

glycan interactions can mediate, among other things, cell adhesion and modulate immune cell functions.

[0005] Activation of T cells, for example, occurs through the simultaneous engagement of the T-cell receptor and a co-stimulatory molecule (*e.g.*, CD28 or ICOS) on CD4⁺ T cells by the major histocompatibility complex (MHCII) peptide and co-stimulatory molecules on the antigen-presenting cell (APC). Both are required for production of an effective immune response. In the absence of co-stimulation, T-cell receptor signaling alone results in anergy. It is believed that the signaling pathways downstream from co-stimulatory molecules usually engage the PI3K pathway generating PIP3 at the plasma membrane and recruiting PH domain-containing signaling molecules like PDK1 that are essential for the activation of PKC- θ , and eventual IL-2 production. In addition, CD4⁺ cells are useful in the initial antigenic activation of naïve CD8 T cells, and sustaining memory CD8⁺ T cells in the aftermath of an acute infection. Therefore, activation of CD4⁺ T cells can be beneficial to the action of CD8⁺ T cells. However, an undesirable T-cell activation can result in the development of an inflammatory and/or autoimmune disorder in a subject.

[0006] Accordingly, there is a need in the art for compositions and methods for treating inflammatory and/or autoimmune disorders, for example, by reducing the number or activity of activated T cells in a subject with an inflammatory and/or autoimmune disorder.

SUMMARY

[0007] The disclosure is based, in part, upon the discovery that Siglec extracellular domains (ECDs) regardless of their “inhibitory” or “activating” cytoplasmic domains, including Siglec ECDs with reduced or no sialic acid binding activity, can act as a ligand and suppress immune responses (such as T cell activation) independent of sialoglycan interaction, by interacting with certain receptors on T cells potentially through protein-protein interaction. This discovery differs from the conventional understanding that Siglecs function as sialoglycan-sensing receptors and signal through their ITIM cytoplasmic domains or through association with DAP-10/DAP-12 to inhibit or activate certain immune functions. As discussed herein, the Siglec ECDs, which include functional fragments and variants of Siglec ECDs, can suppress the activation and/or activity of certain immune cells, *e.g.*, T cells. Accordingly, in certain examples, a protein comprising a Siglec ECD, or a functional fragment or variant thereof as described herein, can be administered to a subject in need thereof to treat an inflammatory and/or autoimmune disorder, such as fibrosis and arthritis.

[0008] In one aspect, the present disclosure provides a pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD comprises a mutation that reduces sialic acid binding activity. Accordingly, in one example, the disclosure provides a pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD comprises at least one (*e.g.*, 1, 2, 3 or 4) mutation that reduces or abolishes sialic acid binding activity. In certain examples, the at least one mutation results in the Siglec ECD having less than 50% (*e.g.*, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the sialic acid binding activity of a corresponding Siglec ECD lacking the mutation, *e.g.*, a corresponding wild-type Siglec ECD.

[0009] In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-2, Siglec-3, Siglec-4, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-12, Siglec-14, Siglec-15, and Siglec-16 ECD. In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-4, Siglec-6, Siglec-9, Siglec-11, and Siglec-15 ECD. In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-4, Siglec-6, Siglec-9, and Siglec-11 ECD.

[0010] In certain examples,

(a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is present in the region from amino acid 106 to 134 of SEQ ID NO: 15 (wild-type human Siglec-1);

(b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is present in the region from amino acid 110 to 135 of SEQ ID NO: 23 (wild-type human Siglec-2);

(c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is present in the region from amino acid 109 to 137 of SEQ ID NO: 25 (wild-type human Siglec-3);

(d) the Siglec ECD is a human Siglec-4 ECD, and the mutation is present in the region from amino acid 108 to 133 of SEQ ID NO: 33 (wild-type human Siglec-4);

(e) the Siglec ECD is a human Siglec-5 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 41 (wild-type human Siglec-5);

- (f) the Siglec ECD is a human Siglec-6 ECD, and the mutation is present in the region from amino acid 112 to 140 of SEQ ID NO: 43 (wild-type human Siglec-6);
- 5 (g) the Siglec ECD is a human Siglec-7 ECD, and the mutation is present in the region from amino acid 114 to 142 of SEQ ID NO: 51 (wild-type human Siglec-7);
- (h) the Siglec ECD is a human Siglec-8 ECD, and the mutation is present in the region from amino acid 115 to 149 of SEQ ID NO: 63 (wild-type human Siglec-8);
- 10 (i) the Siglec ECD is a human Siglec-9 ECD, and the mutation is present in the region from amino acid 110 to 138 of SEQ ID NO: 65 (wild-type human Siglec-9);
- (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 87 (wild-type human Siglec-10);
- 15 (k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 92 (wild-type human Siglec-11);
- (l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 100 (wild-type human Siglec-12);
- 20 (m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 102 (wild-type human Siglec-14);
- (n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is present in the region from amino acid 133 to 161 of SEQ ID NO: 104 (wild-type human Siglec-15); or
- 25 (o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is present in the region from amino acid 110 to 139 of SEQ ID NO: 114 (wild-type human Siglec-16).

[0011] In certain examples,

- (a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 116 of wild-type human Siglec-1 (*e.g.*, R116);
- 5 (b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-2 (*e.g.*, R120);
- (c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-3 (*e.g.*, R119);
- 10 (d) the Siglec ECD is a human Siglec-4 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 118 of wild-type human Siglec-4 (*e.g.*, R118);
- (e) the Siglec ECD is a human Siglec-5 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-5 (*e.g.*, R119);
- 15 (f) the Siglec ECD is a human Siglec-6 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 122 of wild-type human Siglec-6 (*e.g.*, R122);
- (g) the Siglec ECD is a human Siglec-7 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 124 of wild-type human Siglec-7 (*e.g.*, R124);
- 20 (h) the Siglec ECD is a human Siglec-8 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 109 of wild-type human Siglec-8 (*e.g.*, R128);
- (i) the Siglec ECD is a human Siglec-9 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-9 (*e.g.*, R120);
- 25 (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-10 (*e.g.*, R119);
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(k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 132 of wild-type human Siglec-11 (*e.g.*, R132);

(l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 125 of wild-type human Siglec-12 (*e.g.*, R125);

(m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-14 (*e.g.*, R119);

(n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 143 of wild-type human Siglec-15 (*e.g.*, R143); or

(o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-16 (*e.g.*, R120).

[0012] In another aspect, the present disclosure provides a pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD is selected from a human Siglec-6, Siglec-1, Siglec-2, Siglec-3, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD. In another example, the disclosure relates to a pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD is selected from a human Siglec-1, Siglec-2, Siglec-3, Siglec-6, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD. In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-6, and Siglec-11 ECD.

[0013] In certain examples, the Siglec ECD, or the functional fragment or variant thereof, and the serum half-life enhancer are covalently linked together in a fusion protein. In certain examples, the Siglec ECD, or the functional fragment or variant thereof, and the serum half-life enhancer are chemically conjugated together. In certain examples, the serum half-life enhancer is selected from an immunoglobulin Fc domain, transferrin, albumin, XTEN, a

homo-amino acid polymer (HAP), a proline-alanine-serine polymer (PAS), an elastin-like peptide (ELP), albumin binding domain, carboxy-terminal peptide (CTP), gelatin-like protein (GLK) fusion, and a polyethylene glycol (PEG). In certain examples, the serum half-life enhancer is an immunoglobulin Fc domain. In certain examples, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2,

5

IgD, IgE, or IgM Fc domain. In certain examples, a composition of claim 14, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain. In certain examples, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain. In certain examples, the Siglec ECD, or functional fragment or variant thereof, conjugated to the serum half-life enhancer is present as a dimer.

[0014] In certain examples, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 21, 31, 37, 39, 47, 49, 59, 61, 73, 75, 77, 81, 83, 85, 90, 96, 98, 108, 110, 117, 120, 122, 124, 126-135, 139, 141, 143, 145, 147, 149-151, 154, 155, and 158, or a functional fragment thereof.

[0015] In certain examples, the pharmaceutical composition, optionally comprising a pharmaceutically acceptable carrier, is disposed in a sterile container (*e.g.*, a bottle or vial). In certain examples, the pharmaceutical composition is lyophilized in the sterile container. In certain examples, the pharmaceutical composition is present as a solution in the sterile container. In certain examples, the sterile container has a label disposed thereon identifying the pharmaceutical composition contained in the container.

[0016] In certain examples, the disclosure relates to a method of treating an inflammatory and/or autoimmune disorder in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition as described herein.

[0017] In yet another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein in the preparation of a medicament for treating an inflammatory and/or autoimmune disorder in a subject in need thereof. In yet another aspect, the present disclosure provides a use of a Siglec extracellular domain (ECD), or a functional fragment or variant thereof in the preparation of a medicament for treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, wherein the Siglec ECD comprises a mutation that reduces sialic acid binding activity. In yet another aspect, the present disclosure provides a use of a Siglec extracellular domain (ECD) or a functional fragment or variant thereof in the preparation of a medicament for treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, wherein the Siglec ECD is selected from a human Siglec-6, Siglec-1, Siglec-2, Siglec-3, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD. In another example, the disclosure relates to a method of treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, the method comprising administering to the subject a Siglec

extracellular domain (ECD), or a functional fragment or variant thereof, wherein the Siglec ECD comprises at least one (*e.g.*, 1, 2, 3, or 4) mutation that reduces sialic acid binding activity. In certain examples, the at least one mutation results in the Siglec ECD, or the functional fragment or variant thereof, having less than 50% (*e.g.*, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the sialic acid binding activity of a corresponding wild-type Siglec ECD.

[0018] In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-2, Siglec-3, Siglec-4, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-12, Siglec-14, Siglec-15, and Siglec-16 ECD. In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-4, Siglec-6, Siglec-9, Siglec-11, and Siglec-

15 ECD. In certain examples, the Siglec ECD is selected from a human Siglec-1, Siglec-4, Siglec-6, Siglec-9, and Siglec-11 ECD.

[0019] In certain examples:

- 5 (a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is present in the region from amino acid 106 to 134 of SEQ ID NO: 15 (wild-type human Siglec-1);
- (b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is present in the region from amino acid 110 to 135 of SEQ ID NO: 23 (wild-type human Siglec-2);
- 10 (c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is present in the region from amino acid 109 to 137 of SEQ ID NO: 25 (wild-type human Siglec-3);
- (d) the Siglec ECD is a human Siglec-4 ECD, and the mutation is present in the region from amino acid 108 to 133 of SEQ ID NO: 33 (wild-type human Siglec-4);
- 15 (e) the Siglec ECD is a human Siglec-5 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 41 (wild-type human Siglec-5);
- (f) the Siglec ECD is a human Siglec-6 ECD, and the mutation is present in the region from amino acid 112 to 140 of SEQ ID NO: 43 (wild-type human Siglec-6);
- 20 (g) the Siglec ECD is a human Siglec-7 ECD, and the mutation is present in the region from amino acid 114 to 142 of SEQ ID NO: 51 (wild-type human Siglec-7);
- 25 (h) the Siglec ECD is a human Siglec-8 ECD, and the mutation is present in the region from amino acid 115 to 149 of SEQ ID NO: 63 (wild-type human Siglec-8);
- 30 (i) the Siglec ECD is a human Siglec-9 ECD, and the mutation is present in the region from amino acid 110 to 138 of SEQ ID NO: 65 (wild-type human Siglec-9);

- (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 87 (wild-type human Siglec-10);
- 5 (k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 92 (wild-type human Siglec-11);
- (l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 100 (wild-type human Siglec-12);
- 10 (m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 102 (wild-type human Siglec-14);
- (n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is present in the region from amino acid 133 to 161 of SEQ ID NO: 104 (wild-type human Siglec-15); or
- 15 (o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is present in the region from amino acid 110 to 139 of SEQ ID NO: 114 (wild-type human Siglec-16).

[0020] In certain examples:

- 20 (a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 116 of wild-type human Siglec-1 (*e.g.*, R116);
- (b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human
- 25 Siglec-2 (*e.g.*, R120);
- (c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-3 (*e.g.*, R119);
- (d) the Siglec ECD is a human Siglec-4 ECD, and the mutation is a substitution of an
- 30 arginine residue at a position corresponding to position 118 of wild-type human Siglec-4 (*e.g.*, R118);

- (e) the Siglec ECD is a human Siglec-5 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-5 (*e.g.*, R119);
- 5 (f) the Siglec ECD is a human Siglec-6 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 122 of wild-type human Siglec-6 (*e.g.*, R122);
- (g) the Siglec ECD is a human Siglec-7 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 124 of wild-type human Siglec-7 (*e.g.*, R124);
- 10 (h) the Siglec ECD is a human Siglec-8 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 109 of wild-type human Siglec-8 (*e.g.*, R128);
- (i) the Siglec ECD is a human Siglec-9 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-9 (*e.g.*, R120);
- 15 (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-10 (*e.g.*, R119);
- (k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 132 of wild-type human Siglec-11 (*e.g.*, R132);
- 20 (l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 125 of wild-type human Siglec-12 (*e.g.*, R125);
- (m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-14 (*e.g.*, R119);
- 25 (n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 143 of wild-type human Siglec-15 (*e.g.*, R143); or
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(o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-16 (*e.g.*, R120).

[0021] In another example, the disclosure relates to a method of treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, the method comprising administering to the subject a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, wherein the Siglec ECD is selected from a human Siglec-1, Siglec-2, Siglec-3, Siglec-6, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD.

[0022] In certain examples, the Siglec ECD, or the functional fragment or variant thereof, comprises a mutation that reduces sialic acid binding activity.

[0023] In certain examples, the mutation results in the Siglec ECD, or the functional fragment or variant thereof, having less than 50% (*e.g.*, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the sialic acid binding activity of a corresponding Siglec ECD without the mutation, *e.g.*, a corresponding wild-type Siglec ECD.

[0024] In certain examples:

(a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is present in the region from amino acid 106 to 134 of SEQ ID NO: 15 (wild-type human Siglec-1);

(b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is present in the region from amino acid 110 to 135 of SEQ ID NO: 23 (wild-type human Siglec-2);

(c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is present in the region from amino acid 109 to 137 of SEQ ID NO: 25 (wild-type human Siglec-3);

(d) the Siglec ECD is a human Siglec-6 ECD, and the mutation is present in the region from amino acid 112 to 140 of SEQ ID NO: 43 (wild-type human Siglec-6);

- (e) the Siglec ECD is a human Siglec-7 ECD, and the mutation is present in the region from amino acid 114 to 142 of SEQ ID NO: 51 (wild-type human Siglec-7);
- 5 (f) the Siglec ECD is a human Siglec-8 ECD, and the mutation is present in the region from amino acid 115 to 149 of SEQ ID NO: 63 (wild-type human Siglec-8);
- (g) the Siglec ECD is a human Siglec-10 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 87 (wild-type human Siglec-10);
- 10 (h) the Siglec ECD is a human Siglec-11 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 92 (wild-type human Siglec-11);
- (i) the Siglec ECD is a human Siglec-12 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 100 (wild-type human Siglec-12);
- 15 (j) the Siglec ECD is a human Siglec-14 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 102 (wild-type human Siglec-14);
- (k) the Siglec ECD is a human Siglec-16 ECD, and the mutation is present in the region from amino acid 110 to 139 of SEQ ID NO: 114 (wild-type human Siglec-16).
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[0025] In certain examples:

- (a) the Siglec ECD is a human Siglec-1 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 116 of wild-type human Siglec-1 (*e.g.*, R116);
- 25 (b) the Siglec ECD is a human Siglec-2 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-2 (*e.g.*, R120);
- (c) the Siglec ECD is a human Siglec-3 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-3 (*e.g.*, R119);
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- (d) the Siglec ECD is a human Siglec-6 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 122 of wild-type human Siglec-6 (*e.g.*, R122);
- 5 (e) the Siglec ECD is a human Siglec-7 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 124 of wild-type human Siglec-7 (*e.g.*, R124);
- (f) the Siglec ECD is a human Siglec-8 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 109 of wild-type human Siglec-8 (*e.g.*, R128);
- 10 (g) the Siglec ECD is a human Siglec-10 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-10 (*e.g.*, R119);
- (h) the Siglec ECD is a human Siglec-11 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 132 of wild-type human Siglec-11 (*e.g.*, R132);
- 15 (i) the Siglec ECD is a human Siglec-12 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 125 of wild-type human Siglec-12 (*e.g.*, R125);
- (j) the Siglec ECD is a human Siglec-14 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-14 (*e.g.*, R119); or
- 20 (k) the Siglec ECD is a human Siglec-16 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-16 (*e.g.*, R120).
- 25 **[0026]** In certain examples, the Siglec ECD, or the functional fragment or variant thereof, is conjugated to a serum half-life enhancer. In certain examples, the Siglec ECD, or the functional fragment or variant thereof, and the serum half-life enhancer are covalently linked together in a fusion protein. In certain examples, the Siglec ECD, or the functional fragment or variant thereof, and serum half-life enhancer are chemically conjugated together. In
- 30 certain examples, the serum half-life enhancer is selected from an immunoglobulin Fc domain, transferrin, albumin, XTEN, a homo-amino acid polymer (HAP), a proline-alanine-

serine polymer (PAS), an elastin-like peptide (ELP), albumin binding domain, carboxy-terminal peptide (CTP), gelatin-like protein (GLK) fusion, and a polyethylene glycol (PEG). In certain examples, the serum half-life enhancer is an immunoglobulin Fc domain. In certain examples, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain. In certain examples, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain. In certain examples, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

[0027] In certain examples, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 21, 31, 37, 39, 47, 49, 59, 61, 73, 75, 77, 81, 83, 85, 90, 96, 98, 108, 110, 117, 120, 122, 124, 126-135, 139, 141, 143, 145, 147, 149-151, 154, 155, and 158, or a functional fragment thereof.

[0028] In certain examples, the Siglec ECD or the Siglec ECD and the serum half-life enhancer is present as a dimer.

[0029] These and other examples and features of the disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0030] The invention can be more completely understood with reference to the following drawings, in which:

[0031] **FIGURE 1** depicts a schematic representation of certain exemplary native Siglec proteins, including murine proteins, human proteins, and a group of “conserved” protein that are common between murine and human Siglecs.

[0032] **FIGURE 2A** depicts a schematic representation of ITIM-containing Siglec molecules that function like a “PD-1-like receptor” and wherein the ITIM-containing Siglecs function as receptors and suppress immune function by binding to sialoglycan on a target cell, *e.g.*, a cancer cell. **FIGURE 2B** depicts a schematic representation of Siglec molecules that function like a “PD-L1-like ligand” regardless of their intracellular domains being “inhibitory” (ITIM) or “activating” (DAP-10/12) and wherein such Siglecs function as ligands and suppress immune responses by binding to currently unidentified receptor(s) on T cells in a sialoglycan-independent manner.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD comprises a mutation that reduces sialic acid binding activity.
2. The pharmaceutical composition of claim 1, wherein the mutation results in the Siglec ECD having less than 50% of the sialic acid binding activity of a corresponding Siglec ECD without the mutation(s).
3. The pharmaceutical composition of claim 1 or 2, wherein the Siglec ECD is selected from a human Siglec-9, Siglec-1, Siglec-2, Siglec-3, Siglec-4, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, Siglec-15, and Siglec-16 ECD.
4. The pharmaceutical composition of any one of claims 1-3, wherein the Siglec ECD is selected from a human Siglec-9, Siglec-1, Siglec-4, Siglec-6, and Siglec-11 ECD.
5. The pharmaceutical composition of any one of claims 1-4, wherein:
 - (a) the Siglec ECD is a human Siglec-9 ECD, and the mutation is present in the region from amino acid 110 to 138 of SEQ ID NO: 65 (wild-type human Siglec-9);
 - (b) the Siglec ECD is a human Siglec-1 ECD, and the mutation is present in the region from amino acid 106 to 134 of SEQ ID NO: 15 (wild-type human Siglec-1);
 - (c) the Siglec ECD is a human Siglec-2 ECD, and the mutation is present in the region from amino acid 110 to 135 of SEQ ID NO: 23 (wild-type human Siglec-2);
 - (d) the Siglec ECD is a human Siglec-3 ECD, and the mutation is present in the region from amino acid 109 to 137 of SEQ ID NO: 25 (wild-type human Siglec-3);

- (e) the Siglec ECD is a human Siglec-4 ECD, and the mutation is present in the region from amino acid 108 to 133 of SEQ ID NO: 33 (wild-type human Siglec-4);
- (f) the Siglec ECD is a human Siglec-5 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 41 (wild-type human Siglec-5);
- (g) the Siglec ECD is a human Siglec-6 ECD, and the mutation is present in the region from amino acid 112 to 140 of SEQ ID NO: 43 (wild-type human Siglec-6);
- (h) the Siglec ECD is a human Siglec-7 ECD, and the mutation is present in the region from amino acid 114 to 142 of SEQ ID NO: 51 (wild-type human Siglec-7);
- (i) the Siglec ECD is a human Siglec-8 ECD, and the mutation is present in the region from amino acid 115 to 149 of SEQ ID NO: 63 (wild-type human Siglec-8);
- (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 87 (wild-type human Siglec-10);
- (k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 92 (wild-type human Siglec-11);
- (l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is present in the region from amino acid 122 to 151 of SEQ ID NO: 100 (wild-type human Siglec-12);
- (m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is present in the region from amino acid 109 to 138 of SEQ ID NO: 102 (wild-type human Siglec-14);

- (n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is present in the region from amino acid 133 to 161 of SEQ ID NO: 104 (wild-type human Siglec-15); or
 - (o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is present in the region from amino acid 110 to 139 of SEQ ID NO: 114 (wild-type human Siglec-16).
6. The pharmaceutical composition of any one of claims 1-4, wherein:
- (a) the Siglec ECD is a human Siglec-9 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-9 (*e.g.*, R120);
 - (b) the Siglec ECD is a human Siglec-1 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 116, of wild-type human Siglec-1 (*e.g.*, R116);
 - (c) the Siglec ECD is a human Siglec-2 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-2 (*e.g.*, R120);
 - (d) the Siglec ECD is a human Siglec-3 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-3 (*e.g.*, R119);
 - (e) the Siglec ECD is a human Siglec-4 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 118 of wild-type human Siglec-4 (*e.g.*, R118);
 - (f) the Siglec ECD is a human Siglec-5 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-5 (*e.g.*, R119);
 - (g) the Siglec ECD is a human Siglec-6 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 122 of wild-type human Siglec-6 (*e.g.*, R122);

- (h) the Siglec ECD is a human Siglec-7 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 124 of wild-type human Siglec-7 (*e.g.*, R124);
- (i) the Siglec ECD is a human Siglec-8 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 109 of wild-type human Siglec-8 (*e.g.*, R128);
- (j) the Siglec ECD is a human Siglec-10 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-10 (*e.g.*, R119);
- (k) the Siglec ECD is a human Siglec-11 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 132 of wild-type human Siglec-11 (*e.g.*, R132);
- (l) the Siglec ECD is a human Siglec-12 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 125 of wild-type human Siglec-12 (*e.g.*, R125);
- (m) the Siglec ECD is a human Siglec-14 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 119 of wild-type human Siglec-14 (*e.g.*, R119);
- (n) the Siglec ECD is a human Siglec-15 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 143 of wild-type human Siglec-15 (*e.g.*, R143); or
- (o) the Siglec ECD is a human Siglec-16 ECD, and the mutation is a substitution of an arginine residue at a position corresponding to position 120 of wild-type human Siglec-16 (*e.g.*, R120).

7. A pharmaceutical composition comprising a Siglec extracellular domain (ECD), or a functional fragment or variant thereof, conjugated to a serum half-life enhancer that increases the serum half-life of the Siglec ECD when administered to a subject, wherein the Siglec ECD is selected from a human Siglec-6, Siglec-1, Siglec-2, Siglec-3, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD.

8. The pharmaceutical composition of claim 7, wherein the Siglec ECD is selected from a human Siglec-6, Siglec-1, and Siglec-11 ECD.
9. The pharmaceutical composition of any one of claims 1-8, wherein the Siglec ECD, or the functional fragment or variant thereof, and the serum half-life enhancer are covalently linked together in a fusion protein.
10. The pharmaceutical composition of claim 9, wherein the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 21, 31, 37, 39, 47, 49, 59, 61, 73, 75, 77, 81, 83, 85, 90, 96, 98, 108, 110, 117, 120, 122, 124, 126-135, 139, 141, 143, 145, 147, 149-151, 154, 155, and 158, or a functional fragment thereof.
11. The pharmaceutical composition of any one of claims 1-8, wherein the Siglec ECD, or the functional fragment or variant thereof, and the serum half-life enhancer are chemically conjugated together.
12. The pharmaceutical composition of any one of claims 1-9 and 11, wherein the serum half-life enhancer is selected from an immunoglobulin Fc domain, transferrin, albumin, XTEN, a homo-amino acid polymer (HAP), a proline-alanine-serine polymer (PAS), an elastin-like peptide (ELP), albumin binding domain, carboxy-terminal peptide (CTP), gelatin-like protein (GLK), and a polyethylene glycol (PEG).
13. The pharmaceutical composition of claim 12, wherein the serum half-life enhancer is an immunoglobulin Fc domain.
14. The pharmaceutical composition of claim 13, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.
15. The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.
16. The pharmaceutical composition of any one of claims 1-15, wherein the Siglec ECD, or the functional fragment or variant thereof, conjugated to the serum half-life enhancer is present as a dimer.
17. The pharmaceutical composition of any one of claims 1-16, wherein the pharmaceutical composition is disposed in a sterile container.

18. The pharmaceutical composition of claim 17, wherein the pharmaceutical composition is lyophilized or present as a solution in the sterile container.
19. Use of the pharmaceutical composition of any one of claims 1-18 in the preparation of a medicament for treating an inflammatory and/or autoimmune disorder in a subject in need thereof.
20. Use of a Siglec extracellular domain (ECD), or a functional fragment or variant thereof in the preparation of a medicament for treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, wherein the Siglec ECD comprises a mutation that reduces sialic acid binding activity.
21. The use of claim 20, wherein the Siglec ECD is selected from a human Siglec-9, Siglec-1, Siglec-2, Siglec-3, Siglec-4, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, Siglec-15, and Siglec-16 ECD.
22. Use of a Siglec extracellular domain (ECD) or a functional fragment or variant thereof in the preparation of a medicament for treating an inflammatory disorder and/or an autoimmune disorder in a subject in need thereof, wherein the Siglec ECD is selected from a human Siglec-6, Siglec-1, Siglec-2, Siglec-3, Siglec-7, Siglec-8, Siglec-10, Siglec-11, Siglec-12, Siglec-14, and Siglec-16 ECD.
23. The use of claim 22, wherein the Siglec ECD, or the functional fragment or variant thereof, is conjugated to a serum half-life enhancer.