[NPL71] Warmerdam et al., J. Exp. Med. 172:19-25 (1990)

Summary

[0037] The disclosure provides anti-myostatin antibodies and methods of using the same. The disclosure also provides proteins containing a variant Fc region and methods of using the same.

In one aspect, the present disclosure provides for an isolated antibody that binds to latent myostatin, wherein the antibody inhibits activation of myostatin, wherein the antibody binds to the same epitope as an antibody comprising the VH sequence of SEQ ID NO: 12 and the VL sequence of SEQ ID NO: 14, wherein the epitope is within a fragment consisting of amino acids 81-100 of myostatin propeptide (SEQ ID NO: 78), and wherein the antibody blocks the proteolytic release and the spontaneous release of mature myostatin from latent myostatin.

In one example, there is provided an isolated antibody that binds to latent myostatin, wherein the antibody inhibits activation of myostatin, wherein the antibody binds to the same epitope as an antibody comprising the VH sequence of SEQ ID NO: 12 and the VL sequence of SEQ ID NO: 14, and wherein the antibody blocks the proteolytic release and the spontaneous release of mature myostatin from latent myostatin.

In another example, there is provided an isolated nucleic acid encoding the antibody as disclosed herein.

In another example, there is provided a host cell comprising the nucleic acid of disclosed herein.

In another example, there is provided a method of producing an anti-myostatin antibody comprising culturing the host cell as disclosed herein so that the antibody is produced.

In yet another example, there is provided a pharmaceutical formulation comprising the antibody as disclosed herein and a pharmaceutically acceptable carrier.

[0038] In some examples, an isolated anti-myostatin antibody of the present disclosure binds to latent myostatin. In further examples, the antibody binds to an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). In some examples, an isolated anti-myostatin antibody of the present disclosure inhibits activation of myostatin. In further examples, the antibody blocks the release of mature myostatin from latent myostatin. In further examples, the antibody blocks the proteolytic release of mature myostatin. In further examples, the antibody blocks the spontaneous release of mature myostatin. In further examples, the antibody does not bind to mature myostatin. In further examples, the antibody binds to the same epitope as an antibody described in Table 13. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 13. In further examples, the antibody binds to the same epitope as an antibody described in Table 2a. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a. In further examples, the antibody binds to the same epitope as an antibody described in Table 11a. . In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 11a. In further examples, the antibody binds to the same epitope as an antibody described in Table 2a, 11a, or 13. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a, 11a, or 13.

[0039] In some examples, an isolated anti-myostatin antibody of the present disclosure binds to latent myostatin with higher affinity at neutral pH than at acidic pH. In some examples, the anti-myostatin antibody binds to latent myostatin with higher affinity at pH7.4 than at pH5.8.

In some examples, an isolated anti-myostatin antibody of the present disclosure binds to a polypeptide fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78) with higher affinity at pH7.4 than at pH5.8. In some examples, the antibody binds to the same myostatin epitope as an antibody described in Table 13 with a higher affinity at neutral pH than at acidic pH. In additional examples, an anti-myostatin antibody binds to the same epitope as an antibody described in Table 13 with a higher affinity at pH7.4 than at pH5.8. In further examples, the antibody binds to the same epitope as an antibody

tionally comprises (a) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131).

[0045] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131). In a further example, the antibody further comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[0046] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises a heavy chain variable domain framework FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 132-134; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 135-136; FR3 comprising the amino acid sequence of SEQ ID NO: 137; and FR4 comprising the amino acid sequence of SEQ ID NO: 138. In some examples, an isolated anti-myostatin antibody of the present disclosure comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[0047] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99; or (c) a VH sequence as in (a) and a VL sequence as in (b). In further examples, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further examples, the antibody comprises a VL sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99. In some embodimments, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further embodimments, the antibody comprises a

VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; and a VL sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99.

[0048] The disclosure also provides isolated nucleic acids encoding an anti-myostatin antibody of the present disclosure. The disclosure also provides host cells comprising a nucleic acid of the present disclosure. The disclosure also provides a method of producing an antibody comprising culturing a host cell of the present disclosure so that the antibody is produced.

[0049] In some example the disclosure provides a method of producing an anti-myostatin antibody comprising: (a) culturing a host cell of the present disclosure so that the antibody is produced; or (b) immunizing an animal against a polypeptide, wherein the polypeptide comprises the region corresponding to amino acids at positions 21 to 100 of myostatin propeptide (SEQ ID NO: 78).

[0050] The disclosure further provides a method of producing an anti-myostatin antibody. In some examples, the method comprises immunizing an animal against a polypeptide, wherein the polypeptide comprises the region corresponding to amino acids at positions 21-100 of myostatin propeptide (SEQ ID NO: 78).

[0051] The disclosure also provides a pharmaceutical formulation comprising an anti-myostatin antibody of the present disclosure and a pharmaceutically acceptable carrier.

[0052] The disclosure provides polypeptides comprising variant Fc regions and methods of making and using the same.

[0053] In one example, the disclosure provides Fc gamma RIIB-binding polypeptides comprising variant Fc regions and methods of using the same. In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid alteration in a parent Fc region. In further examples, the ratio of [KD value of the parent Fc region for monkey Fc gamma RIIb]/[KD value of the variant Fc region for monkey Fc gamma RIIb] is 2.0 or greater. In further examples, the ratio of [KD value of the parent Fc region for monkey Fc gamma RIIIa]/[KD value of the variant Fc region for monkey Fc gamma RIIIa] is 0.5 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIb]/[KD value of the variant Fc region for human Fc gamma RIIb] is 2.0 or greater. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIIa]/[KD value of the variant Fc region for human Fc gamma RIIIa] is 0.5 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIa (type H)]/[KD value of the variant Fc region for human Fc gamma RIIa (type H)] is 5.0 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIa (type R)]/[KD value of the variant Fc region for human Fc gamma RIIa (type R)] is 5.0 or smaller. In another example, the KD value of the variant Fc region for monkey Fc gamma RIIb is

$1.0x10^{-6}$ M or smaller. In another example, the KD value of the variant Fc region for monkey Fc gamma RIIIa is $5.0x10^{-7}$ M or greater. In another example, the KD value of the variant Fc region for human Fc gamma RIIb is $2.0x10^{-6}$ M or smaller. In another example, the KD value of the variant Fc region for human Fc gamma RIIIa is $1.0x10^{-6}$ M or greater. In another example, the KD value of the variant Fc region for human Fc gamma RIIa (type H) is $1.0x10^{-7}$ M or greater. In another example, the KD value of the variant Fc region for human Fc gamma RIIa (type R) is $2.0x10^{-7}$ M or greater.

[0054] In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 233, 234, 235, 236, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396, according to EU numbering.

[0055] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: (i) position 231, 232, 233, 234, 235, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396; (ii) position: 231, 232, 235, 239, 268, 295, 298, 326, 330, and 396; or (iii) position 268, 295, 326, and 330; according to EU numbering.

[0056] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 233, 234, 235, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396, according to EU numbering.

[0057] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 235, 239, 268, 295, 298, 326, 330, and 396, according to EU numbering.

[0058] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 268, 295, 326, and 330 according to EU numbering.

[0059] In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid selected from the

group consisting of: (a) Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 231; (b) Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 232; (c) Asp at position 233; (d) Trp, Tyr at position 234; (e) Trp at position 235; (f) Ala, Asp, Glu, His, Ile, Leu, Met, Asn, Gln, Ser, Thr, Val at position 236; (g) Asp, Tyr at position 237; (h) Glu, Ile, Met, Gln, Tyr at position 238; (i) Ile, Leu, Asn, Pro, Val at position 239; (j) Ile at position 264; (k) Phe at position 266; (l) Ala, His, Leu at position 267; (m) Asp, Glu at position 268; (n) Asp, Glu, Gly at position 271; (o) Leu at position 295; (p) Leu at position 298; (q) Glu, Phe, Ile, Leu at position 325; (r) Thr at position 326; (s) Ile, Asn at position 327; (t) Thr at position 328; (u) Lys, Arg at position 330; (v) Glu at position 331; (w) Asp at position 332; (x) Asp, Ile, Met, Val, Tyr at position 334; and (y) Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 396; according to EU numbering.

[0060] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least one amino acid selected from the group consisting of: (a) Gly, Thr at position 231; (b) Asp at position 232; (c) Trp at position 235; (d) Asn, Thr at position 236; (e) Val at position 239; (f) Asp, Glu at position 268; (g) Leu at position 295; (h) Leu at position 298; (i) Thr at position 326; (j) Lys, Arg at position 330, and (k) Lys, Met at position 396; according to EU numbering.

[0061] In another example, the disclosure provides a polypeptide comprising an isoelectric point (pI)-increased variant Fc region and a method of using the same. In some examples, a polypeptide comprising a variant Fc region with increased pI comprises at least two amino acid alterations in a parent Fc region. In further examples, each of the amino acid alterations increases the isoelectric point (pI) of the variant Fc region compared with that of the parent Fc region. In further examples, the amino acid can be exposed on the surface of the variant Fc region. In further examples, a polypeptide comprises the variant Fc region and an antigen-binding domain. In further examples, antigen-binding activity of the antigen-binding domain changes according to ion concentration conditions. In further examples, the variant Fc region with increased pI of the present disclosure comprises at least two amino acid alterations of at least two positions selected from the group consisting of: 285, 311, 312, 315, 318, 333, 335, 337, 341, 342, 343, 384, 385, 388, 390, 399, 400, 401, 402, 413, 420, 422, and 431 according to EU numbering. In further examples, the variant Fc region with increased pI comprises Arg or Lys at each of the positions selected.

[0062] In some examples, a variant Fc region of the present disclosure comprises amino acid alterations described in Tables 14-30.

[0063] In some examples, a polypeptide comprises a variant Fc region of the present

CLAIMS

1. An isolated antibody that binds to latent myostatin, wherein the antibody inhibits activation of myostatin, wherein the antibody binds to the same epitope as an antibody comprising the VH sequence of SEQ ID NO: 12 and the VL sequence of SEQ ID NO: 14, wherein the epitope is within a fragment consisting of amino acids 81-100 of myostatin propeptide (SEQ ID NO: 78), and wherein the antibody blocks the proteolytic release and the spontaneous release of mature myostatin from latent myostatin.

2. The antibody of claim 1, which binds to latent myostatin with higher affinity at neutral pH than at acidic pH, or which binds to latent myostatin with higher affinity at pH7.4 than at pH5.8.

3. The antibody of claim 1 or 2, which is (a) a monoclonal antibody, (b) a human, humanized, or chimeric antibody; (c) a full length IgG antibody or (d) an antibody fragment that binds to myostatin.

4. An isolated nucleic acid encoding the antibody of any one of claims 1 to 3.

5. A host cell comprising the nucleic acid of claim 4.

6. A method of producing an anti-myostatin antibody comprising:
culturing the host cell of claim 5 so that the antibody is produced.

7. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 3 and a pharmaceutically acceptable carrier.