stimulatory molecules expressed in T_{reg} cells searches were performed to identify genes specifically expressed in T cells (Abbas, A.R. et al., *Genes Immun* 6, 319-31 (2005)) that had both Ig domains and immunoreceptor tyrosine-based activation or inhibition (ITAM/ITIM) motifs. Through the intersection of these two genome-wide bioinformatics search strategies a novel cell surface-bound protein with the protein encoding an IgV domain, a transmembrane domain, and two putative immunoreceptor tyrosine inhibitory motifs was identified (see US patent publication no. US20040121370, incorporated herein by reference). The protein designated TIGIT (for T-Cell-Ig and ITIM domain) was shown to be expressed on T cells - particularly T_{reg} and memory cell subsets – as well as NK cells. There is a need for new therapeutics and methods of treatment to address immune disorders, particularly autoimmune disorders. Herein, Applicants identify TIGIT binding partners and provide new compositions, detection methods, and methods of treatment for immune disorders modulated by TIGIT interaction with those binding partners and the elucidated TIGIT effects on T cell maturation and activity.

SUMMARY

The present disclosure concerns compositions and methods useful for the diagnosis and treatment of immune related disease in mammals, including humans. The present disclosure is based on the identification of proteins involved in the negative regulation of proliferation and function of certain types of immune cells. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the immune response to an antigen (*e.g.*, neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (*e.g.*, inflammation). Herein, Applicants demonstrate that TIGIT (for "T-Cell-Ig and ITIM domain") protein specifically binds to poliovirus receptor (PVR, also known as CD155) and several other members of a newly elucidated protein family, and that this TIGIT-PVR interaction negatively regulates T cell activation and proliferation. Accordingly, TIGIT polypeptides, agonists thereof, and antagonists thereof, as well as PVR polypeptides, agonists thereof and antagonists thereof are useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. The disclosure also provides methods of treating immune-related and inflammatory diseases and methods and

compositions for detecting and assessing the status of immune-related and inflammatory diseases.

In one embodiment, the disclosure provides an isolated polypeptide comprising an amino acid sequence comprising one or more of the following amino acids: an alanine at amino acid position corresponding to amino acid position 67 of human TIGIT, a glycine at an amino acid position corresponding to amino acid position 74 of human TIGIT, a proline at an amino acid position corresponding to amino acid position 114 of human TIGIT, and a glycine at an amino acid position corresponding to amino acid position 116 of human TIGIT. In one embodiment, the polypeptide is not PVR, PVRL1, PVRL2, PVRL3, PVRL4, TIGIT, CD96, or CD226. In another embodiment, the polypeptide further comprises one or more of: an amino acid selected from valine, isoleucine, and leucine at an amino acid position corresponding to amino acid position 54 of human TIGIT, an amino acid selected from serine and threonine at an amino acid position corresponding to amino acid position 55 of human TIGIT, a glutamine at an amino acid position corresponding to amino acid position 56 of human TIGIT, a threonine at an amino acid position corresponding to amino acid position 112 of human TIGIT, and an amino acid selected from phenylalanine and tyrosine at an amino acid position corresponding to amino acid position 113 of human TIGIT. In another embodiment, the polypeptide further comprises one or more structural submotifs selected from the following:

a. an amino acid selected from valine and isoleucine at amino acid position 54-an amino acid selected from serine and threonine at amino acid position 55-a glutamine at amino acid position 56;
b. an alanine at position 67-any amino acid at each of amino acid positions 68-73-a glycine at amino acid position 74; and
c. a threonine at amino acid position 112-an amino acid selected from phenylalanine and tyrosine at amino acid position 113-a proline at amino acid position 114-any amino acid at amino acid position 115-a glycine at amino acid position 116,

wherein the numbering of the amino acid positions corresponds to the amino acid positions of human TIGIT, although the absolute numbering of the amino acids in the polypeptide may differ.

In another embodiment, the disclosure provides a method of determining whether a test polypeptide is a member of the TLP family of polypeptides comprising aligning the amino acid sequence of the test polypeptide with an amino acid sequence of one or more members of the TLP family of polypeptides and assessing the presence or absence in the test

polypeptide amino acid sequence of one or more of an alanine at amino acid position corresponding to amino acid position 67 of human TIGIT, a glycine at an amino acid position corresponding to amino acid position 74 of human TIGIT, a proline at an amino acid position corresponding to amino acid position 114 of human TIGIT, and a glycine at an amino acid position corresponding to amino acid position 116 of human TIGIT. In another embodiment, the disclosure provides a method for identifying one or more members of the TLP protein family by identifying proteins in one or more sequence databases whose amino acid sequences comprise at least one amino acid selected from an alanine at amino acid position corresponding to amino acid position 67 of human TIGIT, a glycine at an amino acid position corresponding to amino acid position 74 of human TIGIT, a proline at an amino acid position corresponding to amino acid position 114 of human TIGIT, and a glycine at an amino acid position corresponding to amino acid position 116 of human TIGIT.

In another embodiment, the disclosure provides an isolated agent that specifically interacts with one or more conserved or substantially conserved regions of TLP family members. In one aspect, there is provided an isolated TIGIT-like protein (TLP)-interacting agent that specifically interacts with one or more conserved or substantially conserved regions of TLP family members, wherein the TLP-interacting agent is capable of binding to mouse TIGIT and human TIGIT. In one embodiment, the agent is an antagonist of the expression and/or activity of a TLP family member. In another embodiment, the antagonist is selected from a small molecule inhibitor, an inhibitory antibody or antigen-binding fragment thereof, an aptamer, an inhibitory nucleic acid, and an inhibitory polypeptide. In another embodiment, the agent is an agonist of the expression and/or activity of a TLP family member. In another embodiment, the agonist is selected from an agonizing antibody or antigen-binding fragment thereof, an agonizing peptide, and a small molecule or protein that activates TIGIT binding to PVR and/or TIGIT intracellular signaling mediated by PVR. In another embodiment, the disclosure provides a method of identifying or detecting one or more TLP family members by contacting a putative TLP family member polypeptide with at least one of the above agents and determining the binding of the at least one agent to the putative TLP family member.

In another embodiment, the disclosure provides a method of determining whether a test immune cell is an activated or normal T_{reg}, memory T cell, NK cell, or T_{Fh} cell, comprising assessing the level of expression of TIGIT in the test immune cell and comparing it to the level of expression of TIGIT in a known activated or normal Treg, memory T cell, NK cell, or TFh cell, or by comparing the level of expression of TIGIT in the test immune cell to known standard TIGIT expression value(s). In another embodiment, the disclosure provides a method for modulating immune system function and/or activity comprising modulating the binding of TIGIT to one or more of PVR, PVRL3, and PVRL2.

In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof comprising at least one HVR comprising an amino acid sequence selected from the amino acid sequences set forth in SEQ ID NOs: 23-28. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof comprising at least one HVR comprising an amino acid sequence selected from the amino acid sequences set forth in SEQ ID NOs: 31-36. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody light chain comprises the amino acid sequence set forth in SEQ ID NO: 21. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody light chain comprises the amino acid sequence set forth in SEQ ID NO: 29. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 22 or a portion thereof. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 30 or a portion thereof. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody light chain comprises the amino acid sequence set forth in SEQ ID NO: 21 or a portion thereof and the antibody heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 22 or a portion thereof. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody light chain comprises the amino acid sequence set forth in SEQ ID NO: 29 or a portion thereof and the antibody heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 30 or a portion thereof. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody light chain is encoded by the nucleotide sequence of SEQ ID NO: 50 or a portion thereof. In another embodiment, the disclosure provides an anti-TIGIT antibody or a fragment thereof wherein the antibody heavy chain is encoded by the nucleotide sequence of SEQ ID NO: 51
or a portion thereof. In one embodiment, an antibody or antigen-binding fragment thereof of the disclosure is selected from a humanized antibody, a chimeric antibody, a bispecific antibody, a heteroconjugate antibody, and an immunotoxin.

In another embodiment, the at least one HVR of the disclosure is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an HVR set forth in any of SEQ ID NOs: 23-28. In another embodiment, the at least one HVR of the disclosure is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an HVR set forth in any of SEQ ID NOs: 31-36. In another embodiment, the light chain of an antibody or antigen-binding fragment

of the disclosure comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 21. In another embodiment, the light chain of an antibody or antigen-binding fragment of the disclosure comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 29. In another embodiment, the heavy chain of an antibody or antigen-binding fragment of the disclosure comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 22. In another embodiment, the heavy chain of an antibody or antigen-binding fragment of the disclosure comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 30. In another embodiment, an antibody or antigen-binding fragment of the disclosure comprises a light chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 21 and a heavy chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 22. In another embodiment, an antibody or antigen-binding fragment of the disclosure comprises a light chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 29 and a heavy chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 30.

In another embodiment, the disclosure provides a method of modulating a CD226-PVR interaction and/or a CD96-PVR interaction comprising administering at least one of TIGIT, an agonist of TIGIT expression and/or activity, or an antagonist of TIGIT expression and/or activity in vivo or in vitro. In one embodiment, TIGIT or an agonist of TIGIT expression and/or activity is administered and the CD226-PVR interaction and/or the CD96-PVR interaction is inhibited or blocked. In another embodiment, an antagonist of TIGIT expression and/or activity is administered and the CD226-PVR interaction and/or the CD96-PVR interaction is stimulated.

In another embodiment, the disclosure provides a method of modulating immune cell function and/or activity by modulating TIGIT and/or PVR expression and/or activity, or by modulating the intracellular signaling mediated by TIGIT binding to PVR. In one embodiment, the modulating is decreasing or inhibiting proliferation of one or more immune cells or proinflammatory cytokine release by one or more immune cells by treating the cells in vitro

or in vivo with TIGIT, an agonist of TIGIT expression and/or activity, an agonist of PVR expression and/or activity, or by stimulating intracellular signaling mediated by TIGIT binding to PVR. In another embodiment, the modulating is increasing or stimulating proliferation of one or more immune cells or proinflammatory cytokine release by one or more immune cells by treating the cells in vitro or in vivo with an antagonist of TIGIT expression and/or activity, an antagonist of PVR expression and/or activity, or by inhibiting intracellular signaling mediated by TIGIT binding to PVR.

In one aspect, there is provided use of the TLP-interacting agent described herein in the manufacture of a medicament for modulating immune system function and/or activity. In one embodiment, the disclosure provides a method of inhibiting an immune response by administering in vitro or in vivo TIGIT, an agonist of TIGIT expression and/or activity, an agonist of PVR expression and/or activity, or by stimulating intracellular signaling mediated by TIGIT binding to PVR. In another embodiment, the disclosure provides a method of increasing or stimulating an immune response by administering in vitro or in vivo an antagonist of TIGIT expression and/or activity, an antagonist of PVR expression and/or activity, or by inhibiting intracellular signaling mediated by TIGIT binding to PVR. In one aspect, there is provided a method of increasing or stimulating an immune response by administering in vitro the TLP-interacting agent described herein. In another aspect, there is provided use of the TLP-interacting agent described herein in the manufacture of a medicament for stimulating an immune response. In another embodiment, the disclosure provides a method of modulating the type and/or amount of cytokine production from an immune cell by modulating TIGIT or PVR expression and/or activity in vitro or in vivo. In one aspect, there is provided a method of modulating the type and/or amount of cytokine production from an immune cell by administering *in vitro* the TLP-interacting agent described herein. In another aspect, there is provided use of the TLP-interacting agent described herein in the manufacture of a medicament for modulating the type and/or amount of cytokine production from an immune cell. In one embodiment, proinflammatory cytokine production is stimulated and/or increased by administration of an antagonist of TIGIT expression and/or activity, an antagonist of PVR expression and/or activity, or by inhibiting intracellular signaling mediated by TIGIT binding to PVR. In another embodiment, proinflammatory cytokine production is inhibited by administration of an agonist of TIGIT expression and/or activity, an agonist of PVR expression and/or activity, or by stimulating intracellular signaling mediated by TIGIT binding to PVR.

In another embodiment, the disclosure provides a method of stimulating ERK phosphorylation and/or intracellular signaling through the ERK pathway in one or more immune cells comprising treating the one or more immune cells with TIGIT, an agonist of TIGIT expression and/or activity, or an agonist of PVR expression and/or activity.

In another aspect, there is provided a method of diagnosing an immune-related disease relating to aberrant immune cell response in a subject comprising determining the expression and/or activity of TIGIT in a sample from the subject and comparing the expression and/or activity of TIGIT to a reference amount of TIGIT expression and/or activity or the amount of TIGIT expression and/or activity in a sample from a normal subject. In one embodiment, the immune-related disease is selected from psoriasis, arthritis, inflammatory bowel disease or cancer. In another embodiment, the cancer is breast cancer. In another

aspect, there is provided a method of assessing the severity of an immune-related disease relating to aberrant immune cell response in a subject comprising determining the expression and/or activity of TIGIT in a sample from the subject and comparing the expression and/or activity of TIGIT to a reference amount of TIGIT expression and/or activity or the amount of TIGIT expression and/or activity in a sample from a normal subject. In one embodiment, the immune-related disease is selected from psoriasis, arthritis, inflammatory bowel disease or cancer. In another embodiment, the cancer is breast cancer. In another embodiment, the disclosure provides a method of preventing an immune-related disease relating to aberrant immune cell response in a subject comprising modulating the expression and/or activity of TIGIT in the subject. In one aspect, there is provided use of the TLP-interacting agent described herein in the manufacture of a medicament for preventing an immune-related disease relating to aberrant immune cell response in a subject. In one embodiment, the immune-related disease is selected from psoriasis, arthritis, inflammatory bowel disease or cancer. In another embodiment, the cancer is breast cancer. In another embodiment, the disclosure provides a method of treating or lessening the severity of an immune-related disease relating to aberrant immune cell response in a subject comprising modulating the expression and/or activity of TIGIT in the subject. In one aspect, there is provided use of the TLP-interacting agent described herein in the manufacture of a medicament for treating or lessening the severity of an immune-related disease relating to aberrant immune cell response in a subject. In one embodiment, the immune-related disease is selected from psoriasis, arthritis, inflammatory bowel disease or cancer. In another embodiment, the cancer is breast cancer.

In another aspect, there is provided a method of detecting the presence or expression level of TIGIT in a sample from a subject comprising contacting the sample with the TLP-interacting agent described herein and detecting the presence of the bound TLP-interacting agent.

In yet another aspect, there is provided an article of manufacture for use in the diagnosis or treatment of an immune-related disease, wherein the article of manufacture comprises the TLP-interacting agent described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts an alignment of human, mouse, rhesus and dog TIGIT protein sequences. Shading indicates positions containing identical amino acids in three or four species. The signal sequence is indicated by a dashed line, the immunoglobulin V-set domain is indicated by a double line, N-glycosylation sites are indicated by a thin line above the requisite position, the transmembrane domain is indicated by a thick line, and the putative extended ITIM motif is indicated by a double dashed line. Human TIGIT shares 88%, 67%, and 58% identity with rhesus, dog and mouse sequences, respectively.

Figures 2A and 2B depict an alignment of protein sequences of IgV domains of the indicated PVR family proteins. Side chains that share similarity across sequences are marked according to property. V-frame fingerprint residues (black circle) and PVR-related fingerprint residues (thick line boxed residues) are indicated. For comparative purposes, six IgV domain sequences (set forth under the horizontal line) from non-PVR family members are also aligned.

What is claimed:

1. An isolated TIGIT-like protein (TLP)-interacting agent that specifically interacts with one or more conserved or substantially conserved regions of TLP family members, wherein the TLP-interacting agent is capable of binding to mouse TIGIT and human TIGIT.

2. The TLP-interacting agent of claim 1, wherein the one or more conserved or substantially conserved regions of TLP family members comprise one or more of the following structural submotifs:

(a) V/I^{54}- S/T^{55}-Q^{56};

(b) A^{67}- X^{68}- X^{69}- X^{70}- X^{71}- X^{72}- X^{73}-G^{74}, wherein X is any amino acid; and/or

(c) T^{112}- F/Y^{113}- P^{114}- X^{115}- G^{116}, wherein X is any amino acid.

3. The TLP-interacting agent of claim 1 or 2, wherein the TLP-interacting agent is an antagonist of the expression and/or activity of TIGIT and/or PVR.

4. The TLP-interacting agent of claim 3, wherein the antagonist is selected from an antagonizing antibody or antigen-binding fragment thereof, a small molecule inhibitor, an aptamer, an inhibitory nucleic acid, and an inhibitory polypeptide.

5. The TLP-interacting agent of claim 4, wherein the antagonist is an antagonizing antibody or antigen-binding fragment thereof.

6. The TLP-interacting agent of claim 5, wherein the antagonizing antibody or antigen-binding fragment thereof is selected from the group consisting of a humanized antibody, a chimeric antibody, a bispecific antibody, a heteroconjugate antibody, and an immunotoxin.

7. The TLP-interacting agent of any one of claims 3-6, wherein the antagonist specifically binds TIGIT and blocks TIGIT interaction with PVR.

8. The TLP-interacting agent of claim 7, wherein the antagonist inhibits intracellular signaling mediated by TIGIT binding to PVR.

9. The TLP-interacting agent of claim 1 or 2, wherein the TLP-interacting agent is an agonist of the expression and/or activity of PVR.

10. The TLP-interacting agent of claim 9, wherein the agonist is selected from an agonizing antibody or antigen-binding fragment thereof, an agonizing peptide, and a small molecule.

11. The TLP-interacting agent of claim 10, wherein the agonist is an agonizing antibody or antigen-binding fragment thereof.

12. The TLP-interacting agent of claim 11, wherein the agonizing antibody or antigen-binding fragment thereof is selected from the group consisting of a humanized antibody, a chimeric antibody, a bispecific antibody, a heteroconjugate antibody, and an immunotoxin.

13. The TLP-interacting agent of any one of claims 9-12, wherein the agonist specifically binds TIGIT and stimulates the interaction of PVR with CD226 and/or CD96.

14. Use of the TLP-interacting agent of any one of claims 1-8 in the manufacture of a medicament for modulating immune system function and/or activity.

15. The use of claim 14, wherein the modulating is increasing or stimulating proliferation of one or more immune cells or proinflammatory cytokine release by one or more immune cells.

16. A method of increasing or stimulating an immune response by administering *in vitro* the TLP-interacting agent of any one of claims 1-8.

17. Use of the TLP-interacting agent of any one of claims 1-8 in the manufacture of a medicament for stimulating an immune response.

18. A method of modulating the type and/or amount of cytokine production from an immune cell by administering *in vitro* the TLP-interacting agent of any one of claims 1-8.

19. Use of the TLP-interacting agent of any one of claims 1-8 in the manufacture of a medicament for modulating the type and/or amount of cytokine production from an immune cell.

20. The method of claim 18 or the use of claim 19, wherein proinflammatory cytokine production is stimulated and/or increased by the TLP-interacting agent.

21. A method of detecting the presence or expression level of TIGIT in a sample from a subject comprising contacting the sample with the TLP-interacting agent of any one of claims 1-13 and detecting the presence of the bound TLP-interacting agent.

22. The method of claim 21, wherein the TLP-interacting agent is an antibody or antigen-binding fragment thereof.

23. Use of the TLP-interacting agent of any one of claims 1-8 in the manufacture of a medicament for preventing an immune-related disease relating to aberrant immune cell response in a subject.

24. Use of the TLP-interacting agent of any one of claims 1-8 in the manufacture of a medicament for treating or lessening the severity of an immune-related disease relating to aberrant immune cell response in a subject.

25. A method of diagnosing an immune-related disease relating to aberrant immune cell response in a subject comprising determining the expression and/or activity of TIGIT in a sample from the subject and comparing the expression and/or activity of TIGIT to a reference amount of TIGIT expression and/or activity or the amount of TIGIT expression and/or activity in a sample from a normal subject.

26. The method of claim 25, wherein the determining comprises use of the TLP-interacting agent of any one of claims 1-13.

27. A method of assessing the severity of an immune-related disease relating to aberrant immune cell response in a subject comprising determining the expression and/or activity of TIGIT in a sample from the subject and comparing the expression and/or activity of TIGIT to a reference amount of TIGIT expression and/or activity or the amount of TIGIT expression and/or activity in a sample from a normal subject.

28. The method of claim 27, wherein the determining comprises use of the TLP-interacting agent of any one of claims 1-13.

29. An article of manufacture for use in the diagnosis or treatment of an immune-related disease, wherein the article of manufacture comprises the TLP-interacting agent of any one of claims 1-13.

30. The article of manufacture of claim 29, wherein the article of manufacture further comprises an instruction or a label for using the TLP-interacting agent to detect TIGIT in a sample from a subject.

31. The use of claim 23 or 24, the method of any one of claims 25-28, or the article of manufacture of claim 29 or 30, wherein the immune-related disease is selected from the group consisting of psoriasis, arthritis, inflammatory bowel disease, and cancer.