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Application Data Sheet 37 CFR 1.76

Attorney Docket Number 735023000400

Application Number

Title of Invention ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF

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Inventor Information:

Inventor 1

Legal Name

Prefix Given Name Middle Name Family Name Suffix

Arnon ROSENTHAL

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All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.

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☐ An Address is being provided for the correspondence Information of this application.

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Application Information:

Title of the Invention ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF

Attorney Docket Number 735023000400 Small Entity Status Claimed □

Application Type Provisional

Subject Matter Utility

Total Number of Drawing Sheets (if any) 2 Suggested Figure for Publication (if any)
**Application Data Sheet 37 CFR 1.76**

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**Title of Invention:** ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF

**Publication Information:**

- **Request Early Publication (Fee required at time of Request 37 CFR 1.219)**
- **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Application Data Sheet 37 CFR 1.76

| Title of Invention | ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF |

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

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Application Data Sheet 37 CFR 1.76

Title of Invention: ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an assignee under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

○ Assignee ○ Legal Representative under 35 U.S.C. 117 ○ Joint Inventor

○ Person to whom the inventor is obligated to assign. ○ Person who shows sufficient proprietary interest

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Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☐

Mailing Address Information:

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### Application Data Sheet 37 CFR 1.76

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If the Assignee is an Organization check here. [ ]

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ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES
AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] This invention relates to agonist anti-TREM2 antibodies and therapeutic uses of such antibodies.

BACKGROUND OF THE INVENTION

[0002] Triggering receptor expressed on myeloid cells-2 (TREM2) is an immunoglobulin-like receptor that is expressed primarily on myeloid lineage cells, such as macrophages, dendritic cells, osteoclasts, and microglia; and is required for suppression of Toll-like receptor (TLR) signaling, the suppression of inflammatory cytokines, as well as for normal osteoclast development. TREM2 was discovered as a member of the TREM transmembrane glycoproteins, which belong to the single immunoglobulin variable (IgV) domain receptor family. The genes encoding human and mouse TREMs map to human chromosome 6p21.1 and mouse chromosome 17C3, respectively. The TREM cluster includes genes encoding TREM1, TREM2, TREM4, and TREM5, as well as the TREM-like genes in both human and mouse. Additionally TREM3 and plasmacytoid dendritic cell (pDC)-TREM was identified in mouse. The TREM-like genes, TREML1 and TREML2 in humans, and Treml1 and Treml2 in mouse, encode TLT-1 and TLT-2 respectively. The two best characterized of these receptors, TREM1 and TREM2, display some sequence homology with other members of the Ig-SF such as activating NK cells receptors (20% identity with NKp44) and act through association with a DAP12-mediated pathway for signaling.

[0003] TREM2 was originally cloned as a novel cDNA encoding a TREM1 homologue (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). This receptor is a glycoprotein of about 40kDa, which is reduced to 26kDa after N-deglycosylation. The TREM2 gene encodes a 230aa protein consisting of an extracellular domain, a transmembrane region and a short cytoplasmic tail. The extracellular region, encoded by exon 2, is composed of a single type V Ig-
SF domain, containing three potential N-glycosylation sites. The putative transmembrane region contains a charged lysine residue. The cytoplasmic tail of TREM2 lacks signaling motifs and is thought to signal through the signaling adaptor molecule DAP12/TRYROBP.

The signaling adaptor molecule DAP12 is expressed as a homodimer at the surface of a variety of cells participating in innate immune response, including macrophages, granulocytes, and dendritic cells (DC). DAP12 is a member of the type I transmembrane adapter protein family on the basis of homology with the human T-cell receptor (TCR)-associated CD3 chains and the Fc receptor (FcR) γ-chain (Turnbull, IR and Colonna, M, Nat Rev Immunol. 2007. 7(2): p. 155-61). These proteins share many structural and functional characteristics, including one or more ITAM motifs in their cytoplasmic domain, charged acidic residue in transmembrane region (critical for interaction with its partner chain) and the ability to recruit Src homology domain-2 (SH2)-containing proteins following tyrosine phosphorylation. The ITAM motif mediates signal propagation by activation of the ZAP70 or Syk tyrosine kinase. Both kinases phosphorylate several substrates, thereby facilitating the formation of a signaling complex leading to cellular activation. Interestingly, some B-cells and T-cells also express DAP12 under inflammatory conditions. In humans, subsets of CD4⁺CD28⁻ T-cells, αβTCR⁺CD4⁺ T-cells, and CD8⁺ T-cells expressing this protein have been described in patients suffering from chronic inflammatory diseases, in the context of autoimmune T cells (Whittaker, GC et al., J Biol Chem. 285(5): p. 2976-85). In view of the significant level of DAP12 expression in mouse peritoneal macrophages, this protein would also be expected to be expressed in other macrophage-related cells, such as osteoclasts in the bone marrow, Kupffer cells in the liver, alveolar macrophages of the lung, and microglial cells in the brain (Takagi, R et al., Immunol Rev, 2006. 214: p. 118-29).

TREM2 has been identified as expressed on the surface of human monocyte-derived dendritic cells and as an mRNA transcript in the mouse macrophage cell line RAW264 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). Human TREM2 was the first DAP12-associated receptor described on the surface of DCs. Studies have demonstrated that TREM2 cell surface expression is reduced in DAP12-deficient bone marrow-derived dendritic cells (BMDCs) and in DAP12-deficient macrophages, as compared to wild-type cells (Ito, H and Hamerman, JA, Eur J Immunol. 42(1): p. 176-85; Hamerman, JA et al., J Immunol, 2006. 177(4): p. 2051-5; and...

Recent studies have also shown cell-surface expression of TREM2 on macrophages infiltrating tissue from the circulation, as well as on macrophages activated by IL-4 or IL-13 (Tumbull, IR et al., J Immunol, 2006. 177(6): p. 3520-4). However, TREM2 expression was not found in other cell populations, such as tissue-resident macrophages, circulating monocytes, or the corresponding progenitor cells in the bone marrow, suggesting that TREM2 expression is not induced centrally, but locally during tissue infiltration or by cytokine-mediated activation. Moreover, it has also been observed that IFN-γ and LPS abrogate TREM2 expression, and that TREM2−/− macrophages produce increased levels of inflammatory cytokines in response to TLR ligands. Further, it has been recently reported that TREM2 is highly expressed on microglia and infiltrating macrophages in the central nervous system during experimental autoimmune encephalomyelitis (Piccio, L et al., Eur J Immunol, 2007. 37(5): p. 1290-301).

It has been shown that TREM2 signals through DAP12. Downstream this leads to activation of PI3K and other intracellular signals. On myeloid cells, TLR signals are important for activation, such as with infection response, but also play a key role in the pathological inflammatory response, such as with macrophages and dendritic cells (Hamerman, JA et al., (2006) J Immunol 177: 2051-2055; Ito, H et al., Eur J Immunol 42: 176-185; Neumann, H et al., (2007) J Neuroimmunol 184: 92-99; Takahashi, K et al., (2005) J Exp Med 201: 647-657; and Takahashi, K et al., (2007) PLoS Med 4: e124). Deficiency of either TREM2 or DAP12 leads to increased pro-inflammatory signaling. The impact of TREM2-deficiency in vitro has been shown in the context of stimulation with typical TLR ligands, such as LPS, CpG DNA, and Zymosan. TREM-2-deficient dendritic cells show increased release of IL-12p70, TNF, IL-6, and IL-10 in the presence, but not in the absence of stimulation.

Several recent studies have explored the intracellular signaling events induced by the activation of the TREM2/DAP12 pathway. For example, TREM2 activates signaling pathways involved in cell survival (e.g., protein kinase B-Akt), cell activation and differentiation (e.g., Syk, Erk1/2, PLC-γ, etc.), and in the control of the actin cytoskeleton (e.g., Syk, Vav, etc.) (Peng, Q et
al., Sci Signal. 3(122): p. ra38). After ligation of TREM2, the ITAM tyrosines in DAP12 are phosphorylated by SRC-family kinases leading to the recruitment and activation of the Syk kinase and/or ZAP70 kinase. In the mouse, Syk might be the predominant kinase involved, whereas in humans both Syk and ZAP70 seem to couple efficiently with such ITAM-containing subunits, binding them through their tandem SH2 domains.

Studies on TREM2 signaling have shown that, like TREM1, TREM2-mediated signaling through DAP12 also leads to an increase in intracellular calcium ion levels and ERK1/2 phosphorylation of ERK1/2 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22; and Sharif, O and Knapp, S, Immunobiology, 2008. 213(9-10): p. 701-13). Importantly, TREM2 receptor ligation does not induce the degradation of IkB-a and the subsequent nuclear translocation of NF-kB, which demonstrates a clear difference between TREM2 and TREM1 signaling (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). Receptor cross-linking of TREM2 on immature dendritic cells triggers the up-regulation of molecules involved in T-cell co-stimulation, such as CD86, CD40, and MHC class II, as well as the up-regulation of the chemokine receptor CCR7 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). TREM2 is also expressed on microglia, where receptor cross-linking results in an increase in ERK1/2 phosphorylation and CCR7, but not an increase in CD86 or MHC class II expression, suggesting possible cell type-specific differences in TREM2 signaling. Additionally, activation of TREM2 signaling in microglia and myeloid Precursors results in an increase in phagocytosis of apoptotic neurons, nerve and non-nerve tissue debris in the nervous system, disease causing proteins, bacteria and other foreign invaders, which is accompanied by a polarization and re-organization of F-actin in an ERK-dependent manner (Takahashi, K et al., PLoS Med, 2007. 4(4): p. e124; and Neumann, H and Takahashi, K, J Neuroimmunol, 2007. 184(1-2): p. 92-9).

It has also been shown that bone marrow-derived macrophages (BMDM) that have been silenced for TREM2 using shRNAi display increased secretion of TNF in response to the TLR2/6 ligand zymosan and the TLR9 ligand CpG, as compared to control BMDM cells that were treated with a non-specific shRNAi, indicating that TREM2 negatively regulates cytokin synthesis in macrophages (Ito, H and Hamerman, JA, Eur J Immunol. 42(1): p. 176-85; Hamerman, JA et al., J Immunol, 2006. 177(4): p. 2051-5; and Hamerman, JA et al., Nat
Immunol. 2005. 6(6): p. 579-86). These results have been confirmed using BMDM cells from TREM2 knockout mice, and have further shown that levels of TNF and IL-6 were also higher in TREM2−/− BMDM cells in response to LPS, as compared to wild-type BMDM cells (Turnbull, IR, et al., J Immunol. 2006. 177(6): p. 3520-4; and Turnbull, IR and Colonna, M, Nat Rev Immunol, 2007. 7(2): p. 155-61). Additionally, TREM2 overexpression in microglia has been demonstrated to lead to a decrease in TNF and inducible nitric oxide (iNOS) mRNA after culture of these cells with apoptotic neurons, whereas TREM2 knockdown resulted in a modest increase in TNF and iNOS mRNA levels. These results indicate that, in contrast to TREM1, which is a positive regulator of cytokine synthesis, TREM2 is a negative regulator of cytokine synthesis. This effect of TREM2 on inflammation may be independent of the type of macrophage as it occurs in both microglia and BMDM cells.

[0011] It has also been shown that in resident myeloid cells of the central nervous system, activation of microglia can lead to inflammation (Neumann, H et al., (2007) J Neuroimmunol 184: 92-99; Takahashi, K et al., (2005) J Exp Med 201: 647-657; Takahashi, K et al., (2007) PLoS Med 4: e124; and Hsieh, CL et al., (2009) J Neurochem 109: 1144-1156). Moreover, microglia activation has also been implicated in frontotemporal dementia (FTD), Alzheimer’s disease, Parkinson’s disease, stroke/ischemic brain injury, and multiple sclerosis. Whereas reduced TREM2 activation leads to increases in certain activation and inflammation markers, such as NOS2 gene transcription in myeloid cells, increased TREM2 activation leads to reduced NOS2 transcription. It is thought that dying neurons express an endogenous ligand for TREM2. HSP60 has been implicated as a ligand of TREM2 on neuroblastoma cells (Stefani, L et al., (2009) Neurochem 110: 284-294). TREM2 induction also leads to increased phagocytosis of dying neurons by microglia, and similarly increases phagocytosis by other myeloid lineage cells.

TREM2 gene expression has also been shown to be increased in APP23 transgenic mice, an Alzheimer’s disease model in which the mice express a mutant form of the amyloid precursor protein that is associated with familial Alzheimer’s disease (Melchior, B et al., ASN Neuro 2; e00037). Uptake of Amyloid 1-42 has also been shown to be increased in BV-2 microglial cell lines that overexpress TREM2.


Further, exome sequencing of individuals with frontotemporal dementia (FTD) presentation has identified homozygous mutations in TREM2 (Guerreiro, RJ et al., JAMA Neurol 70: 78-84; and Guerreiro, RJ et al., Arch Neurol: 1-7). Some of these mutations lead to truncation and likely loss-of-function of TREM2. These same TREM2 mutations can also cause Naku-Hakula disease in some individuals. Imaging analysis in certain individuals with TREM2 homozygous mutations has also shown evidence of demyelination.

Heterozygous mutations in TREM2, which are the same as the mutation that cause Naku-Hakula and FTD, also increase the risk of Alzheimer’s disease (Guerreiro, R et al., N Engl J Med 368: 117-127; Jonsson, T et al., N Engl J Med 368: 107-116; and Neumann, H et al., N Engl J Med 368: 182-184). Although these TREM2 mutations are rarer than the known risk variants of Alzheimer’s disease (e.g., APOE4), the effect of carrying these mutations is just as serious—around a 3-5 fold increase in the risk of developing Alzheimer’s disease. Moreover, even individuals without Alzheimer’s disease who carry a heterozygous TREM2 mutation show worse cognition as compared to individuals with two normal TREM2 alleles. Further, it has been shown that the R47H variant of TREM2 (arginine to histidine amino acid substitution at position 47 of TREM2), which is most common TREM2 mutation (up to 1 in 200 individuals) is
located within the immunoglobulin domain of TREM2, and may thus alter ligand binding. TREM2 was also shown to be required for survival of microglia in the brain (Otero et al., (2009) \textit{Nat Immunol.};10:734-43).

[0017] In addition an integrative network-based approach to rank-ordered organized structure of molecular networks of gene expression for relevance to late onset developing Alzheimer’s disease (LOAD) identified TYROBP/DAP12 as the signaling molecule for TREM2 as a key regulator of the immune/microglia gene modules that is associated with LOAD. TYROBP was found to be the causal regulator of the highest scoring immune/ microglia module as rank-ordered based on the number of other genes that TREM2 regulated and the magnitude of loss of regulation, as well as differential expression in LOAD brains. TYROBP was significantly upregulated in LOAD brains and there was a progression of TYROBP expression changes across mild cognitive impairment (MCI), which often precedes LOAD (Zhang et al., (2013) \textit{Cell} 153, 707-720). Targeting such causal networks in ways that restore them to a normal state may be a way to treat disease.

[0018] Accordingly, there is a need for antibodies that specifically bind TREM2 and/or its signaling adapter molecule DAP12/TYROBP on a cell surface and activate one or more TREM2 and/or DAP12 activities in order to treat one or more diseases, disorders, and conditions associated with decreased TREM2 and/or DAP12 activity.

[0019] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

\textbf{SUMMARY OF THE INVENTION}

[0020] The invention is generally directed to methods and compositions that include agonist antibodies, \textit{e.g.}, monoclonal antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibody fragments, \textit{etc.}, that specifically bind a TREM2 protein and/or its signaling adaptor molecule DAP12, \textit{e.g.}, a mammalian TREM2, a human TREM2, a mammalian DAP12, or a human DAP12, including wild-type proteins and naturally occurring variants thereof. The methods provided herein find use in preventing, reducing risk, or treating an individual having
dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, or multiple sclerosis.

[0021] In certain aspects, the present disclosure provides an isolated agonist antibody that binds to a TREM2 protein, a DAP12 protein, or both, where the antibody induces one or more TREM2 activities, DAP12 activities, or both.

[0022] In certain embodiments, the TREM2 protein, the DAP12 protein, or both is a mammalian protein or a human protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein, the DAP12 protein, or both is a wild-type protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein, the DAP12 protein, or both is a naturally occurring variant. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities include TREM2 binding to DAP12. In certain embodiments that may be combined with any of the preceding embodiments, the one or more DAP12 activities include DAP12 binding to TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include DAP12 phosphorylation. In certain embodiments that may be combined with any of the preceding embodiments, DAP12 phosphorylation is induced by one or more SRC family tyrosine kinases. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include PI3K activation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include increased expression of one or more anti-inflammatory mediators selected from IL-12p70, IL-6, and IL-10. In certain embodiments that may be combined with any of the preceding embodiments, the increased expression of the one or more anti-inflammatory mediators occurs in one or more cells selected from macrophages, dendritic cells, and microglial cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include reduced expression of one or more pro-inflammatory mediators selected from IFN-a4, IFN-b, IL-6, IL-12 p70, IL-1β and TNF. In certain embodiments that may be combined with any of the preceding embodiments, the reduced expression of the one or more pro-inflammatory
mediators occurs in one or more cells selected from macrophages, dendritic cells, and microglial cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include extracellular signal-regulated kinase (ERK) phosphorylation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include increased expression of C-C chemokine receptor 7 (CCR7). In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include a reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include induction of osteoclast production, increased rate of osteoclastogenesis, or both. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include of increasing the survival of macrophages, microglial cells, or both. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include increasing the function of macrophages, microglial cells, or both. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include induction of one or more types of clearance without inflammation selected from apoptotic neuron clearance without inflammation, nerve tissue debris clearance without inflammation, non-nerve tissue debris clearance without inflammation, bacteria or other foreign body clearance without inflammation, and disease-causing protein clearance without inflammation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include induction of phagocytosis without inflammation of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, or disease-causing proteins without inflammation. In certain embodiments that may be combined with any of the preceding
embodiments, the disease-causing protein is selected from A beta peptide, alpha synuclein protein, Tau protein, TDP-43 protein, prion protein, and huntingtin protein. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include normalization of disrupted TREM2/DAP12-dependent gene expression. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities, DAP12 activities, or both include recruitment of Syk, ZAP70, or both to DAP12. In certain embodiments that may be combined with any of the preceding embodiments, the isolated agonist antibody that binds to a TREM2 protein binds to one or more amino acids within amino acid residues selected from: i) amino acid residues 29-112 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 29-112 of SEQ ID NO: 1; ii) amino acid residues 29-41 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 29-41 of SEQ ID NO: 1; iii) amino acid residues 40-44 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 40-44 of SEQ ID NO: 1; iv) amino acid residues 47-69 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 47-69 of SEQ ID NO: 1; v) amino acid residues 67-76 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 67-76 of SEQ ID NO: 1; vi) amino acid residues 76-86 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 76-86 of SEQ ID NO: 1; vii) amino acid residues 91-100 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 91-100 of SEQ ID NO: 1; viii) amino acid residues 99-115 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 99-115 of SEQ ID NO: 1; ix) amino acid residues 104-112 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 104-112 of SEQ ID NO: 1; and x) amino acid residues 114-118 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 114-118 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the isolated agonist antibody that binds to a TREM2 protein binds to an epitope containing one or more amino acid residues selected from: i) amino acid residue Arg47 or Asp87 of SEQ ID NO: 1; ii) amino acid residues
40-44 of SEQ ID NO: 1; iii) amino acid residues 67-76 of SEQ ID NO: 1; and iv) amino acid residues 114-118 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the isolated agonist antibody that binds to a DAP12 protein binds to one or more amino acids within amino acid residues 22-40 of SEQ ID NO: 2, or amino acid residues on a DAP12 protein corresponding to amino acid residues 22-40 of SEQ ID NO: 2. In certain embodiments that may be combined with any of the preceding embodiments, the isolated agonist antibody is a bispecific antibody that binds to one or more amino acids selected from: i) one or more amino acid residues of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues of SEQ ID NO: 1; and one or more amino acid residues of SEQ ID NO: 2, or amino acid residues on a DAP12 protein corresponding to amino acid residues of SEQ ID NO: 2. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, or a chimeric antibody. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a monoclonal antibody. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an antibody fragment that binds to one or more human proteins selected from human TREM2, a naturally occurring variant of human TREM2, human DAP12, and naturally occurring variant of human DAP12. In certain embodiments that may be combined with any of the preceding embodiments, the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

[0023] Other aspects of the present disclosure provide an isolated nucleic acid encoding the anti-TREM2 antibody, the anti-DAP12 antibody, or both of any one of the preceding embodiments. Other aspects of the present disclosure provide a vector containing the isolated nucleic acid of the preceding embodiment. Other aspects of the present disclosure provide a host cell containing the vector of the preceding embodiment. Other aspects of the present disclosure provide a method of producing an anti-TREM2 antibody, an anti-DAP12 antibody, or both, by culturing the host cell of the preceding embodiment so that the anti-TREM2 antibody, the anti-DAP12 antibody, or both, is produced. In certain embodiments, the method further includes recovering the anti-TREM2 antibody, the anti-DAP12 antibody, or both, produced by the host cell.
[0024] Other aspects of the present disclosure provide a pharmaceutical composition containing the anti-TREM2 antibody, the anti-DAP12 antibody, or both of any of the preceding embodiments, and a pharmaceutically acceptable carrier.

[0025] Other aspects of the present disclosure provide a method of preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and multiple sclerosis, by administering to the individual a therapeutically effective amount of the anti-TREM2 antibody, the anti-DAP12 antibody, or both of any of the preceding embodiments. In certain embodiments, the individual has a heterozygous variant of TREM2, where the variant contains one or more substitutions selected from: i) a glutamic acid to stop codon substitution in the nucleic acid sequence encoding amino acid residue Glu14 of SEQ ID NO: 1; ii) a glutamine to stop codon substitution in the nucleic acid sequence encoding amino acid residue Gln33 of SEQ ID NO: 1; iii) a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp44 of SEQ ID NO: 1; iv) an arginine to histidine amino acid substitution at an amino acid corresponding to amino acid residue Arg47 of SEQ ID NO: 1; v) a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp78 of SEQ ID NO: 1; vi) a valine to glycine amino acid substitution at an amino acid corresponding to amino acid residue Val126 of SEQ ID NO: 1; vii) an aspartic acid to glycine amino acid substitution at an amino acid corresponding to amino acid residue Asp134 of SEQ ID NO: 1; and viii) a lysine to asparagine amino acid substitution at an amino acid corresponding to amino acid residue Lys186 of SEQ ID NO: 1. In certain embodiments, the individual has a heterozygous variant of TREM2, where the variant contains a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G313 of the nucleic acid sequence encoding SEQ ID NO: 1; a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G267 of the nucleic acid sequence encoding SEQ ID NO: 1; or both. In certain embodiments, the individual has a heterozygous variant of DAP12, where the variant contains one or more variants selected from: i) a methionine to threonine substitution at an amino acid corresponding to amino acid residue Met1 of SEQ ID NO: 2; ii) a glycine to arginine amino acid substitution at an amino acid corresponding to amino acid residue Gly49 of SEQ ID NO: 2; iii) a deletion within exons 1-4 of
the nucleic acid sequence encoding SEQ ID NO: 2; iv) an insertion of 14 amino acid residues at exon 3 of the nucleic acid sequence encoding SEQ ID NO: 2; and v) a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G141 of the nucleic acid sequence encoding SEQ ID NO: 2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1A shows an amino acid sequence alignment between the human TREM2 protein and the human NCTR2 protein, depicting the homology between the two proteins.

[0027] Figure 1B shows a structure-based sequence alignment between several TREM proteins and other members of the IgV family. The amino acid residue numbering is consistent with the mature sequence of the human TREM1 protein. The secondary structure elements of TREM1 are illustrated as arrows for the β strands and cylinders for α helices. Amino acid residues involved in homo- and heterodimer formation are shown on black background. Cysteine residues that form disulfide bonds and that are conserved for the V-type Ig fold, are depicted in bold and marked with asterisks. Gaps are indicated by “—”. M-I residues violating antibody-like dimer formation mode are marked with closed triangles as (e.g., Radaev et al., (2003) Structure. 11(12):1527-1535).

[0028] Figure 2 shows an amino acid sequence alignment between the human TREM1 protein and the human TREM2 protein, depicting the homology between the two proteins.

DETAILED DESCRIPTION OF THE INVENTION

General techniques

Definitions

[0030] As used herein, the term “preventing” includes providing prophylaxis with respect to occurrence or recurrence of a particular disease, disorder, or condition in an individual. An individual may be predisposed to, susceptible to a particular disease, disorder, or condition, or at risk of developing such a disease, disorder, or condition, but has not yet been diagnosed with the disease, disorder, or condition.

[0031] As used herein, an individual “at risk” of developing a particular disease, disorder, or condition may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of a particular disease, disorder, or
condition, as known in the art. An individual having one or more of these risk factors has a higher probability of developing a particular disease, disorder, or condition than an individual without one or more of these risk factors.

[0032] As used herein, the term “treatment” refers to clinical intervention designed to alter the natural course of the individual being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of progression, ameliorating or palliating the pathological state, and remission or improved prognosis of a particular disease, disorder, or condition. An individual is successfully “treated”, for example, if one or more symptoms associated with a particular disease, disorder, or condition are mitigated or eliminated.

[0033] An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount can be provided in one or more administrations.

[0034] A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease, disorder, or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the anti-TREM2 and/or anti-DAP12 antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the anti-TREM2 and/or anti-DAP12 antibody are outweighed by the therapeutically beneficial effects.

[0035] As used herein, administration “in conjunction” with another compound or composition includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of administration.

[0036] An “individual” for purposes of treatment, prevention, or reduction of risk refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, and the like. Preferably, the individual is human.
The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein. The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. The pairing of a $V_H$ and $V_L$ together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th Ed., Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha (“α”), delta (“δ”), epsilon (“ε”), gamma (“γ”) and mu (“μ”), respectively. The γ and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al., Cellular and Molecular Immunology, 4th ed. (W.B. Saunders Co., 2000).

“Native antibodies” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ($V_H$) followed by a number of constant domains. Each light
chain has a variable domain at one end ($V_L$) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0041] An “isolated” antibody, such as an isolated anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, is one that has been identified, separated and/or recovered from a component of its production environment (e.g., naturally or recombinantly). Preferably, the isolated polypeptide is free of association with all other contaminant components from its production environment. Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant T-cells since at least one component of the antibody’s natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0042] The “variable region” or “variable domain” of an antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “$V_H$” and “$V_L$”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.
The term “variable” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies, such as anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent-cellular toxicity.

The term “monoclonal antibody” as used herein refers to an antibody, such as a monoclonal anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations, etc.) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal

The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

An “antibody fragment” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab’, F(ab’)2 and Fv fragments; diabodies; linear antibodies (see U.S.

Papain digestion of antibodies, such as anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, produces two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_{H}), and the first constant domain of one heavy chain (C_{H1}). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')_{2} fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_{H1} domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')_{2} antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

“Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.
“Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the $V_H$ and $V_L$ domains, which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Plückthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

“Functional fragments” of antibodies, such as anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the F region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

The term “diabodies” refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the $V_H$ and $V_L$ domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, i.e., a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” sFv fragments in which the $V_H$ and $V_L$ domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., Proc. Nat’l Acad. Sci. USA 90:6444-48 (1993).

As used herein, a “chimeric antibody” refers to an antibody (immunoglobulin), such as a chimeric anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat’l Acad. Sci.
USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest. As used herein, “humanized antibody” is used a subset of “chimeric antibodies.”

[0054]  “Humanized” forms of non-human (e.g., murine) antibodies, such as humanized forms of anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, for example, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.
A “human antibody” is one that possesses an amino-acid sequence corresponding to that of an antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, produced by a human and/or has been made using any of the techniques for making humanized antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries.


The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain, such as that of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., Immunity 13:37-45 (2000); Johnson and Wu in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, e.g., Hamers-Casterman et al., Nature 363:446-448 (1993) and Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability
and are the most commonly used (Kabat et al., supra). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Kabat</th>
<th>AbM</th>
<th>Chothia</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>L24-L34</td>
<td>L24-L34</td>
<td>L26-L32</td>
<td>L30-L36</td>
</tr>
<tr>
<td>L2</td>
<td>L50-L56</td>
<td>L50-L56</td>
<td>L50-L52</td>
<td>L46-L55</td>
</tr>
<tr>
<td>L3</td>
<td>L89-L97</td>
<td>L89-L97</td>
<td>L91-L96</td>
<td>L89-L96</td>
</tr>
<tr>
<td>H1</td>
<td>H31-H35B</td>
<td>H26-H35B</td>
<td>H26-H32</td>
<td>H30-H35B (Kabat numbering)</td>
</tr>
<tr>
<td>H1</td>
<td>H31-H35</td>
<td>H26-H35</td>
<td>H26-H32</td>
<td>H30-H35 (Chothia numbering)</td>
</tr>
<tr>
<td>H2</td>
<td>H50-H65</td>
<td>H50-H58</td>
<td>H53-H55</td>
<td>H47-H58</td>
</tr>
<tr>
<td>H3</td>
<td>H95-H102</td>
<td>H95-H102</td>
<td>H96-H101</td>
<td>H93-H101</td>
</tr>
</tbody>
</table>

HVRs may comprise “extended HVRs” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (a preferred embodiment) (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat et al., supra, for each of these extended-HVR definitions.

“Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

The phrase “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR
or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g., residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0061] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. References to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. References to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (e.g., see United States Patent Publication No. 2010-280227).

[0062] An "acceptor human framework" as used herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferable those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may by 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.
A "human consensus framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, *supra*. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.*, *supra*.

An "amino-acid modification" at a specified position, e.g., of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

An "affinity-matured" antibody, such as an affinity matured anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas *et al.* *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al.* *Gene* 169:147-155 (1995); Yelton *et al.* *J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992).

As use herein, the term "specifically recognizes" or "specifically binds" refers to measurable and reproducible interactions such as attraction or binding between a target and an
antibody, such as between an anti-TREM2 antibody and TREM2, or an anti-DAP12 antibody and DAP12 that is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, that specifically or preferentially binds to a target or an epitope is an antibody that binds this target or epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets or other epitopes of the target. It is also understood by reading this definition that, for example, an antibody (or a moiety) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. An antibody that specifically binds to a target may have an association constant of at least about 10^3 M^{-1} or 10^4 M^{-1}, sometimes about 10^5 M^{-1} or 10^6 M^{-1}, in other instances about 10^6 M^{-1} or 10^7 M^{-1}, about 10^8 M^{-1} to 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher. A variety of immunoassay formats can be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

[0067] As used herein, an “interaction” between a TREM2 protein, or DAP12 protein, and a second protein encompasses, without limitation, protein-protein interaction, a physical interaction, a chemical interaction, binding, covalent binding, and ionic binding. As used herein, an antibody “inhibits interaction” between two proteins when the antibody disrupts, reduces, or completely eliminates an interaction between the two proteins. An antibody of the present disclosure, or fragment thereof, “inhibits interaction” between two proteins when the antibody or fragment thereof binds to one of the two proteins.

[0068] An “agonist” antibody or an “activating” antibody is an antibody, such as an agonist anti-TREM2 antibody or an agonist anti-DAP12 antibody of the present disclosure, that induces (e.g., increases) one or more activities or functions of the antigen after the antibody binds the antigen.
Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype.

The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2, IgG3 and IgG4.

A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most
preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0073] “Fc receptor” or “FcR” describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (“ITAM”) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (“ITIM”) in its cytoplasmic domain. (see, e.g., M. Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. FcRs can also increase the serum half-life of antibodies.

[0074] Binding to FcRn in vivo and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. See also, e.g., Shields et al., J. Biol. Chem. 9(2):6591-6604 (2001).

[0075] As used herein, “percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art,
for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms known in the art needed to achieve maximal alignment over the full length of the sequences being compared.

[0076] An “isolated” nucleic acid molecule encoding an antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0077] The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors,” or simply, “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector.
“Polynucleotide,” or “nucleic acid,” as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, “caps,” substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5’ and 3’ terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2’-O-methyl-, 2’-O-allyl-, 2’-fluoro- or 2’-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein
phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0079] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

[0080] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0081] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0082] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. For example, reference to
an “antibody” is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

[0083] It is understood that aspect and embodiments of the invention described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

**TREM2 proteins**

[0084] In one aspect, the invention provides agonist antibodies that bind to a TREM2 protein of the present disclosure and induce one or more TREM2 activities after binding to a TREM2 protein expressed in a cell.

[0085] TREM2 proteins of the present disclosure include, without limitation, a mammalian TREM2 protein, human TREM2 protein (Uniprot Accession No. Q9NZC2), mouse TREM2 protein (Uniprot Accession No. Q9NH8), rat TREM2 protein (Uniprot Accession No. D3ZZ89), Rhesus monkey TREM2 protein (Uniprot Accession No. F6QVF2), bovine TREM2 protein (Uniprot Accession No. Q05B59), equine TREM2 protein (Uniprot Accession No. F7D6L0), pig TREM2 protein (Uniprot Accession No. H2EZZ3), and dog TREM2 protein (Uniprot Accession No. E2RP46). As used herein “TREM2 protein” refers to both wild-type sequences and naturally occurring variant sequences.

[0086] Triggering receptor expressed on myeloid cells-2 (TREM2) is variously referred to as TREM-2, TREM2a, TREM2b, TREM2c, triggering receptor expressed on myeloid cells-2a, and triggering receptor expressed on monocytes-2. TREM2 is a 230 amino acid membrane protein. TREM2 is an immunoglobulin-like receptor primarily expressed on myeloid lineage cells, including without limitation, macrophages, dendritic cells, osteoclasts, and microglia. In some embodiments, TREM2 forms a receptor signaling complex with DAP12. In some embodiments, TREM2 phosphorylates and signals through DAP12 (an ITAM domain adaptor protein). In some embodiments TREM2 signaling results in the downstream activation of PI3K or other intracellular signals. On Myeloid cells, Toll-like receptor (TLR) signals are important for the activation of TREM2 activities, e.g., in the context of an infection response. TLRs also play a key role in the pathological inflammatory response, e.g., TLRs expressed in macrophages and dendritic cells.
The amino acid sequence of human TREM2 is set forth below as SEQ ID NO: 1:

```
10   20   30   40   50   60
MEPLRLLLILL FVTELSGAHN TTVFQGVAGQ SLQVSCPYDS MKHNGRRKAW CRQLEKEGPC
70   80   90  100  110  120
QRVSTHNLQ LLEFLRBMGW STAITDDTLG GTLTTITLRNL QPHDAGLYQC QSLHGSEADT
130  140  150  160  170  180
LRKVLVEWL A DPLHDRAGD LWFPGESESF EDADHEHSS SRLLEGEPF PPTSILLLLA
190  200  210  220  230
CIFLIKILAA SALWAAAAMG QKPGTHPPSE LDCHDPGYQ LQTLPLGLRDT
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[0088] TREM2 contains an extracellular immunoglobulin-like variable-type (IgV) domain located at amino acid residues 29-112 of human TREM2 (SEQ ID NO: 1); additional extracellular sequences located at amino acid residues 113-174 of human TREM2 (SEQ ID NO: 1); a transmembrane domain located at amino acid residues 175-195 of human TREM2 (SEQ ID NO: 1); and an intracellular domain located at amino acid residues 196-230 of human TREM2 (SEQ ID NO: 1).

[0089] The transmembrane domain of human TREM2 contains a lysine at amino acid residue 186 that can interact with an aspartic acid in DAP12, which is a key adaptor protein that transduces signaling from TREM2, TREM1, and other related IgV family members.

[0090] Homologues of human TREM2 include, without limitation, the natural killer (NK) cell receptor NK-p44 (NCTR2), the polymeric immunoglobulin receptor (pIgR), CD300E, CD300A, CD300C, and TREML1/TLT1. In some embodiments, NCTR2 has similarity with TREM2 within the IgV domain.

**DAP12 proteins**

[0091] In one aspect, the invention provides agonist antibodies that bind to a DAP12 protein of the present disclosure and induce one or more DAP12 activities after binding to a DAP12 protein expressed in a cell.

[0092] DAP12 proteins of the present disclosure include, without limitation, a mammalian DAP12 protein, human DAP12 protein (Uniprot Accession No. O43914), mouse DAP12 protein (Uniprot Accession No. O54885), rat DAP12 protein (Uniprot Accession No. Q6X9T7), Rhesus
monkey DAP12 protein (Uniprot Accession No. Q8WNQ8), bovine DAP12 protein (Uniprot Accession No. Q95J80), and pig DAP12 protein (Uniprot Accession No. Q9TU45). As used herein “DAP12 protein” refers to both wild-type sequences and naturally occurring variant sequences.

[0093] DNAX-activation protein 12 (DAP12) is variously referred to as Killer-activating receptor-associated protein, KAR-associated protein (KARAP), PLOSL, PLO-SL, TYRO protein, and tyrosine kinase-binding protein. DAP12 is a 113 amino acid membrane protein. In some embodiments, DAP12 functions as a transmembrane signaling polypeptide, which contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. It may associate with the killer-cell inhibitory receptor (KIR) family of membrane glycoproteins and may act as an activating signal transduction element. In other embodiments, the DAP12 protein may bind zeta-chain (TCR) associated protein kinase 70kDa (ZAP-70) and spleen tyrosine kinase (SYK), and play a role in signal transduction, bone modeling, brain myelination, and inflammation.

[0094] Mutations within the DAP12-encoding gene have been associated with polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), also known as Nasu-Hakola disease. Without wishing to be bound by theory, it is believed that the DAP12 receptor is TREM2, which also causes PLOSL. Multiple alternative transcript variants encoding distinct isoforms of DAP12 have been identified. DAP12 non-covalently associates with activating receptors of the CD300 family. Cross-linking of CD300-TYROBP/DAP12 complexes results in cellular activation, such as neutrophil activation mediated by integrin. DAP12 is a homodimer; disulfide-linked protein. In some embodiments, DAP12 interacts with SIRPB1, TREM1, CLECSF5, SIGLEC14, CD300LB, CD300E, and CD300D by similarity and via ITAM domain, as well as with SYK via SH2 domain. In other embodiments, DAP12 activates SYK, which mediates neutrophils and macrophages integrin-mediated activation. In other embodiments, DAP12 interacts with KLRC2 and KIR2DS3.

[0095] The amino acid sequence of human DAP12 is set forth below as SEQ ID NO: 2:

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10 20 30 40 50 60
MGGLEFCSR LLLPLLAVS GLRPVQAQAQ SDCSCSTVSP GVLAGIVMGD LVLTVLIALA
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DAP12 is a single-pass type I membrane protein. It contains an extracellular domain located at amino acid residues 22-40 of human DAP12 (SEQ ID NO: 2); a transmembrane domain located at amino acid residues 41-61 of human DAP12 (SEQ ID NO: 2); and an intracellular domain located at amino acid residues 62-113 of human DAP12 (SEQ ID NO: 2). The immunoreceptor tyrosine-based activation motif (ITAM) domain is located at amino acid residues 80-118 of human DAP12 (SEQ ID NO: 2).

In some embodiments, an aspartic acid residue in DAP12 interacts with the transmembrane domain of human TREM2 containing a lysine at amino acid residue 186, and transduces signaling from TREM2, TREM1, and other related IgV family member proteins.

**Anti-TREM2 and anti-DAP12 antibodies**

*Anti-TREM2 antibodies*

Anti-TREM2 antibodies of the present disclosure generally bind to one or more TREM2 proteins expressed in a cell. In some embodiments, the anti-TREM2 antibody is a TREM2 agonist. In some embodiments the anti-TREM2 antibody induces one or more activities of TREM2 after binding to a TREM2 protein that is expressed in a cell. In certain embodiments the TREM2 protein is expressed on a cell surface. The TREM2 activities induced by anti-TREM2 antibodies of the present disclosure may include, without limitation, DAP12 phosphorylation; recruitment of Syk, ZAP70, or both to DAP12; PI3K activation; increased expression of anti-inflammatory mediators; reduced expression of pro-inflammatory mediators; ERK phosphorylation; increased expression of CCR7, induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; increased survival and function of microglial cells and/or macrophages; and induction of apoptotic neuron clearance without inflammation. The anti-TREM2 antibodies of the present disclosure can be used to
prevent, reduce risk of, or treat c dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and multiple sclerosis. In some embodiments, the anti-TREM2 antibodies of the present disclosure are monoclonal antibodies. Anti-TREM2 antibodies of the present disclosure may be tested for inducing one or more TREM2 activities (e.g., DAP12 phosphorylation; recruitment of Syk, ZAP70, or both to DAP12; PI3K activation; increased expression of anti-inflammatory mediators; reduced expression of pro-inflammatory mediators; ERK phosphorylation; increased expression of CCR7, induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; and induction of apoptotic neuron clearance without inflammation) using any suitable method known in the art and/or described herein. For example, the anti-TREM2 antibodies can be assayed in vitro for tyrosine phosphorylation of DAP12 and/or ERK, by assaying for recruitment of Syk and/or ZAP70 to Dap12, by assaying for PI3K activation, by assaying for induction of expression of anti-inflammatory mediators (e.g., IL-12p70, IL-6, and IL-10) or CCR7, or by assaying for reduced expression of pro-inflammatory mediators (e.g., IL1-β and TNF) with TLR stimulation (e.g., LPS, CpG DNA, or Zymosan). Useful assays may include western blots (e.g., for tyrosine-phosphorylated DAP12 or threonine-serine-phosphorylated PI3K-kinase substrates), ELISA (e.g., for secreted interleukin or cytokine secretion), FACS (e.g., for anti-TREM2 binding to TREM2), immunocytochemistry (e.g., for e.g., for tyrosine-phosphorylated DAP12 or threonine-serine-phosphorylated PI3K-kinase substrates), reporter-gene assays (e.g., for TLR activation), increased survival and/or function of microglial cells and/or macrophages, increased phagocytosis of apoptotic neurons, damaged synapses, A beta, and/or other cellular debris by macrophages, dendritic cells, osteoclasts, and/or microglial cells, increased cytoskeleton reorganization, and decreased microglial pro-inflammatory responses, or other assays known in the art.

[0099] Certain aspects of the present disclosure provide anti-TREM2 antibodies that bind to a human TREM2, or a homolog thereof, including without limitation a mammalian TREM2 protein, mouse TREM2 protein (Uniprot Accession No. Q99NH8), rat TREM2 protein (Uniprot
Accession No. D3ZZ89), Rhesus monkey TREM2 protein (Uniprot Accession No. F6QVF2), bovine TREM2 protein (Uniprot Accession No. Q05B59), equine TREM2 protein (Uniprot Accession No. F7D6L0), pig TREM2 protein (Uniprot Accession No. H2EZZ3), and dog TREM2 protein (Uniprot Accession No. E2RP46); and induce at least one TREM2 activity. In some embodiments, the at least one TREM2 activity is DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, and/or reduced expression of one or more pro-inflammatory mediators.

[0100] In some embodiments, anti-TREM2 antibodies of the present disclosure bind to a TREM2 protein of the present disclosure and/or naturally occurring variants. In certain preferred embodiments, the anti-TREM2 antibodies bind to human TREM2.

[0101] In some embodiments, anti-TREM2 antibodies of the present disclosure bind to a TREM2 protein of the present disclosure expressed on the surface of a cell and induce at least one TREM2 activity of the present disclosure after binding to the surface expressed TREM2 protein.

[0102] In certain embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 29-112 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 29-112 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 29-41 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 29-41 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 47-69 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 47-69 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 76-86 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 76-86 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 91-100 of human TREM
2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 91-100 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 99-115 of human TREM2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 99-115 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 104-112 of human TREM2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 104-112 of SEQ ID NO: 1.

[0103] TREM2 proteins of the present disclosure include a complementary determining region 1 (CDR1) located at amino acid residues corresponding to amino acid residues 40-40 of human TREM2 (SEQ ID NO: 1); a complementary determining region 2 (CDR2) located at amino acid residues corresponding to amino acid residues 67-76 of human TREM2 (SEQ ID NO: 1); and a complementary determining region 3 (CDR3) located at amino acid residues corresponding to amino acid residues 114-118 of human TREM2 (SEQ ID NO: 1). Accordingly, in some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 40-44 of human TREM2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 40-44 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 67-76 of human TREM2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 67-76 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 114-118 of human TREM2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 114-118 of SEQ ID NO: 1.

[0104] In other embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residue Arg47 or Asp87 of human TREM2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residues 40-44 of human TREM2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid
residues 67-76 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residues 114-118 of human TREM 2 (SEQ ID NO: 1).

[0105] Additional anti-TREM2 antibodies, e.g., antibodies that specifically bind to a TREM2 protein of the present disclosure, may be identified, screened, and/or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

*Anti-DAP12 antibodies*

[0106] Anti-DAP12 antibodies of the present disclosure generally bind to one or more DAP12 proteins expressed in a cell. In some embodiments, the anti-DAP12 antibody is a DAP12 agonist. In some embodiments the anti-DAP12 antibody induces one or more activities of DAP12 after binding to a DAP12 protein that is expressed in a cell. In certain embodiments the DAP12 protein is expressed on a cell surface. The DAP12 activities induced by anti-DAP12 antibodies of the present disclosure may include, without limitation, binding to TREM2; DAP12 phosphorylation; recruitment of Syk, ZAP70, or both to DAP12; PI3K activation; increased expression of anti-inflammatory mediators; reduced expression of pro-inflammatory mediators; ERK phosphorylation; increased expression of CCR7, induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; increased survival and function of microglial cells and/or macrophages; and induction of apoptotic neuron clearance without inflammation. The anti-DAP12 antibodies of the present disclosure can be used to prevent, reduce risk of, or treat c dementia, frontotemporal dementia, Alzheimer's disease, Nasu-Hakola disease, and multiple sclerosis. In some embodiments, the anti-DAP12 antibodies of the present disclosure are monoclonal antibodies. Anti-DAP12 antibodies of the present disclosure may be tested for inducing one or more DAP12 activities (e.g., DAP12 phosphorylation; recruitment of Syk, ZAP70, or both to DAP12; PI3K activation; increased expression of anti-inflammatory mediators; reduced expression of pro-inflammatory mediators; ERK phosphorylation; increased expression of CCR7, induction of microglial cell chemotaxis toward
CCL19 and CCL21 expressing cells; reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; and induction of apoptotic neuron clearance without inflammation) using any suitable method known in the art and/or described herein. For example, the anti-DAP12 antibodies can be assayed in vitro for tyrosine phosphorylation of DAP12 and/or ERK, by assaying for recruitment of Syk and/or ZAP70 to Dap12, by assaying for PI3K activation, by assaying for induction of expression of anti-inflammatory mediators (e.g., IL-12p70, IL-6, and IL-10) or CCR7, or by assaying for reduced expression of pro-inflammatory mediators (e.g., IL1-β and TNF) with TLR stimulation (e.g., LPS, CpG DNA, or Zymosan). Useful assays may include western blots (e.g., for tyrosine-phosphorylated DAP12 or threonine/serine-phosphorylated PI3K-kinase substrates), ELISA (e.g., for secreted interleukin or cytokine secretion), FACS (e.g., for anti-DAP12 binding to DAP12), immunocytochemistry (e.g., for e.g., for tyrosine-phosphorylated DAP12 or threonine/serine-phosphorylated PI3K-kinase substrates), reporter-gene assays (e.g., for TLR activation), increased survival and/or function of microglial cells and/or macrophages, increased phagocytosis of apoptotic neurons, damaged synapses, A beta, and/or other cellular debris by macrophages, dendritic cells, osteoclasts, and/or microglial cells, increased cytoskeleton reorganization, and decreased microglial pro-inflammatory responses, or other assays known in the art.

[0107] Certain aspects of the present disclosure provide anti-DAP12 antibodies that bind to a human DAP12, or a homolog thereof, including without limitation a mammalian DAP12 protein, mouse DAP12 protein (Uniprot Accession No. Q99NH8), rat DAP12 protein (Uniprot Accession No. D3ZZ89), Rhesus monkey DAP12 protein (Uniprot Accession No. F6QVF2), bovine DAP12 protein (Uniprot Accession No. Q05B59), equine DAP12 protein (Uniprot Accession No. F7D6L0), pig DAP12 protein (Uniprot Accession No. H2EZ3), and dog DAP12 protein (Uniprot Accession No. E2RP46); and induce at least one DAP12 activity. In some embodiments, the at least one DAP12 activity is DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, and/or reduced expression of one or more pro-inflammatory mediators.
[0108] In some embodiments, anti-DAP12 antibodies of the present disclosure bind to a DAP12 protein of the present disclosure and/or naturally occurring variants. In certain preferred embodiments, the anti-DAP12 antibodies bind to human DAP12.

[0109] In some embodiments, anti-DAP12 antibodies of the present disclosure bind to a DAP12 protein of the present disclosure expressed on the surface of a cell and induce at least one DAP12 activity of the present disclosure after binding to the surface expressed DAP12 protein.

[0110] In certain embodiments, anti-DAP12 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 22-40 of human DAP12 (SEQ ID NO: 2), or within amino acid residues on a DAP12 protein corresponding to amino acid residues 22-40 of SEQ ID NO: 2.

[0111] Additional anti-DAP12 antibodies, e.g., antibodies that specifically bind to a DAP12 protein of the present disclosure, may be identified, screened, and/or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Bispecific antibodies

[0112] Certain aspects of the present disclosure relate to bispecific antibodies that bind to both TREM2 and DAP12 proteins of the present disclosure. Methods of generating bispecific antibodies are well known in the art and described herein. In some embodiments, bispecific antibodies of the present disclosure bind to one or more amino acid residues of human TREM2 (SEQ ID NO: 1), or amino acid residues on a TREM2 protein corresponding to amino acid residues of SEQ ID NO: 1. In other embodiments, bispecific antibodies of the present disclosure also bind to one or more amino acid residues of human DAP12 (SEQ ID NO: 2), or amino acid residues on a DAP12 protein corresponding to amino acid residues of SEQ ID NO: 2.

DAP12 binding and phosphorylation

[0113] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce binding of TREM2 to DAP12. In other embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce DAP12 phosphorylation after binding to a TREM2 and/or DAP12 protein expressed in a cell. In other embodiments, TREM2-
mediated DAP12 phosphorylation is induced by one or more SRC family tyrosine kinases. Examples of Src family tyrosine kinases include, without limitation, Src, Yes, Fyn, Fgr, Lck, Hck, Blk, Lyn, and Frk.

[0114] DAP12 is variously referred to as TYRO protein tyrosine kinase-binding protein, TYROBP, KARAP, and PLOSL. DAP12 is a transmembrane signaling protein that contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. In certain embodiments, the anti-TREM2 and/or anti-DAP12 antibody may induce DAP12 phosphorylation in its ITAM motif. Any method known in the art for determining protein phosphorylation, such as DAP12 phosphorylation, may be used.

[0115] In some embodiments, DAP12 is phosphorylated by SRC family kinases, resulting in the recruitment and activation of the Syk kinase, ZAP70 kinase, or both, to DAP12. Thus, in certain embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may recruit Syk, ZAP70, or both to DAP12.

[0116] Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of DAP12 activity, DAP12 phosphorylation, or recruitment of Syk, ZAP70, or both to DAP12, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

**PI3K activation**

[0117] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce PI3K activation after binding to a TREM2 and/or DAP12 protein expressed in a cell.

[0118] PI3Ks are a family of related intracellular signal transducer kinases capable of phosphorylating the 3-position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). The PI3K family is divided into three different classes (Class I, Class II, and Class III) based on primary structure, regulation, and in vitro lipid substrate specificity.
Activated PI3K produces various 3-phosphorylated phosphoinositides, including without limitation, PtdIns3P, PtdIns(3,4)P2, PtdIns(3,5)P2, and PtdIns(3,4,5)P3. These 3-phosphorylated phosphoinositides function in a mechanism by which signaling proteins are recruited to various cellular membranes. These signaling proteins contain phosphoinositide-binding domains, including without limitation, PX domains, pleckstrin homology domains (PH domains), and FYVE domains. Any method known in the art for determining PI3K activation may be used.

Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of PI3K activity, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

**Increased expression of anti-inflammatory mediators**

In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure have an anti-inflammatory activity in the brain after binding to a TREM2 and/or DAP12 protein expressed on a cell surface. It has recently been reported that TREM2 has an anti-inflammatory role in the brain. In certain embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure increase the expression of anti-inflammatory mediators and/or reduce the expression of pro-inflammatory mediators after binding to a TREM2 and/or DAP12 protein expressed in a cell.

Inflammation is part of a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, and irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by an organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be classified as either acute inflammation or chronic inflammation. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory
response, involving the local vascular system, the immune system, and various cells within the injured tissue. Chronic inflammation is prolonged inflammation that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

[0123] As used herein, anti-inflammatory mediators are proteins involved either directly or indirectly (e.g., by way of an anti-inflammatory signaling pathway) in a mechanism that reduces, inhibits, or inactivates an inflammatory response. Any method known in the art for identifying and characterizing anti-inflammatory mediators may be used. Examples of anti-inflammatory mediators include, without limitation, IL-12p70, IL-6, and IL-10.

[0124] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may increase expression of anti-inflammatory mediators, such as IL-12p70, IL-6, and IL-10. In certain embodiments, increased expression of the anti-inflammatory mediators occurs in macrophages, dendritic cells, and/or microglial cells. Increased expression may include, without limitation, in increase in gene expression, an increase in transcriptional expression, or an increase in protein expression. Any method known in the art for determining gene, transcript (e.g., mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine anti-inflammatory mediator gene expression levels, RT-PCR may be used to determine the level of anti-inflammatory mediator transcription, and Western blot analysis may be used to determine anti-inflammatory mediator protein levels.

[0125] As used herein, an anti-inflammatory mediator may have increased expression if its expression in one or more cells of a subject treated with an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure is greater than the expression of the same anti-inflammatory mediator expressed in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In some embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase anti-inflammatory mediator expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to anti-inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In other embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure increases anti-inflammatory mediator expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to anti-inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody.

[0126] Without wishing to be bound by theory, it is believed that, in some embodiments, anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of one or more anti-inflammatory mediators, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

Reduced expression of pro-inflammatory mediators

[0127] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may decrease the expression of pro-inflammatory mediators after binding to a TREM2 and/or DAP12 protein expressed in a cell.

[0128] As used herein, pro-inflammatory mediators are proteins involved either directly or indirectly (e.g., by way of pro-inflammatory signaling pathways) in a mechanism that induces, activates, promotes, or otherwise increases an inflammatory response. Any method known in the art for identifying and characterizing pro-inflammatory mediators may be used. Examples of
pro-inflammatory mediators include, without limitation, IFN-a4, IFN-b, IL-6, IL-12 p70, IL-1β and TNF.

[0129] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may decrease functional expression and/or secretion of pro-inflammatory mediators, such as IFN-a4, IFN-b, IL-6, IL-12 p70, IL-1β and TNF. Decreased expression may include, without limitation, a decrease in gene expression, a decrease in transcriptional expression, or a decrease in protein expression. Any method known in the art for determining gene, transcript (e.g., mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine pro-inflammatory mediator gene expression levels, RT-PCR may be used to determine the level of pro-inflammatory mediator transcription, and Western blot analysis may be used to determine pro-inflammatory mediator protein levels.

[0130] As used herein, a pro-inflammatory mediator may have decreased expression if its expression in one or more cells of a subject treated with an agonist anti-TREM2 and/or anti-DAP12 antibody of the present disclosure is less than the expression of the same pro-inflammatory mediator expressed in one or more cells of a corresponding subject that is not treated with the agonist anti-TREM2 and/or anti-DAP12 antibody. In some embodiments, the agonist anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may decrease pro-inflammatory mediator expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to pro-inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the agonist anti-TREM2 and/or anti-DAP12 antibody. In other embodiments, the agonist anti-TREM2 and/or anti-DAP12 antibody may decrease pro-inflammatory mediator expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45
fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to pro-inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody.

[0131] Without wishing to be bound by theory, it is believed that some anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may be useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with increased levels of one or more pro-inflammatory mediators, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

**ERK phosphorylation**

[0132] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce extracellular signal-regulated kinase (ERK) phosphorylation after binding to a TREM2 and/or DAP12 protein expressed in a cell.

[0133] Extracellular-signal-regulated kinases (ERKs) are widely expressed protein kinase intracellular signaling kinases that are involved in, for example, the regulation of meiosis, mitosis, and postmitotic functions in differentiated cells. Various stimuli, such as growth factors, cytokines, virus infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents, and carcinogens, activate ERK pathways. Phosphorylation of ERKs leads to the activation of their kinase activity.

[0134] Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of ERK phosphorylation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.
Increased expression of C-C chemokine receptor 7

[0135] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may increase expression of C-C chemokine receptor 7 (CCR7) after binding to a TREM2 and/or DAP12 protein expressed in a cell. Increased expression may include, without limitation, an increase in gene expression, an increase in transcriptional expression, or an increase in protein expression. Any method known in the art for determining gene, transcript (e.g., mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine anti-inflammatory mediator gene expression levels, RT-PCR may be used to determine the level of anti-inflammatory mediator transcription, and Western blot analysis may be used to determine anti-inflammatory mediator protein levels.

[0136] C-C chemokine receptor 7 (CCR7) is a member of the G protein-coupled receptor family. CCR7 is expressed in various lymphoid tissues and can activate B-cells and T-cells. In some embodiments, CCR7 may modulate the migration of memory T-cells to secondary lymphoid organs, such as lymph nodes. In other embodiments, CCR7 may stimulate dendritic cell maturation. CCR7 is a receptor protein that can bind the chemokine (C-C motif) ligands CCL19/ELC and CCL21.

[0137] As used herein, CCR7 may have increased expression if its expression in one or more cells of a subject treated with an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure is greater than the expression of CCR7 expressed in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In some embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase CCR7 expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to CCR7 expression in one or more cells of a corresponding subject that is not treated with the anti-
TREM2 and/or anti-DAP12 antibody. In other embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure increases CCR7 expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to CCR7 expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody.

[0138] In some embodiments, increased expression of CCR7 occurs in macrophages, dendritic cells, and/or microglial cells. Increased expression of CCR7 may induce microglial cell chemotaxis toward cells expressing the chemokines CCL19 and CCL21. Accordingly, in certain embodiments, anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce microglial cell chemotaxis toward CCL19 and CCL21 expressing cells.

[0139] Without wishing to be bound by theory, it is believed that, in some embodiments, anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of CCR7, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

Reduction or normalization of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation

[0140] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may reduce and/or normalize the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation after binding to a TREM2 and/or DAP12 protein expressed in a cell.

[0141] In some embodiments, agonist anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may reduce and/or normalize the ability of bone marrow-derived dendritic
cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a corresponding subject that is not treated with the agonist anti-TREM2 and/or anti-DAP12 antibody. In other embodiments, the agonist anti-TREM2 and/or anti-DAP12 antibody may reduce and/or normalize the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a corresponding subject that is not treated with the agonist anti-TREM2 and/or anti-DAP12 antibody.

Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with an increased or disregulated ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.
Osteoclast production

[0143] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce osteoclast production and/or increase the rate of osteoclastogenesis after binding to a TREM2 and/or DAP12 protein expressed in a cell.

[0144] As used herein, an osteoclast is a type of bone cell that can remove bone tissue by removing its mineralized matrix and breaking up the organic bone (e.g., bone resorption). Osteoclasts can be formed by the fusion of cells of the monocyte-macrophage cell line. In some embodiments, osteoclasts may be characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K.

[0145] As used herein, the rate of osteoclastogenesis may be increased expression if the rate of osteoclastogenesis in a subject treated with an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure is greater than the rate of osteoclastogenesis in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In some embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase the rate of osteoclastogenesis in a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In other embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase the rate of osteoclastogenesis in a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold.
fold, for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody.

Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with a reduction in osteoclast production and/or the rate of osteoclastogenesis, including dementia, frontotemporal dementia, Alzheimer's disease, Nasu-Hakola disease, and/or multiple sclerosis.

Proliferation and survival of macrophages and microglial cells

In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may increase the proliferation, survival, and/or function of macrophages and microglial cells after binding to TREM2 and/or DAP12 protein expressed in a cell.

Microglial cells are a type of glial cell that are the resident macrophages of the brain and spinal cord, and thus act as the first and main form of active immune defense in the central nervous system (CNS). Microglial cells constitute 20% of the total glial cell population within the brain. Microglial cells are constantly scavenging the CNS for plaques, damaged neurons and infectious agents. The brain and spinal cord are considered "immune privileged" organs in that they are separated from the rest of the body by a series of endothelial cells known as the blood–brain barrier, which prevents most infections from reaching the vulnerable nervous tissue. In the case where infectious agents are directly introduced to the brain or cross the blood–brain barrier, microglial cells must react quickly to decrease inflammation and destroy the infectious agents before they damage the sensitive neural tissue. Due to the unavailability of antibodies from the rest of the body (few antibodies are small enough to cross the blood brain barrier), microglia must be able to recognize foreign bodies, swallow them, and act as antigen-presenting cells activating T-cells. Since this process must be done quickly to prevent potentially fatal damage, microglial cells are extremely sensitive to even small pathological changes in the CNS. They achieve this sensitivity in part by having unique potassium channels that respond to even small changes in extracellular potassium.
As used herein, the rate of proliferation, survival, and/or function of macrophages and/or microglia may include increased expression if the rate of proliferation, survival, and/or function of macrophages and/or microglia in a subject treated with an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure is greater than the rate of proliferation, survival, and/or function of macrophages and/or microglia in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In some embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase the rate of proliferation, survival, and/or function of macrophages and/or microglia in a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to the rate of proliferation, survival, and/or function of macrophages and/or microglia in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody. In other embodiments, an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may increase the rate of proliferation, survival, and/or function of macrophages and/or microglia in a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to the rate of proliferation, survival, and/or function of macrophages and/or microglia in a corresponding subject that is not treated with the anti-TREM2 and/or anti-DAP12 antibody.

Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with a reduction in proliferation, survival, and/or
function of macrophages and/or microglial cells, including dementia, frontotemporal dementia, Alzheimer's disease, Nasu-Hakola disease, and/or multiple sclerosis.

**Clearance and phagocytosis without inflammation**

[0151] In some embodiments, the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may induce clearance and/or phagocytosis without inflammation after binding to a TREM2 and/or DAP12 protein expressed in a cell of one or more of apoptotic neurons, nerve tissue debris of the nervous system, non-nerve tissue debris of the nervous system, bacteria, other foreign bodies, or disease-causing proteins. In certain embodiments, disease-causing protein include, without limitation, A beta peptide, alpha synuclein protein, Tau protein, TDP-43 protein, prion protein, and huntingtin protein.

[0152] Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with apoptotic neurons, nerve tissue debris of the nervous system, non-nerve tissue debris of the nervous system, bacteria, other foreign bodies, or disease-causing proteins, including dementia, frontotemporal dementia, Alzheimer's disease, Nasu-Hakola disease, and/or multiple sclerosis.

**Antibody preparation**

[0153] Anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure can encompass polyclonal antibodies, monoclonal antibodies, humanized and chimeric antibodies, human antibodies, antibody fragments (e.g., Fab, Fab'-SH, Fv, scFv, and F(ab')2), bispecific and polyspecific antibodies, multivalent antibodies, library derived antibodies, antibodies having modified effector functions, fusion proteins containing an antibody portion, and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site, such as an epitope having amino acid residues of a TREM2 and/or DAP12 protein of the present disclosure, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The anti-TREM2 and/or anti-DAP12 antibodies may be human, murine, rat, or of any other origin (including chimeric or humanized antibodies).
(1) Polyclonal antibodies

[0154] Polyclonal antibodies, such as anti-TREM2 and/or anti-DAP12 polyclonal antibodies, are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (e.g., a purified or recombinant TREM2 and/or DAP12 protein of the present disclosure) to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R¹N=C=NR, where R and R¹ are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund’s complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0155] The animals are immunized against the desired antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg (for rabbits) or 5 μg (for mice) of the protein or conjugate with 3 volumes of Freund’s complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund’s complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant-cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response.

(2) Monoclonal antibodies

[0156] Monoclonal antibodies, such as anti-TREM2 and/or anti-DAP12 monoclonal antibodies, are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (e.g., isomerizations, amidations)
that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies.

[0157] For example, the anti-TREM2 and/or anti-DAP12 monoclonal antibodies may be made using the hybridoma method first described by Köhler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0158] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinafter described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization (e.g., a purified or recombinant TREM2 and/or DAP12 protein of the present disclosure). Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

[0159] The immunizing agent will typically include the antigenic protein (e.g., a purified or recombinant TREM2 and/or DAP12 protein of the present disclosure) or a fusion variant thereof. Generally peripheral blood lymphocytes (“PBLs”) are used if cells of human origin are desired, while spleen or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice, Academic Press (1986), pp. 59-103.

[0160] Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine or human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which are substances that prevent the growth of HGPRT-deficient-cells.
Preferred immortalized myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors (available from the Salk Institute Cell Distribution Center, San Diego, California USA), as well as SP-2 cells and derivatives thereof (e.g., X63-Ag8-653) (available from the American Type Culture Collection, Manassas, Virginia USA). Human myeloma and mouse-human heteromyeloma cell lines have also been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen (e.g., a TREM2 and/or DAP12 protein of the present disclosure). Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen (e.g., a TREM2 and/or DAP12 protein of the present disclosure). Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the art. For example, binding affinity may be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as tumors in a mammal.
The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, affinity chromatography, and other methods as described above.

Anti-TREM2 and/or anti-DAP12 monoclonal antibodies may also be made by recombinant DNA methods, such as those disclosed in U.S. Patent No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that specifically bind to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host-cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, in order to synthesize monoclonal antibodies in such recombinant host-cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opin. Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Rev.* 130:151-188 (1992).

In certain embodiments, anti-TREM2 and/or anti-DAP12 antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990). Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991) described the isolation of murine and human antibodies, respectively, from phage libraries. Subsequent publications describe the production of high affinity (nanomolar (“nM”) range) human antibodies by chain shuffling (Marks et al., *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies of desired specificity (e.g., those that bind a TREM2 protein of the present disclosure).

The DNA encoding antibodies or fragments thereof may also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains
in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., Proc. Natl Acad. Sci. USA, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

The monoclonal antibodies described herein (e.g., anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure or fragments thereof) may be monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues may be substituted with another amino acid residues or are deleted so as to prevent crosslinking. In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

Chimeric or hybrid anti-TREM2 and/or anti-DAP12 antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopbutyrimidate.

(3) Humanized antibodies

Anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure or antibody fragments thereof may further include humanized or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fab, Fab'-SH, Fv, scFv, F(ab')2 or other antigen-binding subquences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody)
in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Jones et al., *Nature* 321: 522-525 (1986); Riechmann et al., *Nature* 332: 323-329 (1988) and Presta, *Curr. Opin. Struct. Biol.* 2: 593-596 (1992).

[0172] Methods for humanizing non-human anti-TREM2 and/or anti-DAP12 antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization can be essentially performed following the method of Winter and co-workers, Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988), or through substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0173] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the
entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies. Carter et al., *Proc. Nat’l Acad. Sci. USA* 89:4285 (1992); Presta et al., *J. Immunol.* 151:2623 (1993).

Furthermore, it is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analyzing the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen or antigens (e.g., TREM2 proteins of the present disclosure), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Various forms of the humanized anti-TREM2 and/or anti-DAP12 antibody are contemplated. For example, the humanized anti-TREM2 and/or anti-DAP12 antibody may be an antibody fragment, such as an Fab, which is optionally conjugated with one or more TREM2 ligand, such as HSP60. Alternatively, the humanized anti-TREM2 and/or anti-DAP12 antibody may be an intact antibody, such as an intact IgG1 antibody.
(4) Human antibodies

[0176] Alternatively, human anti-TREM2 and/or anti-DAP12 antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. The homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Nat’l Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immunol., 7:33 (1993); U.S. Patent Nos. 5,591,669 and WO 97/17852.

[0177] Alternatively, phage display technology can be used to produce human anti-TREM2 and/or anti-DAP12 antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. McCafferty et al., Nature 348:552-553 (1990); Hoogenboom and Winter, J. Mol. Biol. 227: 381 (1991). According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, e.g., Johnson, Kevin S. and Chiswell, David J., Curr. Opin Struct. Biol. 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). See also U.S. Patent. Nos. 5,565,332 and 5,573,905. Additionally, yeast

[0178] The techniques of Cole et al., and Boerner et al., are also available for the preparation of human anti-TREM2 and/or anti-DAP12 monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., \textit{J. Immunol.} 147(1): 86-95 (1991). Similarly, human anti-TREM2 and/or anti-DAP12 antibodies can be made by introducing human immunoglobulin loci into transgenic animals, \textit{e.g.}, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126, 5,633,425; 5,661,016 and in the following scientific publications: Marks et al., \textit{BioTechnology} 10: 779-783 (1992); Lonberg et al., \textit{Nature} 368: 856-859 (1994); Morrison, \textit{Nature} 368: 812-13 (1994), Fishwild et al., \textit{Nature Biotechnology} 14: 845-51 (1996), Neuberger, \textit{Nature Biotechnology} 14: 826 (1996) and Lonberg and Huszar, \textit{Intern. Rev. Immunol}. 13: 65-93 (1995).

[0179] Finally, human anti-TREM2 and/or anti-DAP12 antibodies may also be generated in \textit{vitro} by activated B-cells (see U.S. Patent Nos. 5,567,610 and 5,229,275).

\textit{(5) Antibody fragments}

[0180] In certain embodiments there are advantages to using anti-TREM2 and/or anti-DAP12 antibody fragments, rather than whole anti-TREM2 and/or anti-DAP12 antibodies. In some embodiments, smaller fragment sizes allow for rapid clearance and better brain penetration.
[0181] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *J. Biochem. Biophys. Method.*, 24:107-117 (1992); and Brennan et al., *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host-cells, for example, using nucleic acids encoding anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the straightforward production of large amounts of these fragments. Anti-TREM2 and/or anti-DAP12 antibody fragments can also be isolated from the antibody phage libraries as discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')$_2$ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')$_2$ fragments can be isolated directly from recombinant host-cell culture. Production of Fab and F(ab')$_2$ antibody fragments with increased *in vivo* half-lives are described in U.S. Patent No. 5,869,046. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894 and U.S. Patent No. 5,587,458. The anti-TREM2 and/or anti-DAP12 antibody fragment may also be a “linear antibody,” e.g., as described in U.S. Patent 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

(6) Bispecific and polyspecific antibodies

[0182] Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes, including those on the same or another protein (e.g., one or more TREM2 proteins of the present disclosure). Alternatively, one part of a BsAb can be armed to bind to the target TREM2 and/or DAP12 antigen, and another can be combined with an arm that binds to a second protein. Such antibodies can be derived from full length antibodies or antibody fragments (e.g., F(ab')$_2$ bispecific antibodies).

[0183] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light chain pairs, where the two chains have different specificities. Millstein et al., *Nature*, 305:537-539 (1983). Because of the random assortment of
immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

[0184] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0185] In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only half of the bispecific molecules provides for an easy way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies, see, for example, Suresh et al., *Methods in Enzymology* 121: 210 (1986).

[0186] According to another approach described in WO 96/27011 or U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the
percentage of heterodimers which are recovered from recombinant-cell culture. The preferred interface comprises at least a part of the C_{H3} region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chains(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0187] Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab’)_{2} fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab’ fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab’-TNB derivatives is then reconverted to the Fab’-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0188] Fab’ fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175: 217-225 (1992) describes the production of fully humanized bispecific antibody F(ab’)_{2} molecules. Each Fab’ fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T-cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0189] Various techniques for making and isolating bivalent antibody fragments directly from recombinant-cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab’

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portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The “diabody” technology described by Hollinger et al., *Proc. Nat’l Acad. Sci. USA*, 90: 6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (V\textsubscript{H}) connected to a light-chain variable domain (V\textsubscript{L}) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V\textsubscript{H} and V\textsubscript{L} domains of one fragment are forced to pair with the complementary V\textsubscript{L} and V\textsubscript{H} domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).

**[0190]** Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

**[0191]** Exemplary bispecific antibodies may bind to two different epitopes on a given molecule (*e.g.*, a TREM2 and/or DAP12 protein of the present disclosure). In some embodiments a bispecific antibody binds to a first antigen, such as a TREM2 or DAP12 protein of the present disclosure, and a second antigen facilitating transport across the blood-brain barrier. Numerous antigens are known in the art that facilitate transport across the blood-brain barrier (*see, e.g.*, Gabathuler R., Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases, Neurobiol. Dis. 37 (2010) 48-57). Such second antigens include, without limitation, transferrin receptor (TR), insulin receptor (HIR), Insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, including CRM197 (a non-toxic mutant of diphtheria toxin), llama single domain antibodies such as TMEM 30(A) (Flippase), protein transduction domains such as TAT, Syn-B, or penetratin, poly-arginine or generally positively charged peptides, Angiopep peptides such as ANG1005 (*see, e.g.*, Gabathuler, 2010), and other cell surface proteins that are enriched on blood-brain barrier endothelial cells (*see, e.g.*, Daneman et al., PLoS One. 2010 Oct 29;5(10):e13741). In some embodiments, second antigens for an anti-TREM2 antibody may include, without limitation, a DAP12 antigen of the present disclosure. In other embodiments, second antigens for an anti-DAP12 antibody may include, without limitation, a TREM2 antigen.
of the present disclosure. In other embodiments, bispecific antibodies that bind to both TREM2 and DAP12 may facilitate and enhance one or more TREM2 and/or DAP12 activities.

(7) Multivalent antibodies

[0192] A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure or antibody fragments thereof can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g., tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein contains three to about eight, but preferably four, antigen binding sites. The multivalent antibody contains at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain or chains comprise two or more variable domains. For instance, the polypeptide chain or chains may comprise VD1-(X1)n-VD2-(X2)n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. Similarly, the polypeptide chain or chains may comprise V_{H\_1}-C_{H\_1}-flexible linker-V_{H\_2}-C_{H\_2}-Fc region chain; or V_{H\_1}-C_{H\_1}-V_{H\_2}-C_{H\_2}-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contempleted here comprise a light chain variable domain and, optionally, further comprise a CL domain.

(8) Effector function engineering

[0193] It may also be desirable to modify an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure to modify effector function and/or to increase serum half-life of the antibody. For example, the Fc receptor binding site on the constant region may be modified or mutated to
remove or reduce binding affinity to certain Fc receptors, such as FcγRI, FcγRII, and/or FcγRIII. In some embodiments, the effector function is impaired by removing N-glycosylation of the Fc region (e.g., in the CH2 domain of IgG) of the antibody. In some embodiments, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., Molecular Immunology 40: 585-593 (2003); Reddy et al., J. Immunology 164:1925-1933 (2000).

[0194] To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term “salvage receptor binding epitope” refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

(9) Other amino acid sequence modifications

[0195] Amino acid sequence modifications of anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, or antibody fragments thereof, are also contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antibody fragments. Amino acid sequence variants of the antibodies or antibody fragments are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding the antibodies or antibody fragments, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics (i.e., the ability to bind or physically interact with a TREM2 and/or DAP12 protein of the present disclosure). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0196] A useful method for identification of certain residues or regions of the anti-TREM2 and/or anti-DAP12 antibody that are preferred locations for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells in Science, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as
arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the target antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0197] Amino acid sequence insertions include amino- ("N") and/or carboxy- ("C") terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0198] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the Table A below under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

**TABLE A: Amino Acid Substitutions**

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>val; leu; ile</td>
<td>val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>lys; gln; asn</td>
<td>lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>gln; his; asp, lys; arg</td>
<td>gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>glu; asn</td>
<td>glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>ser; ala</td>
<td>ser</td>
</tr>
<tr>
<td>Original Residue</td>
<td>Exemplary Substitutions</td>
<td>Preferred Substitutions</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>asn; glu</td>
<td>asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>asp; gln</td>
<td>asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>ala</td>
<td>ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>asn; gln; lys; arg</td>
<td>arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>leu; val; met; ala; phe; norleucine</td>
<td>leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>norleucine; ile; val; met; ala; phe</td>
<td>ile</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>arg; gln; asn</td>
<td>arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>leu; phe; ile</td>
<td>leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>leu; val; ile; ala; tyr</td>
<td>tyr</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>ala</td>
<td>ala</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>thr</td>
<td>thr</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Ser</td>
<td>ser</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>tyr; phe</td>
<td>tyr</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>trp; phe; thr; ser</td>
<td>phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>ile; leu; met; phe; ala; norleucine</td>
<td>leu</td>
</tr>
</tbody>
</table>

[0199] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

1. hydrophobic: norleucine, met, ala, val, leu, ile;
2. neutral hydrophilic: cys, ser, thr;
3. acidic: asp, glu;
4. basic: asn, gln, his, lys, arg;
5. residues that influence chain orientation: gly, pro; and
6. aromatic: trp, tyr, phe.

[0200] Non-conservative substitutions entail exchanging a member of one of these classes for another class.

[0201] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the
molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment, such as an Fv fragment).

[0202] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human anti-TREM2 and/or anti-DAP12 antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen (e.g., a TREM2 protein of the present disclosure). Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0203] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0204] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate
moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0205] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0206] Nucleic acid molecules encoding amino acid sequence variants of the anti-IgE antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibodies (e.g., anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure) or antibody fragments.

(10) Other antibody modifications

[0207] Anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, or antibody fragments thereof, can be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidione, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol,
and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc. Such techniques and other suitable formulations are disclosed in *Remington: The Science and Practice of Pharmacy*, 20th Ed., Alfonso Gennaro, Ed., Philadelphia College of Pharmacy and Science (2000).

**Nucleic acids, vectors, and host cells**

[0208] Anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acids having a nucleotide sequence encoding any of the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are provided. Such nucleic acids may encode an amino acid sequence containing the VL and/or an amino acid sequence containing the VH of the anti-TREM2 and/or anti-DAP12 antibody (*e.g.*, the light and/or heavy chains of the antibody). In some embodiments, one or more vectors (*e.g.*, expression vectors) containing such nucleic acids are provided. In some embodiments, a host cell containing such nucleic acid is also provided. In some embodiments, the host cell contains (*e.g.*, has been transduced with): (1) a vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and an amino acid sequence containing the VH of the antibody, or (2) a first vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and a second vector containing a nucleic acid that encodes an amino acid sequence containing the VH of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell).
[0209] Methods of making an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure are provided. In some embodiments, the method includes culturing a host cell of the present disclosure containing a nucleic acid encoding the anti-TREM2 and/or anti-DAP12 antibody, under conditions suitable for expression of the antibody. In some embodiments, the antibody is subsequently recovered from the host cell (or host cell culture medium).

[0210] For recombinant production of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, a nucleic acid encoding the anti-TREM2 and/or anti-DAP12 antibody is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0211] Suitable vectors containing a nucleic acid sequence encoding any of the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, or fragments thereof polypeptides (including antibodies) described herein include, without limitation, cloning vectors and expression vectors. Suitable cloning vectors can be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mpl8, mpl9, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

[0212] Expression vectors generally are replicable polynucleotide constructs that contain a nucleic acid of the present disclosure. The expression vector may replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated
viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression \textit{(i.e., translation)}, one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

\[0213\] The vectors containing the nucleic acids of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection \textit{(e.g., where the vector is an infectious agent such as vaccinia virus)}. The choice of introducing vectors or polynucleotides will often depend on features of the host cell. In some embodiments, the vector contains a nucleic acid containing one or more amino acid sequences encoding an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure.

\[0214\] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria \textit{(e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523; and Charlton, \textit{Methods in Molecular Biology}, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in \textit{E. coli}.). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

\[0215\] In addition to prokaryotes, eukaryotic microorganisms, such as filamentous fungi or yeast, are also suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern \textit{(e.g., Gerngross, \textit{Nat. Biotech}. 22:1409-1414 (2004); and Li et al., \textit{Nat. Biotech}. 24:210-215 (2006))}. 
[0216] Suitable host cells for the expression of glycosylated antibody can also be derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts (e.g., U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429, describing PLANTIBODIES™ technology for producing antibodies in transgenic plants.).

[0217] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

**Pharmaceutical compositions**

[0218] Anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure can be incorporated into a variety of formulations for therapeutic administration by combining the antibodies with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms. Examples of such formulations include, without limitation, tablets, capsules, powders, granules, ointments,
solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Pharmaceutical compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers of diluents, which are vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents include, without limitation, distilled water, buffered water, physiological saline, PBS, Ringer’s solution, dextrose solution, and Hank’s solution. A pharmaceutical composition or formulation of the present disclosure can further include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

[0219] A pharmaceutical composition of the present disclosure can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well-known compounds that enhance the in vivo stability of the polypeptide, or otherwise enhance its pharmacological properties (e.g., increase the half-life of the polypeptide, reduce its toxicity, and enhance solubility or uptake). Examples of such modifications or complexing agents include, without limitation, sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their in vivo attributes. Such molecules include, without limitation, carbohydrates, polyamines, amino acids, other peptides, ions (e.g., sodium, potassium, calcium, magnesium, manganese), and lipids.


[0221] For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with
inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0222] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0223] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (e.g., at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions intended for in vivo use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under GMP conditions.

[0224] Formulations may be optimized for retention and stabilization in the brain or central nervous system. When the agent is administered into the cranial compartment, it is desirable for the agent to be retained in the compartment, and not to diffuse or otherwise cross the blood brain barrier. Stabilization techniques include cross-linking, multimerizing, or linking to groups such
as polyethylene glycol, polyacrylamide, neutral protein carriers, *etc.* in order to achieve an increase in molecular weight.

[0225] Other strategies for increasing retention include the entrapment of the antibody, such as an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure, in a biodegradable or bioerodible implant. The rate of release of the therapeutically active agent is controlled by the rate of transport through the polymeric matrix, and the biodegradation of the implant. The transport of drug through the polymer barrier will also be affected by compound solubility, polymer hydrophilicity, extent of polymer cross-linking, expansion of the polymer upon water absorption so as to make the polymer barrier more permeable to the drug, geometry of the implant, and the like. The implants are of dimensions commensurate with the size and shape of the region selected as the site of implantation. Implants may be particles, sheets, patches, plaques, fibers, microcapsules and the like and may be of any size or shape compatible with the selected site of insertion.

[0226] The implants may be monolithic, *i.e.* having the active agent homogenously distributed through the polymeric matrix, or encapsulated, where a reservoir of active agent is encapsulated by the polymeric matrix. The selection of the polymeric composition to be employed will vary with the site of administration, the desired period of treatment, patient tolerance, the nature of the disease to be treated and the like. Characteristics of the polymers will include biodegradability at the site of implantation, compatibility with the agent of interest, ease of encapsulation, a half-life in the physiological environment.

[0227] Biodegradable polymeric compositions which may be employed may be organic esters or ethers, which when degraded result in physiologically acceptable degradation products, including the monomers. Anhydrides, amides, orthoesters or the like, by themselves or in combination with other monomers, may find use. The polymers will be condensation polymers. The polymers may be cross-linked or non-cross-linked. Of particular interest are polymers of hydroxylaliphatic carboxylic acids, either homo- or copolymers, and polysaccharides. Included among the polyesters of interest are polymers of D-lactic acid, L-lactic acid, racemic lactic acid, glycolic acid, polycaprolactone, and combinations thereof. By employing the L-lactate or D-
lactate, a slowly biodegrading polymer is achieved, while degradation is substantially enhanced with the racemate. Copolymers of glycolic and lactic acid are of particular interest, where the rate of biodegradation is controlled by the ratio of glycolic to lactic acid. The most rapidly degraded copolymer has roughly equal amounts of glycolic and lactic acid, where either homopolymer is more resistant to degradation. The ratio of glycolic acid to lactic acid will also affect the brittleness of in the implant, where a more flexible implant is desirable for larger geometries. Among the polysaccharides of interest are calcium alginate, and functionalized celluloses, particularly carboxymethylcellulose esters characterized by being water insoluble, a molecular weight of about 5 kD to 500 kD, etc. Biodegradable hydrogels may also be employed in the implants of the subject invention. Hydrogels are typically a copolymer material, characterized by the ability to imbibe a liquid. Exemplary biodegradable hydrogels which may be employed are described in Heller in: Hydrogels in Medicine and Pharmacy, N. A. Peppes ed., Vol. III, CRC Press, Boca Raton, Fla., 1987, pp 137-149.

**Pharmaceutical dosages**

[0228] Pharmaceutical compositions of the present disclosure containing an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure may be administered to an individual in need of treatment with the anti-TREM2 and/or anti-DAP12 antibody, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, intracranial, intraspinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0229] Dosages and desired drug concentration of pharmaceutical compositions of the present disclosure may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles described in Mordenti, J. and Chappell, W. “The Use of Interspecies Scaling in Toxicokinetics,”

[0230] For *in vivo* administration of any of the anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of an individual’s body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. For repeated administrations over several days or longer, depending on the severity of the disease, disorder, or condition to be treated, the treatment is sustained until a desired suppression of symptoms is achieved.

[0231] An exemplary dosing regimen may include administering an initial dose of an anti-TREM2 and/or anti-DAP12 antibody, of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg every other week. Other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the physician wishes to achieve. For example, dosing an individual from one to twenty-one times a week is contemplated herein. In certain embodiments, dosing ranging from about 3 μg/kg to about 2 mg/kg (such as about 3 μg/kg, about 10 μg/kg, about 30 μg/kg, about 100 μg/kg, about 300 μg/kg, about 1 mg/kg, and about 2/mg/kg) may be used. In certain embodiments, dosing frequency is three times per day, twice per day, once per day, once every other day, once weekly, once every two weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or once monthly, once every two months, once every three months, or longer. Progress of the therapy is easily monitored by conventional techniques and assays. The dosing regimen, including the anti-TREM2 and/or anti-DAP12 antibody administered, can vary over time independently of the dose used.

[0232] Dosages for a particular anti-TREM2 and/or anti-DAP12 antibody may be determined empirically in individuals who have been given one or more administrations of the anti-TREM2 and/or anti-DAP12 antibody. Individuals are given incremental doses of an anti-TREM2 and/or anti-DAP12 antibody. To assess efficacy of an anti-TREM2 and/or anti-DAP12 antibody, a clinical symptom of any of the diseases, disorders, or conditions of the present disclosure (e.g.,
dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and multiple sclerosis) can be monitored.

[0233] Administration of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can be continuous or intermittent, depending, for example, on the recipient’s physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an anti-TREM2 and/or anti-DAP12 antibody may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

[0234] Guidance regarding particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Therapeutic uses

[0235] Further aspects of the present disclosure provide methods for activating DAP12, activating PI3K, increasing expression of one or more anti-inflammatory mediators (e.g., IL-12p70, IL-6, and IL-10), or reducing expression of one or more pro-inflammatory mediators (e.g., L-1β and TNF) in an individual in need thereof, by administering to the individual a therapeutically effective amount of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure to induce one or more TREM2 and/or DAP12 activities in the individual.

[0236] As disclosed herein, anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure may be used for preventing, reducing risk, or treating dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis. In some
embodiments, the present disclosure provides methods of preventing, reducing risk, or treating an individual having dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis, by administering to the individual a therapeutically effective amount of an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure to induce one or more TREM2 activities, including without limitation, DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators (e.g., IL-12p70, IL-6, and IL-10), or reduced expression of one or more pro-inflammatory mediators (e.g., L-1β and TNF). In certain embodiments, the individual has a heterozygous TREM2 variant allele having an arginine to histidine amino acid substitution at amino acid residue 47 of the human TREM2 protein (SEQ ID NO: 1).

[0237] In some embodiments, the methods of the present disclosure may involve the coadministration of anti-TREM2 and/or anti-DAP12 antibodies, or bispecific antibodies that bind to both TREM2 and DAP12, with TLR antagonists or with agents neutralizing TLR agonist (e.g., neutralizing cytokine or interleukin antibodies).

[0238] In some embodiments, the methods of the present disclosure may involve the administration of chimeric constructs, including an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure in conjunction with a TREM2 ligand, such as HSP60.

Dementia

[0239] Dementia is a non-specific syndrome (i.e., a set of signs and symptoms) that presents as a serious loss of global cognitive ability in a previously unimpaired person, beyond what might be expected from normal ageing. Dementia may be static as the result of a unique global brain injury. Alternatively, dementia may be progressive, resulting in long-term decline due to damage or disease in the body. While dementia is much more common in the geriatric population, it can also occur before the age of 65. Cognitive areas affected by dementia include, without limitation, memory, attention span, language, and problem solving. Generally, symptoms must be present for at least six months to before an individual is diagnosed with dementia.
Exemplary forms of dementia include, without limitation, frontotemporal dementia, Alzheimer's disease, vascular dementia, semantic dementia, and dementia with Lewy bodies.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can prevent, reduce the risk, and/or treat dementia. In some embodiments, administering an anti-TREM2 and/or anti-DAP12 antibody may induce one or more TREM2 and/or DAP12 activities in an individual having dementia (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Frontotemporal dementia

Frontotemporal dementia (FTD) is a condition resulting from the progressive deterioration of the frontal lobe of the brain. Over time, the degeneration may advance to the temporal lobe. Second only to Alzheimer's disease (AD) in prevalence, FTD accounts for 20% of pre-senile dementia cases. The clinical features of FTD include memory deficits, behavioral abnormalities, personality changes, and language impairments (Cruts, M. & Van Broeckhoven, C., Trends Genet. 24:186-194 (2008); Neary, D., et al., Neurology 51:1546-1554 (1998); Ratnavalli, E., Brayne, C., Dawson, K. & Hodges, J. R., Neurology 58:1615-1621 (2002)).

A substantial portion of FTD cases are inherited in an autosomal dominant fashion, but even in one family, symptoms can span a spectrum from FTD with behavioral disturbances, to Primary Progressive Aphasia, to Cortico-Basal Ganglionic Degeneration. FTD, like most neurodegenerative diseases, can be characterized by the pathological presence of specific protein aggregates in the diseased brain. Historically, the first descriptions of FTD recognized the presence of intraneuronal accumulations of hyperphosphorylated Tau protein in neurofibrillary tangles or Pick bodies. A causal role for the microtubule associated protein Tau was supported by the identification of mutations in the gene encoding the Tau protein in several families (Hutton, M., et al., Nature 393:702-705 (1998). However, the majority of FTD brains show no accumulation of hyperphosphorylated Tau but do exhibit immunoreactivity to ubiquitin (Ub) and TAR DNA binding protein (TDP43) (Neumann, M., et al., Arch. Neurol. 64:1388-1394 (2007)).
A majority of those FTD cases with Ub inclusions (FTD-U) were shown to carry mutations in the progranulin gene.

[0244] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can prevent, reduce the risk, and/or treat FTD. In some embodiments, administering an anti-TREM2 and/or anti-DAP12 antibody may induce one or more TREM2 and/or DAP12 activities in an individual having FTD (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Alzheimer's disease

[0245] Alzheimer’s disease (AD) is the most common form of dementia. There is no cure for the disease, which worsens as it progresses, and eventually leads to death. Most often, AD is diagnosed in people over 65 years of age. However, the less-prevalent early-onset Alzheimer's can occur much earlier.

[0246] Common symptoms of Alzheimer’s disease include, behavioral symptoms, such as difficulty in remembering recent events; cognitive symptoms, confusion, irritability and aggression, mood swings, trouble with language, and long-term memory loss. As the disease progresses bodily functions are lost, ultimately leading to death. Alzheimer’s disease develops for an unknown and variable amount of time before becoming fully apparent, and it can progress undiagnosed for years.

[0247] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can prevent, reduce the risk, and/or treat Alzheimer’s disease. In some embodiments, administering an anti-TREM2 and/or anti-DAP12 antibody may induce one or more TREM2 and/or DAP12 activates in an individual having Alzheimer’s disease (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).
Nasu-Hakola disease

Nasu-Hakola disease (NHD), which may alternatively be referred to as polycystic lipomembranous osteodysplasia with sclerosing leukencephalopathy (PLOSL), is a rare inherited leukodystrophy characterized by progressive presenile dementia associated with recurrent bone fractures due to polycystic osseous lesions of the lower and upper extremities. NHD disease course is generally divided into four stages: latent, osseous, early neurologic, and late neurologic. After a normal development during childhood (latent stage), NHD starts manifesting during adolescence or young adulthood (typical age of onset 20-30 years) with pain in the hands, wrists, ankles, and feet. Patients then start suffering from recurrent bone fractures due to polycystic osseous and osteoporotic lesions in the limb bones (osseous stage). During the third or fourth decade of life (early neurologic stage), patients present with pronounced personality changes (e.g., euphoria, lack of concentration, loss of judgment, and social inhibitions) characteristic of a frontal lobe syndrome. Patients also typically suffer from progressive memory disturbances. Epileptic seizures are also frequently observed. Finally (late neurologic stage), patients progress to a profound dementia, are unable to speak and move, and usually die by the age of 50.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can prevent, reduce the risk, and/or treat Nasu-Hakola disease (NHD). In some embodiments, administering an anti-TREM2 and/or anti-DAP12 antibody may induce one or more TREM2 and/or DAP12 activates in an individual having NHD (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Multiple sclerosis

Multiple sclerosis (MS) can also be referred to as disseminated sclerosis or encephalomyelitis disseminata. MS is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms. MS affects the ability of nerve cells in the
brain and spinal cord to communicate with each other effectively. Nerve cells communicate by sending electrical signals called action potentials down long fibers called axons, which are contained within an insulating substance called myelin. In MS, the body’s own immune system attacks and damages the myelin. When myelin is lost, the axons can no longer effectively conduct signals. MS onset usually occurs in young adults, and is more common in women.

[0251] Symptoms of MS include, without limitation, changes in sensation, such as loss of sensitivity or tingling; pricking or numbness, such as hypoesthesia and paresthesia; muscle weakness; clonus; muscle spasms; difficulty in moving; difficulties with coordination and balance, such as ataxia; problems in speech, such as dysarthria, or in swallowing, such as dysphagia; visual problems, such as nystagmus, optic neuritis including phosphenes, and diplopia; fatigue; acute or chronic pain; and bladder and bowel difficulties; cognitive impairment of varying degrees; emotional symptoms of depression or unstable mood; Uhthoff’s phenomenon, which is an exacerbation of extant symptoms due to an exposure to higher than usual ambient temperatures; and Lhermitte’s sign, which is an electrical sensation that runs down the back when bending the neck.

[0252] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 and/or anti-DAP12 antibody of the present disclosure can prevent, reduce the risk, and/or treat multiple sclerosis. In some embodiments, administering an anti-TREM2 and/or anti-DAP12 antibody may induce one or more TREM2 and/or DAP12 activates in an individual having multiple sclerosis (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, and reduced expression of one or more pro-inflammatory mediators).

[0253] The invention will be more fully understood by reference to the following Examples. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.
EXEMPLARYs

Example 1: Production, Identification, and Characterization of agonist anti-TREM2 and anti-DAP12 antibodies

[0254] The amino acid sequence of human TREM2 is set forth below in SEQ ID NO: 1. Human TREM2 contains an extracellular immunoglobulin-like variable-type (IgV) domain located at amino residues 29-112 of SEQ ID NO: 1; additional extracellular sequences located at amino residues 113-174 of SEQ ID NO: 1; a transmembrane domain located at amino residues 175-195 of SEQ ID NO: 1; and an intracellular domain located at amino residues 196-230 of SEQ ID NO: 1.

[0255] TREM2 amino acid sequence (SEQ ID NO: 1):

```
10  20  30  40  50  60
MEPLLILLFVTLSGAGNVTVFQSAGVQQLQSCFPYESMKSSGRRKANCRQLGKGP
70  80  90 100 110 120
QRVSTHNLLLSLRRWNGSTATDDILGGLTITLRLNQPHDAGLYQCQSLHSEADTT
130 140 150 160 170 180
LRKVLVEVLAQPLHRDAGDLWFGESSESFEADAHVHSISRSLEGIEIPFPPTISRLLLA
190 200 210 220 230
CIFLIKILAAALWAAAWHDKHGTHPPSELDGCHDGFYQQLTLPGLRDT
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[0256] A known feature of human TREM2 is that the transmembrane domain contains a lysine (aa186) that can interact with an aspartic acid in DAP12, a key adaptor protein that transduces signaling from TREM2, TREM1, and other related IgV family members.

[0257] A BLAST analysis of human TREM2 identified 18 related homologues. These homologues included the Natural Killer (NK) cell receptor NK-p44 (NCTR2), the polymeric immunoglobulin receptor (plgR), CD300E, CD300A, CD300C, and TREML1/TLT1. The closest homologue was identified as NCTR2, having similarity with TREM2 within the IgV domain (Fig. 1A). A BLAST analysis also compared TREM proteins with other IgV family proteins (Fig. 1B).
[0258] TREM2 is also closely related to TREM1. An alignment of the amino acid sequences of TREM1 and TREM2 was generated by 2-way blast (Fig. 2). This is limited to the IgV domain as well.

[0259] Agonistic antibodies for TREM1, NK-p44, and other members of this family have been previously described. Without wishing to be bound by theory, it is believed that agonist antibodies specific for TREM2 can be produced using similar techniques. Antibodies that bind the extracellular domain of TREM2, particularly the IgV domain (amino acid residues 29-112 of SEQ ID NO: 1) will be generated using mouse hybridoma technology, phage display technology, and yeast display technology. Antibodies are then screened for their ability to activate TREM2 signaling and functions in cells and in a whole animal in vivo as described in Examples 2-22 below.

[0260] For example, agonist anti-TREM2 antibodies can be produced that target the IgV domain (amino acid residues 29-112). IgV domains bind to targets, and through multimerization of receptors, such as IgG itself or NKp44, lead to activation. Thus these domains are rational targets for agonistic antibodies. They are also highly divergent.

[0261] Agonist anti-TREM2 antibodies can also be produced that target amino acid residues 99-115 of human TREM2. Without wishing to be bound by theory it is believed that amino acid residues 99-115 correspond to a peptide that block binding of TREM2 to its endogenous target, as the corresponding peptide in mouse TREM1 (amino acid residues 83-99) block binding of TREM1 to its endogenous target (Gibot et al., Infect. Immunity 2004). The mouse TREM1 peptide is called LP17 (LQVTDSGLYRCV1YHPP). The equivalent region in human TREM2 is located within the CD3 domain and is located at amino acid residues 99-115 of SEQ ID NO: 1 (LQPHDAGLYQCQSLHG).

[0262] Another approach for predicting a relevant (e.g., agonistic) site within the human TREM2 protein is by targeting the sites where the mutations are found in Alzheimer’s disease (e.g., R47H and D87N), Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), or Nasu–Hakola disease. Also relevant is the site of the major mutations associated with human disease, which are generally found within the IgV domain.
The crystal structures of the TREM2-related structures of TREM1 (Kelker, MS et al., J Mol Biol, 2004. 344(5): p. 1175-81; Kelker, MS et al., J Mol Biol, 2004. 342(4): p. 1237-48; and Radaev, S et al., Structure, 2003. 11(12): p. 1527-35), TLT1 (Gattis, JL et al., J Biol Chem, 2006. 281(19): p. 13396-403), and NKp44 have been described, and thus structural regions/features are identified within the IgV domain that are particularly likely to play a central role in interacting with natural agonists. These studies support the belief that the complementary determining regions (CDR1, CDR2, CDR3) play a major role in ligand binding. TREM1 has been reported to be either monomeric (Gattis, JL et al., J Biol Chem, 2006. 281(19): p. 13396-403) or dimeric (Radaev, S et al., Structure, 2003. 11(12): p. 1527-35) in vitro under cell-free conditions, but its oligomeric state in vivo remains unclear, as well as that of TREM2.

The amino acid sequence of human DAP12 is set forth below as SEQ ID NO: 2:

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MGGLEPCSRL LLLPLLLAVS GLRPVQAQQ SDSCSCSTVSP GVLAGIVMDL LVLTVLIALA
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DAP12 is a single-pass type I membrane protein. It contains an extracellular domain located at amino acid residues 22-40 of human DAP12 (SEQ ID NO: 2); a transmembrane domain located at amino acid residues 41-61 of human DAP12 (SEQ ID NO: 2); and an intracellular domain located at amino acid residues 62-113 of human DAP12 (SEQ ID NO: 2). The immunoreceptor tyrosine-based activation motif (ITAM) domain of DAP12 is located at amino acid residues 80-118 of human DAP12 (SEQ ID NO: 2). An aspartic acid in DAP12 interacts with the transmembrane domain of human TREM2 containing a lysine at amino acid residue 186, and transduces signaling from TREM2, TREM1, and other related IgV family member proteins.

Agonist anti-DAP12 antibodies can be produced that target amino acid residues 22-40 of the human DAP12. Without wishing to be bound by theory, it is believed that DAP12 is a disulfide-bonded dimer, associating with TREM2, and that dimerizing DAP12 with an antibody
against the extra cellular domain encompassing amino acid residues 22-40 will activate one or more TREM2 and/or DAP12 activities.

Example 2: Normalization and reduction of Toll-like receptor (TLR) responses in dendritic cells by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0267] Bone marrow-derived dendritic cells (BMDC) are stimulated by culturing with TLR ligands, such as LPS, CpG DNA, and zymosan, for 16 h. Conditioned media is collected and ELISA assays are performed in order to evaluate secretion of the cytokines IFN-a4, IFN-b, IL-6, IL-12 p70, and TNF. Without wishing to be bound by theory, it is believed that BMDC cells that do not have active TREM2 will secrete significantly more IL-12, p70, and TNF than BMDC cells that have activated TREM2 after stimulation. It is further believed that anti-TREM2 agonistic antibodies will reduce the expression levels of IL-12, p70, and TNF. Bone marrow-derived dendritic cells from wild-type and from TREM2-heterozyous mice, which would have partially inactive TREM2, will serve as positive controls for determining expression levels of the cytokines IL-12, p70, and TNF, as well as their modulation by agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies.

[0268] Cytokine concentrations in the culture supernatants are determined using mouse IFN-a4, IFN-b, IL-6, IL-12 p70, TNF, and IL-10 ELISA kits (eBioscience) and VeriKine Mouse IFN-b ELISA kit (PBL interferon source) according to manufacturer’s protocol. Levels of mRNA for these cytokines are also measured by Quantitative RT-PCR (qRT-PCR). Total RNA is prepared by using RNeasy plus mini kit (QIAGEN) is reverse-transcribed with Superscript III Reverse Transcriptase (Invitrogen) using oligo dT primer according to manufacturer’s protocol. Quantitative PCR is performed using the Power SYBR Green PCR Master Mix (Applied Biosystems) and 7900HT (Applied Biosystems) according to manufacturer’s protocol. The sequences of IFN-a4, IFN-b, IL-6, IL-12 p70, and TNF primers are as described. (e.g., Hamerman, JA, Eur. J. Immunol. 2012. 42: 176–185).
Example 3: Normalization and reduction of the ability of BMDCs to induce antigen-specific T-cell proliferation by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0269] Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies will reduce and normalize the ability of bone marrow-derived dendritic cells (BMDC) to induce antigen-specific T-cell proliferation.

[0270] Ovalbumin (OVA)-specific T-cell response induced by BMDCs can be determined by CFSE dilution. BMDCs are isolated by MACS after 6 days of culture and plated at 1 X 10^4 cells per well of a round bottom 96 well plate with OVA (2 or 0.5 mg/mL) and CpG DNA (100 or 25 nM) in the presence of GM-CSF (10 ng/mL) for 4 h. CD4 T-cells from the spleen and lymph nodes of OT-II transgenic mice are isolated by using Dynal Mouse CD4 Negative Isolation Kit (Invitrogen) and stained with CFSE (final 0.8 mM). After 4 h of DC culture, 1X 10^5 CFSE-labeled CD4 OT-II T-cells are added into each well and incubated for 72 h. After culturing, cells are stained with an anti-CD4 monoclonal antibody and flow cytometry is performed to detect CFSE dilution of gated CD4 OT-II T-cells. Data analysis to calculate the percentage of divided and division index is performed by Flowjo software (Treestar) (Eur. J. Immunol. 2012. 42: 176–185).

Example 4: Normalization and reduction of Toll-like receptor (TLR) responses in macrophages by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0271] Bone marrow-derived macrophages (BMDM) or primary peritoneal macrophage responses are altered to TLR signaling by deficiency of TREM2 (Tumbull, IR et al., J Immunol 2006; 177:3520-3524). Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies will reduce and normalize TLR responses in macrophages.

[0272] To elicit primary macrophages, mice are treated with 1.5 ml of 2% thioglycollate medium by intraperitoneal injection, and cells are then isolated by peritoneal lavage. To generate BMDM, total bone marrow is cultured in DMEM supplemented with 10% bovine calf serum, 5% horse serum, and 6 ng/ml recombinant human CSF-1 (R&D Systems). Cells are cultured for 5-6 days, and adherent cells are detached with 1m MEDTA in PBS. Cells are stained with
commercially available antibodies: anti-CD11b, anti-CD40, anti-GR1 (BD Pharmingen), and F4/80 (Caltag Laboratories).

[0273] BMDM are re-plated and allowed to adhere for 4 h at 37°C, and then TLR agonists, such as LPS (Salmonella abortus equi), zymosan (Saccharomyces cerevisiae), and CpG 1826 DNA (purchased from e.g., Sigma-Aldrich) are added. Cell culture supernatant is collected 24 h after stimulation and the levels of IFN-a4, IFN-b, IL-6, IL-12 p70, and TNF cytokines are measured by ELISA or by cytometric bead array (BD Biosciences mouse inflammation kit).

Example 5: Induction of the anti-inflammatory cytokine IL-10 in bone marrow-derived myeloid precursor cells by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0274] Without wishing to be bound by theory, it is believed that bone marrow-derived myeloid precursor cells that are transduced with TREM2 virus will show an increase in the anti-inflammatory cytokine IL-10 following treatment with agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies and stimulation with 100 ng/ml LPS (Sigma), by co-culturing with apoptotic cells, or by a similar stimulus.

[0275] Isolation of bone marrow-derived myeloid precursor cells is performed as follows. Bone marrow cells are isolated from adult 6-8 week-old female C57BL/6 mice (Charles River, Sulzfeld, Germany) from the medullary cavities of the tibia and femur of the hind limbs. Removal of erythrocytes is performed by lysis with hypotonic solution. Cells are cultured in DMEM medium (Invitrogen) containing 10% fetal calf serum (Pan Biotech) and 10 ng/ml of GM-CSF (R&D Systems) in 75 cm<sup>2</sup> culture flasks (Greiner Bio-One). After 24 h, non-adherent cells are collected and re-seeded in fresh 75 cm<sup>2</sup> culture flasks. Medium is changed after 5 d and cells are cultured for an additional 10-11 d. The remaining cells are bone marrow-derived myeloid precursor cells, and are transduced with TREM2 virus. The transduced cells are then examined for the level of IL-10 in conditioned media in both the presence and absence of anti-TREM2 agonistic antibodies and LPS. Supernatant is collected after 24 h, and the level of IL-10 released from the cells is determined by IL-10 ELISA according to manufacturer’s instructions (QuantikineM mouse IL-10, R&D Systems) (JEM (2005), 201; 647–657; and PLoS Medicine (2004), 41 Issue 41 e124).
Example 6: Induction of phagocytosis of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, and disease-causing proteins in cells from the myeloid lineage by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0276] Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies induce phagocytosis of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, and disease-causing proteins, such as A beta peptide, alpha synuclein protein, Tau protein, TDP-43 protein, prion protein, and huntingtin protein, in cells from the myeloid lineage, such as monocytes and microglia.

[0277] Monocytes are isolated from peripheral blood that is collected from adult C57BL/6 mice. Hypotonic lysis buffer depletes erythrocytes. Cells are plated on culture dishes in RPMI medium (Invitrogen) containing 10% fetal calf serum (Pan Biotech). Cells are cultured for several hours at 37°C in 10% CO₂. After trypsinization, adherent cells are collected and used for phagocytosis experiments.

[0278] Microglial cells are prepared from the brains of post-natal day 3 to 5 (P3 to P5) C57BL/6 mice. In brief, meninges are removed mechanically, and the cells are dissociated by trituration and cultured in basal medium (BME; Gibco BRL) supplemented with 10% FCS (PAN Biotech GmbH), 1% glucose (Sigma-Aldrich), 1% L-glutamine (GIBCO BRL), and 1% penicillin/streptomycin (GIBCO BRL), for 14 d to form a confluent glial monolayer. To collect microglial cells, the cultures are shaken on a rotary shaker (200 rpm) for 2 h. The attached astrocytes are used for immunohistochemistry. The detached microglial cells are seeded in normal culture dishes for 1 h, and then all non-adherent cells are removed and discarded. Purity of the isolated microglial cells is about 95% as determined by flow cytometry analysis with antibody directed against CD11b (BD Biosciences). Microglial cells are cultured in basal medium.

[0279] Oligodendrocytes (i.e., neurons) and neuron-enriched cells are prepared from the brain of C57BL/6 mouse embryos (E15-16). In brief, brain tissue is isolated and mechanically dispersed and seeded in culture dishes pre-coated with 0.01 mg/ml poly-L-ornithin (Sigma-
Aldrich) and 10 μg/ml laminin (Sigma-Aldrich). Cells are cultured in neuronal condition medium (BME; Gibco BRL) supplemented with 2% B-27 supplement (Gibco BRL), 1% glucose (Sigma-Aldrich), and 1% FCS (PAN Biotech GmbH). Cells are cultured for 5-10 d to obtain morphologically mature oligodendrocytes.

To conduct phagocytosis assays of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, and disease-causing proteins, microglia are transduced with sh-TREM2 RNA, sh-control RNA, wTREM2, GFP1 control, mtDAP12-GFP, and GFP2 control vector. After transduction, microglia are cultured for 72 h to achieve effective knockdown of TREM2 by RNA interference. Neurons are cultured for 5-10 d, and okadaic acid is then added at the final concentration of 30 nM for 3 h to induce apoptosis. Neuronal cell membranes are labeled with CellTracker CM-DiI membrane dye (Molecular Probes). After incubation, apoptotic neurons or other targets of phagocytosis are washed two times and added to the transduced microglial culture at an effector/target ratio of 1:20. At 1 and 24 h after addition of apoptotic neurons, the number of microglia having phagocytosed neuronal cell membranes is counted under a confocal fluorescence microscope (Leica). Apoptotic cells are counted in three different areas at a magnification of 60. The amount of phagocytosis is confirmed by flow cytometry. Moreover, 24, 48, or 72 h after the addition of apoptotic neurons, cells are collected and used for RT-PCR of cytokines.

To conduct microsphere bead or bacterial phagocytosis assay, microglia are transduced with a TREM2 expression vector or a GFP control vector. Cells are then treated with anti-TREM2 agonistic antibodies. After 24 h, 1.00 μm of red fluorescent microsphere beads (Fluoresbrite Polychromatic Red Microspheres; Polysciences Inc.) or, fluorescent labeled bacteria are added for 1 h. Phagocytosis of microsphere beads or, fluorescent labeled bacteria, by microglia is analyzed by fluorescence microscopy. Furthermore, microglia are collected from the culture plates and analyzed by flow cytometry. The percentage of microglia having phagocytosed beads is determined. Because phagocytosis varies from one experiment to the other, the relative change in phagocytosis is also determined. Data are shown as the relative change in phagocytosis between microglia cultured with agonistic antibodies and control antibody.
[0282] To conduct RT-PCR for analysis of inflammatory gene transcripts, microglia are transduced with a TREM2 vector or a GFP1 control vector. Cells are then cultured on dishes and treated with anti-TREM2 agonistic antibodies. After 24, 48, and 72 h, RNA is isolated from microglia using an RNeasy Mini Kit (QIAGEN). RNA is also collected from microglia that have been transduced with sh-TREM2 RNA, sh-control RNA, wTREM2, GFP2, mtDAP12-GFP, and GFP1 vector and co-cultured with apoptotic neurons for 48 h.

[0283] Reverse transcription of RNA is then performed. Quantitative RT-PCR by SYBR Green is performed on an ABI Prism 5700 Sequence Detection System (PerkinElmer). Amplification of GAPDH is used for sample normalization. The amplification protocol followed the GeneAmp 5700 Sequence Detection System Software (version 1.3). For detection of GAPDH, TNF-alpha, IL-1, NOS2, and TGF-beta transcripts, the following forward and reverse primers were used at final concentrations of 200 nM:

- GAPDH forward primer: 5′-CTCCACTCACGGAATTCGA-3′, and GAPDH reverse primer: 5′-GATGACAAAGCTTCCATTCTCG-3′;
- TNF-α forward primer: 5′-CCGTCAGCCGGATTTGCTATCT-3′, and TNF-α reverse primer: 5′-ACGCGCAGAGGAGGAGTTGACTT-3′;
- IL-1α forward primer: 5′-ACAA- CAAAAAGCCTCGTGCT-3′, and IL-1α reverse primer: 5′-CCATTGAGGTGGAGAGCTTTCA-3′;
- NOS2 forward primer: 5′-GGCAAAACCAAGTGCTACGTCT-3′, NOS2 reverse primer: 5′-TACCTCATTGGCCAGCTT-3′; and
- TGF-β1 forward primer: 5′-AGGACCTGGGTTGAAGTGG-3′, and TGF-β1 reverse primer: 5′-AGTTGGCATGGTAGCCCTTG-3′.

[0284] To conduct amyloid phagocytosis assay, HiLyteFluo\textsuperscript{TM} 647 (Anaspec)-Abeta-(1–40) was resuspended in Tris/EDTA (pH 8.2) at 20 mM and then incubated in the dark for 3 d at 37°C to promote aggregation. Microglial cells are pretreated in low serum (0.5% FBS supplemented with insulin), LPS (50 ng/ml), IFNc (100 units/ml), and anti-TREM2 agonistic antibodies for 24 h prior to the addition of aggregated fluorescently labeled a beta peptide.
Amyloid phagocytosis and surface expression of TREM2 are determined by flow cytometric analysis 5 h post-addition of 100 nM aggregated HiLyteFluor™ 647–Ab-(1–40) (ASN NEURO (2010) 2(3): 157-170). Phagocytosis of other disease-causing proteins is conducted in a similar manner.

Example 7: Induction of ERK activation by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies induce ERK activation.

Microglia are transduced with a TREM2 vector, and 2 X 10⁵ cells are exposed to agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies for 1 h. After stimulation, cells are lysed in reducing sample buffer for Western blot analysis. Phosphorylation of ERK and total amount of ERK are determined by immuno-detection with anti-phospho-ERK and anti-ERK antibodies, respectively (both from Cell Signaling Technology) by Western blot analysis (JEM (2005), 201, 647–657).

Example 8: Induction of CCR7 and migration toward CCL19 and CCL21 in microglia, macrophages, and dendritic cells by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies induce CCR7 and migration toward CCL19 and CCL21 in microglial cells, macrophages, and dendritic cells.

Microglial cells are transduced with a TREM2 vector or a GFP1 control vector. The transduced microglial cells are then either cultured with agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies, or with a control antibody. Cells are collected after 72 h, immuno-labeled with CCR7 specific anti-bodies, and analyzed by flow cytometry.

To determine any functional consequences of increased CCR7 expression, a chemotaxis assay is performed. Microglial cells are stimulated via TREM2 with the agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies and placed in a two-
chamber system. The number of microglial cells migrating toward the chemokine ligands CCL19 and CCL21 is quantified (JEM (2005), 201, 647–657).

[0290] For the chemotaxis assay, microglial cells are exposed to the agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies and treated with 1 μg/ml LPS. Microglia are transferred into the upper chamber of a transwell system (3 μm pore filter; Millipore) containing 450 μl medium with 100 ng/ml CCL19 or CCL21 (both from PeproTech) in the lower chamber. After a 1 h incubation period, the number of microglial cells that have migrated to the lower chamber is counted in three independent areas by microscopy (JEM (2005), 201, 647–657).

Example 9: Induction of F-actin in microglia, macrophages, and dendritic cells by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0291] Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies induce F-actin in microglial cells, macrophages, and dendritic cells.

[0292] Microglia and other cells of interest that are transduced with TREM2 or that express TREM2 are added to culture plates and then exposed to agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies, or a control antibody. Cells are fixed, blocked, and then stained with Alexa Fluor 546-conjugated phalloidin (Molecular Probes) after 1 h and F-actin is labeled with a fluorescence dye. Images are collected by confocal laser scanning microscopy with a 40x objective lens (Leica). (JEM (2005), 201, 647–657).

Example 10: Induction of osteoclast production and increased rate of osteoclastogenesis by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

[0293] Without wishing to be bound by theory, it is believed that agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies induce osteoclast production and increase the rate of osteoclastogenesis.

[0294] RAW264.7 cells that make osteoclasts or bone marrow-derived monocyte/macrophage (BMM) precursor cells are maintained in RPMI-1640 medium
(Mediatech), or another appropriate medium, supplemented with 10% FBS (Atlantic Biologics, Atlanta, GA, USA) and penicillin-streptomycin-glutamine (Mediatech). TREM2B cDNA with a FLAG epitope added to the N terminus is inserted into the retroviral vector pMXpie upstream of an IRES, followed by an eGFP cDNA sequence. Cells are transfected with pMXpie-FLAG TREM2B, using Fugene 6 (Roche) according to manufacturer’s protocol. Cells are selected in puromycin (Sigma) at 2 μg/ml. Stable puromycin-resistant clones are screened for anti-FLAG M2 monoclonal antibody (Sigma) binding by using flow cytometry, and then subcloned and maintained on puromycin selection media.

[0295] RAW264.7 cells expressing TREM2B are seeded in 96-well plates with 3000 cells/well in alpha-MEM medium supplemented with 10% FBS, penicillin-streptomycin-glutamine, 50 ng/ml RANKL, and 20 ng/ml M-CSF. The medium is changed every 3 days, exposed to anti-TREM2 agonistic antibodies and the number of multinucleated (at least three nuclei) TRACP+ osteoclasts are counted and scored by light microscopy. To determine complexity and size, osteoclasts are counted by number of nuclei (>10 or 3–10 nuclei). The surface area of osteoclasts is also measured by using Image J software (NIH). In addition, expression levels of osteoclasts genes are determined. Total RNA is extracted from osteoclastogenic cultures at different time points using TRIzol reagent (Invitrogen). After first-strand cDNA synthesis using a SuperScript III kit (Invitrogen), real-time quantitative PCR reactions are performed for Nfatc1, Acp5, Ctsk, Calcr, and Cend1. Relative quantification of target mRNA expression is calculated and normalized to the expression of cyclophilin and expressed as (mRNA of the target gene/mRNA of cyclophilin) 3 X10^6. (J. OF BONE AND MINERAL RESEARCH (2006), 21, 237–245; J Immunol 2012; 188:2612-2621).

Example 11: In vivo protection from EAE in a whole animal

[0296] Adult 7-9 week-old female C57BL/6 mice (obtained from Charles River Laboratories) are injected in the tail base bilaterally with 200 μl of an inoculum containing 100 μg of myelin oligodendrocyte glycoprotein peptide 35–55 (amino acids MEVGWYRSPFSRVHLYRNGK; Seqlab) and 1 mg of Mycobacterium tuberculosis H37 Ra (Difco) in incomplete Freund adjuvant (Difco). Pertussis toxin (200 ng; List Bio- logical Laboratories) is injected at day 0 and at day 2
after immunization. Clinical signs are scored as follows: 0, no clinical signs; 1, complete limp tail; 2, complete limp tail and abnormal gait; 3, one hind-limb paraparesis; 4, complete hindlimb paraparesis; and 5, fore- and hind-limb paralysis or moribund.

[0297] Only mice having disease onset (clinical score of 1 or more) at day 14 are used for experiments. Agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies are injected intraperitoneally or intravenously in EAE-diseased mice at the day of the first clinical symptoms or at any other desired time (PLoS Med (2007) 4(4): e124).

Example 12: Characterization of the therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in established animal models of traumatic brain injury

[0298] The therapeutic utility of agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies is tested in established animal models of traumatic brain injury (Tanaka, Y et al. (2013) Neuroscience 231 49-60).

[0299] For example, a model of traumatic brain injury that induces the activation of microglia and astrocytes is used. Eight or nine week-old male C57BL/6J WT mice or progranulin heterozygous mice are used (purchased from Charles River Laboratories or Jackson Laboratories). Mice are anesthetized by intraperitoneal administration of xylazine hydrochloride (8 mg/kg) and chloral hydrate (300 mg/kg) dissolved in sterile saline, and subsequently placed in a stereotaxic apparatus (Narishige, Tokyo, Japan). An incision is made in the scalp and the cranium is exposed. The periosteum is cleaned from the skull, a hole is drilled over the right cerebral hemisphere with a dental drill, and the duramater is removed with a needle tip. A stainless steel cannula, with a 0.5 mm outer diameter, is used to make a longitudinal stab wound in the right hemisphere. The cannula is positioned at 1.3 mm lateral to the midline, and 1 mm posterior to bregma, and introduced into the brain until the tip reaches a depth of 2 mm. The cannula is then shifted 2 mm caudally (bregma 3 mm), and then shifts back 2 mm rostrally to its initial position. Finally, the cannula is removed from the brain, and the scalp wound is sutured. Mice are then treated with agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies according to standard procedures and then analyzed by histology and immunofluorescence staining and behavioral tests.
Example 13: Characterization of therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in a model of neuro-inflammation and neuron loss following toxin-induced injury


[0301] Three-month-old mice are treated with 4 intraperitoneal injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) per day for 2 days (4 µg/g body weight) (Sigma-Aldrich) or PBS. Mice are treated with agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies according to standard protocols and then analyzed using Stereological counting to quantify dopamine neurons and microglia in the substantia nigra pars compacta (SNpc), as described.

Example 14: Characterization of the therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in animal models of aging, seizures, spinal cord injury, retinal dystrophy, frontotemporal dementia, and Alzheimer’s disease


Example 15: Characterization of the therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in models of atherosclerosis

[0303] The therapeutic utility of agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies is tested in models of atherosclerosis, as previously described (e.g., Lance, A et al., (2011) Diabetes, 60, 2285; and Kjolby, M et al., (2012) Cell Metabolism 12, 213-223).

Example 16: Characterization of the therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in a model of infection

[0304] The therapeutic utility of agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies is tested in a model of infection. For example, Listeria monocytogenes or other infection in normal mice or progranulin heterozygous mice can be used, as previously described (e.g., Yin, F et al., (2009) J. Exp. Med, 207, 117-128).

Example 17: Characterization of the therapeutic use of agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies in a model of inflammatory diseases


Example 18: Screening for anti-TREM2, anti-DAP12, and/or TEM2/DAP12 bispecific antibodies that induce phosphorylation of DAP12, ERK, and AKT which indicate activation of the PI3K pathway

[0306] Cells (J774, RAW 264.7, BMM cells, or osteoclasts) are removed from tissue culture dishes with PBS-EDTA, washed with PBS, and counted. J774 (40 × 10^6) or RAW 264.7 cells (10 × 10^6 BMM or osteoclasts) are incubated with an anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibody or with an isotype-matched control antibody at 1 μg/10^6 cells.
cells for 20 min on ice or under other conditions. Cells are lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer [50 mM tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton-100, 1 mM NaF, 1 mM phenylmethylsulfonly fluoride, 1 mM NaVO₄, 0.25% sodium deoxycholate, aprotinin (1 μg/ml), leupeptin (1 μg/ml), pepstatin (1 μg/ml)] for 20 min followed by centrifugation at 16,000 g for 10 min at 4°C to remove insoluble materials. The resulting supernatant is subjected to immunoprecipitation reactions with the indicated antibodies (DAP12, ERK, or AKT) and protein A- or protein G-agarose (Sigma). The beads are extensively washed with RIPA buffer and the proteins are separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins are then transferred to nitrocellulose membranes by Western blotting, incubated with the appropriate antibodies (antibodies that specifically recognize the phosphorylated form of DAP12, ERK, or AKT) and visualized with the enhanced chemiluminescence (ECL) system (Pierce), as described (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38).

Example 19: Screening for anti-TREM2, anti-DAP12, and/or TEM2/DAP12 bispecific antibodies that induce calcium flux

[0307]  BMM cells are washed twice with HEPES-containing buffer [20 mM HEPES (pH 7.3), 120 mM NaCl, 1 mM CaCl, 1 mM MgCl, 5 mM KCl, glucose (1 mg/ml), bovine serum albumin (1 mg/ml)] followed by incubation in 0.05% Pluronic F-127 (Invitrogen) and 1 μM Indo-1 AM (Invitrogen) for 20 min at 37°C. Cells are washed twice with HEPES buffer and are then stimulated with an anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibody (16 μg/ml) or with a control antibody (16 μg/ml) and monitored by spectrophotometer (PTL Photon Technology International). The Indo-1 fluorescence emission is converted to calcium (Ca²⁺) according to manufacturer’s instructions (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38).

Example 20: Screening for anti-TREM2, anti-DAP12, and/or TEM2/DAP12 bispecific antibodies that prevent apoptosis
Mature osteoclast cell cultures are differentiated in 24-well dishes with RANKL and M-CSF. After 4 days, complete medium is substituted with serum-free medium to induce apoptosis. Cells are treated with RANKL, PBS, and an anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibody, or an isotype-matched control antibody, during the overnight serum starvation. Cells are fixed in 1% paraformaldehyde and stained with a TUNEL-based kit (Millipore Corporation) according to manufacturer’s instructions. Apoptotic nuclei are counted with a Nikon TE2000-E microscope with 20x magnification. Results are expressed as the percentage of apoptotic cells relative to the total number of cells in six randomly selected fields of the two wells, as described (e.g., Peng et al., 2010 Sci Signal., 3(122): ra38). Similar assays are performed with primary microglial cells.

Example 21: Screening for anti-TREM2, anti-DAP12, and/or TEM2/DAP12 bispecific antibodies that induce osteoclast differentiation

BMM cells are seeded onto the plates in triplicate wells and treated with RANKL, M-CSF, and with an anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibody, or an isotype-matched control monoclonal antibody. The medium is changed every 3 days until large multinucleated cells are visible. After 3 to 5 days in culture, cells are fixed with 3.7% formaldehyde in PBS for 10 min. Plates are then washed twice in PBS, incubated for 30 s in a solution of 50% acetone and 50% ethanol, and washed with PBS. Cells are stained for tartrate-resistant acid phosphatase (TRAP) with a kit from Sigma (product 435). Multinucleated (more than two nuclei), TRAP-positive cells are then counted by light microscopy, as described (e.g., Peng et al., 2010 Sci Signal., 3(122): ra38).

Example 22: Screening for anti-TREM2, anti-DAP12, and/or TEM2/DAP12 bispecific antibodies that normalize TREM2/TYROBP-dependent changes in gene expression within the immune/microglia regulatory module

Microglial cells derived from mouse embryonic stem cells are genetically modified by lentiviral vectors to overexpress either full-length or a truncated version of Tyrobp that lacks both intracellular immunoreceptor tyrosine-based activation motif (ITAM) motifs. Microglia cells are
also derived from mouse embryonic stem cells that are heterozygous for TREM2. To assess the genome-wide gene-expression changes in response to the perturbation of Tyrobp or TREM2, gene-expression data is derived from the RNA sequencing of mouse microglial cell lines overexpressing: (1) vehicle, (2) full-length Tyrobp, or (3) dominant-negative truncated Tyrobp; or (4) overexpressing a knockdown construct for TREM2, such as SiRNA and cells which are heterozygous for TREM2. Approximately 2,638 and 3,415 differentially expressed genes for the overexpression of full-length Tyrobp and truncated Tyrobp are identified, respectively (Zhang et al., 2013 Cell 153, 707–720). Approximately 99% of the differentially expressed genes from the microglia overexpressing intact Tyrobp are downregulated compared to the control vehicle. For example, 658 genes, related to the vacuole/autophagy, as well as genes involved with RNA metabolism and cell-cycle mitosis are downregulated by active Tyrobp, but upregulated in cells expressing dominant-negative truncated Tyrobp. Conversely, some 2,856 genes for the vacuole/autophagy pathway and for mitochondrion are selectively upregulated in microglia expressing the dominant-negative truncated Tyrobp.

[0311] Agonistic anti-TREM2, anti-DAP12, and/or TREM2/DAP12 bispecific antibodies are screened for their ability to elicit gene expression profiles similar to that observed in normal microglial cells and in microglial cells overexpressing intact Tyrobp in cells that express dominant-negative truncated Tyrobp (Zhang et al., 2013 Cell 153, 707–720), in cells that express the knockdown construct for TREM2, or in cells that are heterozygous for TREM2. Antibodies that are capable of changing the gene expression network are selected.
Figure 2

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. N/A

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<tr>
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<tr>
<td>Arnon</td>
<td>ROSENTHAL</td>
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Additional inventors are being named on the separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max):
ANTI-TREM2 AND ANTI-DAP12 ANTIBODIES AND METHODS OF USE THEREOF

Direct all correspondence to:

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ENCLOSED APPLICATION PARTS (check all that apply)

| X | Application Data Sheet, See 37 CFR 1.76 (6 pages). |
|   | CD(s), Number of CDs |
|   | Other (specify) |
| X | Drawing(s) Number of Sheets |
|   | 2 |

Specification (e.g. description of the invention) Number of Pages 116

Fees Due: Filing Fee of $220 ($130 for small entity) ($65 for micro entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $400 ($200 for small entity) ($100 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(A) and 37 CFR 1.16(a).

METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

| X | Applicant asserts small entity status. See 37 CFR 1.27. |
|   | Applicant certifies micro entity status. See 37 CFR 1.29. |
|   | Applicant must attach form PTO/SB/15A or B or equivalent. |
|   | A check or money order made payable to the Director of the United States Patent and Trademark Office is enclosed to cover the filing fee and application size fee (if applicable). |
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sf-3446141
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SIGNATURE /Roberto K. Rodriguez/ DATE August 8, 2014

TYPED or PRINTED NAME Roberto K. Rodriguez REGISTRATION NO. 66,244

TELEPHONE (415) 268-7215 DOCKET NUMBER 735023000400
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- **Payment Type**: Deposit Account
- **Payment was successfully received in RAM**: $130
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- **Deposit Account**: 031952

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**New Applications Under 35 U.S.C. 111**

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

**National Stage of an International Application under 35 U.S.C. 371**

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

**New International Application Filed with the USPTO as a Receiving Office**

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.
WHAT IS CLAIMED IS:

1. An isolated agonist antibody that binds to a TREM2 protein, a DAP12 protein, or both, wherein the antibody induces one or more TREM2 activities, DAP12 activities, or both.

2. The isolated antibody of claim 1, wherein the TREM2 protein, the DAP12 protein, or both is a mammalian protein or a human protein.

3. The isolated antibody of claim 2, wherein the TREM2 protein, the DAP12 protein, or both is a wild-type protein.

4. The isolated antibody of claim 2, wherein the TREM2 protein, the DAP12 protein, or both is a naturally occurring variant.

5. The isolated antibody of any one of claims 1-4, wherein the one or more TREM2 activities comprise TREM2 binding to DAP12.

6. The isolated antibody of any one of claims 1-4, wherein the one or more DAP12 activities comprise DAP12 binding to TREM2.

7. The isolated antibody of any one of claims 1-6, wherein the one or more TREM2 activities, DAP12 activities, or both comprise DAP12 phosphorylation.

8. The isolated antibody of claim 7, wherein DAP12 phosphorylation is induced by one or more SRC family tyrosine kinases.

9. The isolated antibody of any one of claims 1-8, wherein the one or more TREM2 activities, DAP12 activities, or both comprise PI3K activation.

10. The isolated antibody of any one of claims 1-9, wherein the one or more TREM2 activities, DAP12 activities, or both comprise increased expression of one or more anti-inflammatory mediators selected from the group consisting of IL-12p70, IL-6, and IL-10.
11. The isolated antibody of claim 10, wherein the increased expression of the one or more anti-inflammatory mediators occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, and microglial cells.

12. The isolated antibody of any one of claims 1-11, wherein the one or more TREM2 activities, DAP12 activities, or both comprise reduced expression of one or more pro-inflammatory mediators selected from the group consisting of IFN-a4, IFN-b, IL-6, IL-12 p70, IL-1β and TNF.

13. The isolated antibody of claim 12, wherein the reduced expression of the one or more pro-inflammatory mediators occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, and microglial cells.

14. The isolated antibody of any one of claims 1-13, wherein the one or more TREM2 activities, DAP12 activities, or both comprise extracellular signal-regulated kinase (ERK) phosphorylation.

15. The isolated antibody of any one of claims 1-14, wherein the one or more TREM2 activities, DAP12 activities, or both comprise increased expression of C-C chemokine receptor 7 (CCR7).

16. The isolated antibody of any one of claims 1-15, wherein the one or more TREM2 activities, DAP12 activities, or both comprise induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells.

17. The isolated antibody of any one of claims 1-16, wherein the one or more TREM2 activities, DAP12 activities, or both comprise a reduction, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation.

18. The isolated antibody of any one of claims 1-17, wherein the one or more TREM2 activities, DAP12 activities, or both comprise induction of osteoclast production, increased rate of osteoclastogenesis, or both.
19. The isolated antibody of any one of claims 1-18, wherein the one or more TREM2 activities, DAP12 activities, or both comprise of increasing the survival of macrophages, microglial cells, or both.

20. The isolated antibody of any one of claims 1-19, wherein the one or more TREM2 activities, DAP12 activities, or both comprise increasing the function of macrophages, microglial cells, or both.

21. The isolated antibody of any one of any one of claims 1-20, wherein the one or more TREM2 activities, DAP12 activities, or both comprise induction of one or more types of clearance without inflammation selected from the group consisting of apoptotic neuron clearance without inflammation, nerve tissue debris clearance without inflammation, non-nerve tissue debris clearance without inflammation, bacteria or other foreign body clearance without inflammation, and disease-causing protein clearance without inflammation.

22. The isolated antibody of any one of claims 1-21, wherein the one or more TREM2 activities, DAP12 activities, or both comprise induction of phagocytosis without inflammation of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, or disease-causing proteins without inflammation.

23. The isolated antibody of claim 21 or claim 22, wherein the disease-causing protein is selected from the group consisting of A beta peptide, alpha synuclein protein, Tau protein, TDP-43 protein, prion protein, and huntingtin protein.

24. The isolated antibody of any one of claims 1-23, wherein the one or more TREM2 activities, DAP12 activities, or both comprise normalization of disrupted TREM2/DAP12-dependent gene expression.

25. The isolated antibody of any one of claims 1-24, wherein the one or more TREM2 activities, DAP12 activities, or both comprise recruitment of Syk, ZAP70, or both to DAP12.
26. The isolated antibody of any one of claims 1-25, wherein the isolated agonist antibody that binds to a TREM2 protein binds to one or more amino acids within amino acid residues selected from the group consisting of:

i. amino acid residues 29-112 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 29-112 of SEQ ID NO: 1;

ii. amino acid residues 29-41 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 29-41 of SEQ ID NO: 1;

iii. amino acid residues 40-44 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 40-44 of SEQ ID NO: 1;

iv. amino acid residues 47-69 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 47-69 of SEQ ID NO: 1;

v. amino acid residues 67-76 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 67-76 of SEQ ID NO: 1;

vi. amino acid residues 76-86 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 76-86 of SEQ ID NO: 1;

vii. amino acid residues 91-100 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 91-100 of SEQ ID NO: 1;

viii. amino acid residues 99-115 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 99-115 of SEQ ID NO: 1;

ix. amino acid residues 104-112 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 104-112 of SEQ ID NO: 1; and

x. amino acid residues 114-118 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 114-118 of SEQ ID NO: 1.
27. The isolated antibody of any one of claims 1-25, wherein the isolated agonist antibody that binds to a TREM2 protein binds to an epitope comprising one or more amino acid residues selected from the group consisting of:

i. amino acid residue Arg47 or Asp87 of SEQ ID NO: 1;
ii. amino acid residues 40-44 of SEQ ID NO: 1;
iii. amino acid residues 67-76 of SEQ ID NO: 1; and
iv. amino acid residues 114-118 of SEQ ID NO: 1.

28. The isolated antibody of any one of claims 1-25, wherein the isolated agonist antibody that binds to a DAP12 protein binds to one or more amino acids within amino acid residues 22-40 of SEQ ID NO: 2, or amino acid residues on a DAP12 protein corresponding to amino acid residues 22-40 of SEQ ID NO: 2.

29. The isolated antibody of any one of claims 1-25, wherein the isolated agonist antibody is a bispecific antibody that binds to one or more amino acids selected from the group consisting of:

i. one or more amino acid residues of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues of SEQ ID NO: 1; and
ii. one or more amino acid residues of SEQ ID NO: 2, or amino acid residues on a DAP12 protein corresponding to amino acid residues of SEQ ID NO: 2.

30. The isolated antibody of any of the preceding claims, wherein the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, or a chimeric antibody.

31. The isolated antibody of any of the preceding claims, wherein the antibody is a monoclonal antibody.

32. The isolated antibody of any of the preceding claims, wherein the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting
of human TREM2, a naturally occurring variant of human TREM2, human DAP12, and naturally occurring variant of human DAP12.

33. The isolated antibody of claim 32, wherein the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

34. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

35. A vector comprising the nucleic acid of claim 34.

36. A host cell comprising the vector of claim 35.

37. A method of producing an antibody, comprising culturing the host cells of claim 36 so that the antibody is produced.

38. The method of claim 37, further comprising recovering the antibody produced by the host cell.

39. A pharmaceutical composition comprising the antibody of any one of claims 1-33, and a pharmaceutically acceptable carrier.

40. A method of preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and multiple sclerosis, comprising administering to the individual a therapeutically effective amount of the isolated antibody of any one of claims 1-33.

41. The method of claim 40, wherein the individual has a heterozygous variant of TREM2, wherein the variant comprises one or more substitutions selected from the group consisting of:
   i. a glutamic acid to stop codon substitution in the nucleic acid sequence encoding amino acid residue Glu14 of SEQ ID NO: 1;
   ii. a glutamine to stop codon substitution in the nucleic acid sequence encoding amino acid residue Gln33 of SEQ ID NO: 1;
iii. a tryptophan to stop codon substitution in the nucleic acid sequence encoding 
amino acid residue Trp44 of SEQ ID NO: 1;

iv. an arginine to histidine amino acid substitution at an amino acid corresponding to 
amino acid residue Arg47 of SEQ ID NO: 1

v. a tryptophan to stop codon substitution in the nucleic acid sequence encoding 
amino acid residue Trp78 of SEQ ID NO: 1;

vi. a valine to glycine amino acid substitution at an amino acid corresponding to 
amino acid residue Val126 of SEQ ID NO: 1;

vii. an aspartic acid to glycine amino acid substitution at an amino acid corresponding 
to amino acid residue Asp134 of SEQ ID NO: 1; and

viii. a lysine to asparagine amino acid substitution at an amino acid corresponding to 
amino acid residue Lys186 of SEQ ID NO: 1.

42. The method of claim 40, wherein the individual has a heterozygous variant of TREM2, 
wherein the variant comprises a guanine nucleotide deletion at a nucleotide corresponding to 
nucleotide residue G313 of the nucleic acid sequence encoding SEQ ID NO: 1; a guanine 
nucleotide deletion at a nucleotide corresponding to nucleotide residue G267 of the nucleic acid 
sequence encoding SEQ ID NO: 1; or both.

43. The method of claim 40, wherein the individual has a heterozygous variant of DAP12, 
wherein the variant comprises one or more variants selected from the group consisting of:

i. a methionine to threonine substitution at an amino acid corresponding to amino 
acid residue Met1 of SEQ ID NO: 2;

ii. a glycine to arginine amino acid substitution at an amino acid corresponding to 
amino acid residue Gly49 of SEQ ID NO: 2;

iii. a deletion within exons 1-4 of the nucleic acid sequence encoding SEQ ID NO: 2;

iv. an insertion of 14 amino acid residues at exon 3 of the nucleic acid sequence 
encoding SEQ ID NO: 2; and
v. a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G141 of the nucleic acid sequence encoding SEQ ID NO: 2.
ABSTRACT

The invention is generally directed to methods and compositions that include agonist antibodies, e.g., monoclonal, chimeric, humanized antibodies, antibody fragments, etc., that specifically bind a TREM2 and/or DAP12 protein, e.g., a mammalian TREM2, human TREM2, a mammalian DAP12, and/or human DAP12. The methods provided herein find use in preventing, reducing risk, or treating an individual having dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, or multiple sclerosis.