binding have been developed.

[0006] Therefore, what is needed are specific inhibitors of fibrinogen/Mac-1 binding that reduce the pro-inflammatory affects of fibrinogen in the brain and elsewhere in a subject while at the same time retaining the beneficial affects of fibrinogen in blood clotting.

SUMMARY

[0007] The present disclosure~~invention~~ provides an isolated antibody that binds a fibrin or fibrinogen γC domain. In certain aspects of the disclosure~~invention~~, the antibody exhibits greater than 20% inhibition of microglial adhesion to the fibrin or fibrinogen γC domain. In another aspect, the anbibody exhibits greater than 50% inhibition of Mac-1 binding to the fibrin or fibrinogen γC domain. In yet another aspect, the antibody suppresses clinical symptoms of Experimental Autoimmune Encephalomyelitis (EAE) at the time of the relapse.

[0008] In various embodiments, the antibody binds an $\gamma^{377-395}$ epitope of the fibrin or fibrinogen γC domain. The antibody of the disclosure~~invention~~ can alternatively bind an $\gamma^{190-202}$ epitope of the fibrin or fibrinogen γC domain. Such antibodies are monoclonal antibodies, and in various aspects humanized antibodies or human antibodies.

[0009] In various aspects of the disclosure~~invention~~, the antibody comprises a light chain with three complementarity determining regions comprising an amino acid sequence including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4). In various aspects, the antibody comprises a heavy chain with three complementarity determining regions comprising an amino acid sequence including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8). In certain instances, the antibody comprises a light chain with three complementarity determining regions comprising an amino acid sequence including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain with three complementarity determining regions comprising an amino acid sequence including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8).

[0010] In various aspects, the antibodies above comprise a light chain variable amino acid sequence of SEQ ID NO:1. In various aspects, the antibodies above comprise a heavy chain variable amino acid sequence of SEQ ID NO:5. In yet another aspect, the antibodies above comprise both a light chain variable amino acid sequence of SEQ ID NO:1 and a heavy chain variable amino acid sequence of SEQ ID NO:5.

[0011] In yet another aspect of the present disclosure~~invention~~, the antibodies above comprise, individually, a light chain with three complementarity determining regions comprising an amino acid sequence having at least 80% sequence identity to the

sequences including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain with three complementarity determining regions comprising an amino acid sequence having at least 80% sequence identity to the sequences including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8); and wherein the light chain complementarity domains and heavy chain complementarity domains retain Mac-1 binding ability.

[0012] In yet another aspect of the present disclosure~~invention~~, the antibodies above comprise, individually, a light chain with three complementarity determining regions comprising an amino acid sequence having at least 90% sequence identity to the sequences including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain with three complementarity determining regions comprising an amino acid sequence having at least 90% sequence identity to the sequences including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8); and wherein the light chain complementarity domains and heavy chain complementarity domains retain Mac-1 binding ability.

[0013] In yet another aspect of the present disclosure~~invention~~, the antibodies above comprise, individually, a light chain with three complementarity determining regions comprising an amino acid sequence having at least 95% sequence identity to the sequences including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain with three complementarity determining regions comprising an amino acid sequence having at least 95% sequence identity to the sequences including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8); and wherein the light chain complementarity domains and heavy chain complementarity domains retain Mac-1 binding ability.

[0014] In yet another aspect of the present disclosure~~invention~~, the antibodies above comprise, individually, a light chain with three complementarity determining regions comprising an amino acid sequence having at least 99% sequence identity to the sequences including RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain with three complementarity determining regions comprising an amino acid sequence having at least 99% sequence identity to the sequences including GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8); and wherein the light chain complementarity domains and heavy chain complementarity domains retain Mac-1 binding ability.

[0014a] In yet another aspect, there is provided an isolated antibody that binds a fibrin or fibrinogen γC domain, wherein said antibody comprises, individually, a light chain comprising an amino acid sequence having at least 80% sequence identity to the sequence consisting of RSSKSLLHSSGITYLS (SEQ ID NO:2), QMSNLAS (SEQ ID NO:3), and AQNLELPLT (SEQ ID NO:4) and a heavy chain comprising an amino acid sequence having at least 80% sequence identity to the sequence consisting of GYTFTSYWIH (SEQ ID NO:6), LIDPSDSYTNYNQKFRG (SEQ ID NO:7), and SDPTGC (SEQ ID NO:8); and wherein the antibody exhibits at least 20% inhibition of microglial adhesion to the fibrin or fibrinogen γC domain.

[0014b] In yet another aspect, there is provided the use of the antibody as defined herein for the manufacture of a medicament to alleviate a symptom of a pathology associated with Mac-1 binding to fibrin or Mac-1 binding with fibrinogen, wherein said antibody is formulated for administration to said subject.

[0015] A method is also provided for alleviating a symptom of a pathology associated with Mac-1 binding to fibrin or Mac-1 binding with fibrinogen, the method comprising administering the antibody of claim 1 to a subject in which such alleviation is desired in an amount sufficient to alleviate the symptom of the pathology in the subject.

In various aspects of this method, the subject is a human. In various aspects, the pathology includes multiple sclerosis, spinal cord injury, Alzheimer's Disease, stroke, Rheumatoid Arthritis and cancer.

[0016] A pharmaceutical composition is also provided comprising the antibodies above and a pharmaceutically acceptable carrier. In another aspect, a kit is provided comprising the the antibodies above. In yet another aspect, a vector is provided comprising a nucleic acid sequence encoding a fibrin $\gamma^{377-395}$ epitope, CKKTTMKIIPFNRLTIG (SEQ ID NO:18), or a biologically active derivative thereof. In another aspect, a cell is provided comprising the vector.

[0017] In yet another aspect of the disclosure~~invention~~, a method is provided for generating an antibody that immunospecifically binds to a fibrin $\gamma^{377-395}$ epitope, or a biologically active derivative thereof, the method comprising: administering to a subject a first dosage of the cell of claim 23, wherein the first dosage is sufficient to generate an immune response in said subject. In various aspects, the method can further comprise the step of administering to said subject a second dosage of said cell, wherein said second dosage is sufficient to generate an immune response in said subject. In various aspects, the antibody produced inhibits fibrin/Mac-1 binding in said subject.

[0018] In another aspect, a method is provided for screening for a ligand that binds a Mac-1 receptor and modulates Mac-1 receptor activity, the method comprising: (a) providing the antibody of claim 1; (b) contacting a fibrin $\gamma^{377-395}$ epitope CKKTTMKIIPFNRLTIG (SEQ ID NO:18), or a biologically active derivative thereof, and forming an antibody/polypeptide complex; (c) contacting the antibody/polypeptide complex with a composition comprising a candidate compound; and (d) determining whether the candidate compound binds the monoclonal antibody; whereby, binding of the candidate compound indicates that said candidate compound is a ligand that modulates Mac-1 receptor activity.

[0019] These and other features, aspects and advantages of the present teachings will become better understood with reference to the following description, examples and appended claims.

DRAWINGS

[0020] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0021] Figure 1. Monoclonal antibody binding as assessed by absorbance measurements at 595nm compared to commercially available blocking antibody to Mac-1 (M1/70).

[0022] Figure 2. Results of ELISA measuring monoclonal antibody binding to