof, ~~comprising administering the antibody of any one of claims 1 to 48, the bispecific molecule of claim 49, the nucleic acid of claim 50, the vector of claim 51, the cell of claim 52, or the immunoconjugate of claim 53 to the subject.~~

~~58.~~

29. The ~~method~~ use of claim ~~57~~ 28, wherein the inflammatory disease or the autoimmune disease is selected from the group consisting of an inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome, rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, systemic lupus erythematosus (SLE), lupus nephritis, vasculitis, sepsis, systemic inflammatory response syndrome (SIRS), type I diabetes, Grave's disease, multiple sclerosis (MS), autoimmune myocarditis, Kawasaki disease, coronary artery disease, chronic obstructive pulmonary disease, interstitial lung disease, autoimmune thyroiditis, scleroderma, systemic sclerosis, osteoarthritis, atopic dermatitis, vitiligo, graft versus host disease, Sjogren's syndrome, autoimmune nephritis, Goodpasture's syndrome, chronic inflammatory demyelinating polyneuropathy, allergy, asthma, other autoimmune diseases that are a result of either acute or chronic inflammation, and any combinations thereof.

30. ~~59.~~ The ~~method of any one of claims 56 to 58, further comprising administering one or more additional therapeutics.~~

~~60. The method~~use of claim ~~59~~29, wherein the ~~additional therapeutics~~inflammatory disease or autoimmune disease is ~~an anti-IP-10 antibody or an anti-TNF- α antibody~~Crohn's disease.

31. The use of claim 29, wherein the inflammatory disease or autoimmune disease is ulcerative colitis.

32. Use of an isolated antibody in the manufacture of a medicament for the treatment of Crohn's disease in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85 and wherein the light chain comprises SEQ ID NO: 54.

33. Use of an isolated antibody in the manufacture of a medicament for the treatment of ulcerative colitis in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 85 and wherein the light chain comprises SEQ ID NO: 54.

34. Use of an isolated antibody in the manufacture of a medicament for the treatment of Crohn's disease in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.

35. Use of an isolated antibody in the manufacture of a medicament for the treatment of ulcerative colitis in a human subject in need thereof, wherein said isolated antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 50 and wherein the light chain comprises SEQ ID NO: 54.

36. The antibody of any one of claims 4 to 21 for use in therapy.