Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration

Jae Kyu Ryu1, Victoria A. Rafalski1,13, Anke Meyer-Franke1,13, Ryan A. Adams2, Suresh B. Poda3, Pamela E. Rios Coronado1, Lars Østergaard Pedersen4, Veena Menon5, Kim M. Baeten1, Shoana L. Sikorski2, Catherine Bedard1, Kristina Hanspers1, Sophia Bardehle1, Andrew S. Mendiola1, Dimitrios Davalos1,12, Michael R. Machado1, Justin P. Chan2, Ioanna Plastira3,1,5, Mark A. Petersen1,6, Samuel J. Pfaff7, Kenny K. Ang7, Kenneth K. Hallenbeck7, Catriona Syme1, Hiroyuki Hakoizaki8, Mark H. Ellisman8,9, Raymond A. Swanson10,11, Scott S. Zamvil11, Michelle R. Arkin7, Stevin H. Zorn3, Alexander R. Pico1, Lennart Mucke1,11, Stephen B. Freedman1, Jeffrey B. Stavenhagen4, Robert B. Nelson3 and Katerina Akassoglou1,2,11*

Activation of innate immunity and deposition of blood-derived fibrin in the central nervous system (CNS) occur in autoimmune and neurodegenerative diseases, including multiple sclerosis (MS) and Alzheimer’s disease (AD). However, the mechanisms that link disruption of the blood-brain barrier (BBB) to neurodegeneration are poorly understood, and exploration of fibrin as a therapeutic target has been limited by its beneficial clotting functions. Here we report the generation of monoclonal antibody SBB, targeted against the cryptic fibrin epitope γ377−395, to selectively inhibit fibrin-induced inflammation and oxidative stress without interfering with clotting. SBB suppressed fibrin-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and the expression of proinflammatory genes. In animal models of MS and AD, SBB entered the CNS and bound to parenchymal fibrin, and its therapeutic administration reduced the activation of innate immunity and neurodegeneration. Thus, fibrin-targeting immunotherapy inhibited autoimmune- and amyloid-driven neurotoxicity and might have clinical benefit without globally suppressing innate immunity or interfering with coagulation in diverse neurological diseases.

Activation of innate immunity is a key feature of neurological diseases with different etiologies, including autoimmune and neurodegenerative CNS diseases1. Increasing evidence indicates that pathogenic activation of CNS innate immunity contributes to neuronal damage and modulates the onset and progression of neurodegenerative diseases2. Oxidative injury and release of free radicals have been proposed as common mechanisms for innate immunity–driven neurodegeneration and demyelination in MS and AD1–3. Chronic activation of innate immunity and oxidative injury are key elements that drive neurodegeneration in both relapsing–remitting MS and progressive MS1,4–6. In progressive MS, there is robust microglial activation, oxidative stress and neurodegeneration1,4,6. Pathogenic activation of innate immunity contributes to oxidative stress and cognitive decline in AD5. Little is known about the pathogenetic signals that activate innate immune cells toward neurotoxic phenotypes. Understanding the mechanisms of the activation of CNS innate immunity is essential for deciphering how neuroinflammation contributes to neuronal damage and for designing treatments for the selective suppression of pathogenic functions of innate immunity.

The activation of innate immunity, disruption of the BBB and deposition of fibrin are intimately linked in neurological diseases1,11. The blood-coagulation factor fibrinogen extravasates into the CNS parenchyma after disruption of the BBB and is converted into insoluble fibrin, a key proinflammatory matrix that activates innate immune responses1,12. The conversion of fibrinogen into fibrin exposes amino acids 377–395 in the fibrinogen γ-chain (γ377–395) that bind to the CD11b I-domain of the CD11b–CD18 integrin receptor (also known as Mac-1, complement receptor 3 (CR3), or αMβ2) and induces the activation of microglia and macrophages13−16. Fibrin is deposited in AD and MS lesions at sites of microglial activation and macrophage infiltration (reviewed in ref. 11). Fibrin is detected in progressive MS and in active and chronic lesions (reviewed in ref. 11). In progressive MS, deposition of fibrin in the cortex correlates with neuronal loss and inhibition of fibrinolysis17. Disruption of the BBB and deposition of fibrin also occur early in MS and precede demyelination18,19. Fibrinogen has been proposed as a cerebrospinal fluid and plasma biomarker for AD and mild cognitive impairment, and increased concentrations of fibrinogen are considered a predictor of brain atrophy in AD (reviewed in refs 11,20). Depletion of fibrin...
either genetically in fibrinogen-deficient mice or by anticoagulants decreases neuroinflammation, demyelination and axonal damage in animal models of MS and reduces the activation of microglia, damage to white matter and cognitive decline in animal models of AD (reviewed in ref. 13). Fibrin induces rapid and sustained microglial responses and infiltration of macrophages into the CNS 13,14. Although increased disruption of the BBB and deposition of fibrin correlate with neurodegeneration, the molecular links between the leakage of blood into the CNS and neuronal damage are poorly understood. Furthermore, whether and how fibrin-induced activation of innate immunity is neurotoxic remains largely unknown.

Here we report an unanticipated role for fibrin as an activator of the NADPH oxidase complex that induced the release of reactive oxygen species (ROS) and innate immunity–driven neurotoxicity in autoimmunity- and amyloid-driven neurodegeneration. Although activation of innate immunity is an attractive candidate for therapeutic intervention, selective therapies that inhibit the neurotoxic effects of innate immune responses are not widely available. By targeting the cryptic fibrin epitope γ377–395, we developed the first, to our knowledge, fibrin-targeting immunotherapy (monoclonal antibody 5B8) that selectively targets the inflammatory form of fibrin without interfering with clotting or the activation of innate immune cells by other ligands, such as lipopolysaccharide (LPS). 5B8 selectively bound to fibrin, but not to soluble fibrinogen, and inhibited binding of fibrin to CR3 without interfering with fibrin polymerization, in vivo clotting time or activated partial thromboplastin time (aPTT) in human plasma. 5B8 reduced the activation of NADPH oxidase, the release of ROS, the activation of microglia and neurodegeneration in animal models of MS and AD. These studies identify fibrin as a blood-derived signal that activates NADPH oxidase to promote innate immunity–driven neurotoxicity and identify fibrin-targeted immunotherapy as a novel therapeutic strategy that suppresses innate immunity–driven neurodegeneration at sites of increased vascular permeability without interfering with clotting or globally suppressing innate immunity.

**Results**

**Design of fibrin-targeting immunotherapy.** The carboxyl terminus of the fibrinogen γ-chain contains two distinct non-overlapping sites at γ400–411 and γ377–395, which mediate platelet engagement and inflammation, respectively (Fig. 1a). Peptide γ377–395 is the binding site for the platelet integrin receptor αIIbβ3, and is required for platelet aggregation. Peptide γ377–395 is the binding site for the CD11b I-domain of CD11b–CD18 14 and is required for fibrin-induced activation of innate immunity–driven neurotoxicity and microglia; the greatest inhibition (~87%) was by 5B8 (Supplementary Fig. 2a). Antibody 1E3, directed against γ390–402, also bound fibrin but did not inhibit microglial activation (Supplementary Fig. 2a), suggesting that inhibition of fibrin’s proinflammatory functions depends on targeting γ377–395. The F(ab) fragment of 5B8 also inhibited fibrin-induced microglial activation (Supplementary Fig. 2b). 5B8 inhibited fibrin-induced microglial activation in a concentration-dependent manner and reduced the expression of genes encoding proinflammatory molecules (Fig. 1c and Supplementary Fig. 2c,d). The isotype-matched control antibody IgG2b, with endotoxin concentrations of <0.002 EU/μg (Supplementary Fig. 2e), was used as control for all experiments in the study. 5B8 did not alter the polymerization of fibrinogen into fibrin, did not inhibit the aPTT in human plasma and had no effect on the clotting time of mouse plasma in vivo (Fig. 1f–h). ~80% of 5B8 was recovered 5 d after a single intraperitoneal injection into healthy wild-type mice (Supplementary Fig. 2f), which suggested that 5B8’s preferential binding to fibrin and minimal binding to soluble fibrinogen resulted in a minimal sink effect.

In microglia and macrophages, fibrin induces the expression of genes encoding proinflammatory molecules associated with immune responses and cell recruitment15. In bone marrow–derived macrophages (BMDMs), 5B8 reduced the fibrin-induced transcriptional activation of genes encoding molecules related to the migration of immune cells, adhesion, inflammatory responses, regulation of T cell proliferation and chemotaxis, as shown by whole-genome microarray and gene-ontology (GO) analysis (Fig. 2a–c). A heat map of the 20 most-affected transcripts showed that 5B8 inhibited the fibrin-induced transcription of a group of genes encoding molecules that regulate innate and/or adaptive immune responses (Fig. 2c). The reduction in the expression of Cxcl3, Tnfs18, Il12b, Cxcl10 and Ifng (which encode proinflammatory molecules) by 5B8 was confirmed by quantitative RT-PCR (Fig. 2d). 5B8 did not affect the LPS-induced expression of genes encoding proinflammatory molecules (Fig. 2e), which suggested that the suppression of the activation of innate immunity by 5B8 might have been selective for fibrin. Furthermore, 5B8 or its F(ab) fragment alone inhibited microglial activation and the expression of chemokine-encoding genes in fibrinogen-induced encephalomyelitis (Supplementary Fig. 3), an acute neuroinflammatory model that results from fibrin-induced microglial activation14. Thus, we had devised a paradigm for the development of a fibrin epitope–specific immunotherapy. The result was 5B8, a highly selective monoclonal antibody that blocked fibrin-induced, CD11b-mediated activation of innate immunity without affecting fibrin polymerization, in vivo clotting time or aPTT in human plasma.

**Fibrin induces NADPH oxidase–mediated neurodegeneration.** A consequence of the activation of innate immunity in CNS autoimmune and neurodegenerative diseases is neurotoxicity; its successful treatment remains a major unmet clinical need. Intriguingly, fibrin induced the expression of several genes encoding molecules that regulate oxidative stress and the release of ROS (Fig. 3a) and
that are linked to neurotoxicity\textsuperscript{7,21}. Ncf4, which encodes the p40\textsuperscript{phox} subunit of the NADPH oxidase Nox2 complex, was among the genes most upregulated by fibrin (Fig. 3a). NADPH oxidase is a multicomponent enzyme system that is upregulated in human genes most upregulated by fibrin (Fig. 3a). NADPH oxidase is a subunit of the NADPH oxidase Nox2 complex, was among the 1214 brain injury and brain aging\textsuperscript{6,24,25}. Intriguingly, fibrin increased eases with fibrin deposition, including MS, AD, stroke, traumatic stress are common mechanisms of neurodegeneration in dis-

![Image](https://example.com/image.jpg)

**Fig. 1 | Generation and characterization of monoclonal antibody 5B8, which targets the fibrin epitope γ\textsubscript{377–395}. a, Structural map of the carboxy-terminal γ-chain of fibrinogen, showing the cryptic epitope γ\textsubscript{377–395} (red), which binds to the CD11b I-domain, as well as epitopes γ\textsubscript{400–411} (blue) and γ\textsubscript{200–205} (gray). b, ELISA of the binding of 5B8 to γ\textsubscript{377–395}, presented as absorbance at 450 nm (A\textsubscript{450}), plus the dissociation constant (K\textsubscript{D}). Data are from four independent experiments (mean ± s.e.m.). c, ELISA of the binding of 5B8 to fibrin and fibrinogen (key), presented as relative chemiluminescent light units (RLU). Data are from three independent experiments (mean ± s.e.m.). d, Competitive ELISA of 5B8 versus the CD11b I-domain for binding to fibrin. Data are from one experiment. e, Microscopy (left) of microglia left unstimulated (US) or stimulated with fibrin alone or in the presence of 5B8 (40 μg/ml) (above images), and quantification of microglial activation assessed as increased cell size (right) at 48 h after treatment with various combinations (below plots) of the presence (+) or absence (–) of fibrin and 5B8 (40 μg/ml (+) or 10, 20 or 40 μg/ml (wedge)). Scale bar (left), 10 μm. Each symbol (right) represents an independent experiment. Data are from four independent experiments (untreated, fibrin, fibrin + 5B8 (40 μg/ml)) and two independent experiments (fibrin + 5B8 (10, 20 μg/ml)) with similar results by manual and automated quantification (mean ± s.e.m.). ***P < 0.0001 (one-way analysis of variance (ANOVA) with Sidak’s multiple comparisons test). f, In vitro fibrin polymerization in the presence of buffer or fibrinogen (FN) alone or with 5B8, IgG2b or various concentrations of the fibrin polymerization inhibitor (positive control) GPRP (key). Data are representative of two independent experiments with similar results. g, aPTT assay of human plasma in the presence or absence of 5B8, IgG2b or GPRP (key). Data are representative of two independent experiments with similar results. h, In vivo clotting time of blood from mice given injection of 5B8 (+) or not (–). Each symbol represents an individual mouse. Data are from n = 8 mice per treatment (mean ± s.e.m.). NS, not significant (P = 0.7768) (two-tailed Mann-Whitney test).
and progression of disease and disability. To investigate the role of fibrin in neurotoxicity in vivo, we first assessed the effects of fibrin-stimulated BMDMs with 5B8 in three models of experimental autoimmune encephalomyelitis (EAE) that simulate key aspects of MS: relapsing–remitting EAE induced by the epitope of amino acids 139–151 of proteolipid protein (PLP) (‘PLP139–151 EAE’); chronic EAE induced by the epitope of amino acids 139–151 of proteolipid protein (‘MOG35–55 EAE’); and adoptive transfer of CD4+ T cells differentiated under conditions that polarize cells into the TH1 subset (‘Tnfrsf18’).

Changes in gene expression induced by the treatment of fibrin-stimulated BMDMs with 5B8. a, Whole-genome microarray analysis of BMDMs stimulated for 6 h with fibrin and 5B8 or IgG2b (above plot), presented as an MA plot of the ratio of expression in 5B8-treated BMDMs relative to that in IgG2b-treated BMDMs (log2 fold values). Each symbol represents an individual experiment. Data are from three independent experiments. b, Whole-genome microarray analysis of the expression (key below) of various immunity-related genes (left margin) in BMDMs treated as in a (above plot), identifying the top ten biological processes (right margin). Each column represents one sample from each experiment. c, GO-Elite analysis of transcripts in BMDMs treated as in a (above plot), identifying the top ten biological processes (left margin) with the most significantly downregulated transcripts in 5B8-treated BMDMs relative to their expression in IgG2b-treated BMDMs, GO-Elite analysis of transcripts in BMDMs treated as in a (above plot), identifying the top ten biological processes (left margin) with the most significantly downregulated transcripts in 5B8-treated BMDMs relative to their expression in IgG2b-treated BMDMs (z-score > 2.0; P < 0.001) (unpaired two-tailed t-test). d, qRT-PCR analysis of transcripts from genes encoding proinflammatory molecules in BMDMs treated as in e (grid below plot) of fibrin, 5B8, and IgG2b, identifying the top 20 most significantly downregulated transcripts in fibrin-stimulated BMDMs treated with 5B8. Data are from three independent experiments. Each column represents one sample from each experiment. d, qRT-PCR analysis of transcripts from genes encoding proinflammatory molecules in BMDMs treated as in e (grid below plot) of fibrin, 5B8, and IgG2b, identifying the top 20 most significantly downregulated transcripts in fibrin-stimulated BMDMs treated with 5B8. Data are from three independent experiments. Each column represents one sample from each experiment. e, qRT-PCR analysis of transcripts from genes encoding proinflammatory molecules in BMDMs treated as in e (grid below plot) of fibrin, 5B8, and IgG2b, identifying the top 20 most significantly downregulated transcripts in fibrin-stimulated BMDMs treated with 5B8. Data are from three independent experiments.
5B8 reduced the proportion of paralyzed mice (Fig. 4c). Fibrin is a cross-linked polymer of fibrinogen molecules. Consequently, antibodies to fibrinogen detect both fibrinogen and fibrin11. Fibrinogen is converted to fibrin at sites of increased coagulation activity11. In the spinal cord during EAE, coagulation activity promotes the formation of the cross-linked polymer of fibrinogen molecules. Consequently, anti-fibrinogen antibodies detect both fibrinogen and fibrin11. Fibrinogen is converted to fibrin at sites of increased coagulation activity11.

Given prophylactically, 5B8 reduced the mean maximum clinical score and delayed the first day of onset relative to those of groups treated with IgG2b (Supplementary Tables 1 and 2). Given therapeutically, 5B8 reduced the severity of relapses in PLP139–151 EAE (Fig. 4b and Supplementary Table 1). In all EAE treatment groups, 5B8 reduced the proportion of paralyzed mice (Fig. 4c). Fibrin is a cross-linked polymer of fibrinogen molecules. Consequently, antibodies to fibrinogen detect both fibrinogen and fibrin11. Fibrinogen is converted to fibrin at sites of increased coagulation activity11. In the spinal cord during EAE, coagulation activity promotes the

**Fig. 3** 5B8 blocks fibrin-induced ROS production and axonal damage. **a.** Expression (key below) of ROS-related genes (left margin) in microglia (left) and BMDMs (right) left untreated (UT) or treated with fibrin (above plots), showing genes with significantly differential expression in fibrin-treated cells relative to that in untreated cells (Δlog2 fold > 0.585, and false-discovery rate < 0.05 (two-tailed moderated t-test)). Each column represents one sample from two independent experiments (microglia) or three independent experiments (BMDMs). **b.** Immunoblot analysis of gp91phox, phosphorylated (p-) p40phox and GAPDH (loading control) in BMDMs left untreated (far left lane) or stimulated for various times (above lanes) with fibrin in the presence of 5B8 or IgG2b (grid above blots); right margin, molecular size in kilodaltons (kDa); numbers below lanes indicate densitometry, presented as band intensity of gp91phox* or phosphorylated p40phox* (bottom) relative to that of GAPDH (cropped blot images; full blots, Supplementary Fig. 9). Data are from one experiment representative of three independent experiments. **c.** NADPH oxidase activity in BMDMs left untreated or stimulated for 12 h with fibrin in the presence of 5B8 or IgG2b (grid below plot). Data are from three independent experiments (mean ± s.e.m.). **d.** Microscopy of ROS production (assessed via DHE) in BMDMs (left) and human macrophages derived from peripheral blood mononuclear cells (PBMC) (right) left unstimulated or stimulated for 48 h with fibrin in the presence of 5B8 or IgG2b (grid below plot), presented in arbitrary units (AU). Data are from four (left) or two (right) independent experiments (mean ± s.e.m.). **e.** Quantification of ROS production (assessed via DHE) in BMDMs left untreated (US) or stimulated with fibrin or 5B8 (grid below plot), presented in arbitrary units (AU). Data are from four (left) or two (right) independent experiments (mean ± s.e.m.). **f.** Quantification of ROS production in BMDMs isolated from wild-type mice (WT) and mice with genetic ablation of p47phox (p47phox−/−; called ‘p47phox−/−’ here) (key) and left unstimulated (US) or stimulated for 24 h with fibrin (horizontal axis). Data are from n = 4 mice per group (mean ± s.e.m.). **g.** Quantification of ROS production (assessed via DHE in mouse BMDMs left unstimulated or stimulated for 24 h with fibrin in the presence or absence of apocynin (grid below plot). Data are from three independent experiments (mean ± s.e.m.). **h.** Microscopy (left) of MAP-2 (top row) and synapsin-RFP (bottom row) in cortical neurons co-cultured with BMDMs left unstimulated (US) or stimulated with fibrin in the presence of 5B8 or IgG2b (above images); right, quantification of MAP-2 neurite coverage (top) and neurite fragmentation assessed with synapsin-RFP (bottom) among co-cultures as at left (grid below plot). Scale bars (left), 50 μm (top) or 30 μm (bottom). Data are from four independent experiments (mean ± s.e.m.). **i.** Immunoblot analysis of gp91phox, phosphorylated (p-) p40phox and GAPDH (loading control) in BMDMs left untreated (far left lane) or stimulated for various times (above lanes) with fibrin in the presence of 5B8 or IgG2b (grid above blots); right margin, molecular size in kilodaltons (kDa); numbers below lanes indicate densitometry, presented as band intensity of gp91phox* or phosphorylated p40phox* (bottom) relative to that of GAPDH (cropped blot images; full blots, Supplementary Fig. 9). Data are from one experiment representative of three independent experiments. **j.** Immunoblot analysis of gp91phox, phosphorylated (p-) p40phox and GAPDH (loading control) in BMDMs left untreated (far left lane) or stimulated for various times (above lanes) with fibrin in the presence of 5B8 or IgG2b (grid above blots); right margin, molecular size in kilodaltons (kDa); numbers below lanes indicate densitometry, presented as band intensity of gp91phox* or phosphorylated p40phox* (bottom) relative to that of GAPDH (cropped blot images; full blots, Supplementary Fig. 9). Data are from one experiment representative of three independent experiments. **k.** Immunoblot analysis of gp91phox, phosphorylated (p-) p40phox and GAPDH (loading control) in BMDMs left untreated (far left lane) or stimulated for various times (above lanes) with fibrin in the presence of 5B8 or IgG2b (grid above blots); right margin, molecular size in kilodaltons (kDa); numbers below lanes indicate densitometry, presented as band intensity of gp91phox* or phosphorylated p40phox* (bottom) relative to that of GAPDH (cropped blot images; full blots, Supplementary Fig. 9). Data are from one experiment representative of three independent experiments.
conversion of fibrinogen to fibrin, which leads to parenchymal deposition of fibrin at areas of microglial activation and demyelination. After intraperitoneal injection of biotinylated 5B8 into mice with EAE, 5B8 spatially correlated with fibrin(ogen)-rich areas in the spinal cord (Fig. 4d), which demonstrated engagement of the target.

To determine whether 5B8 inhibited the recruitment of inflammatory cells into the CNS, we induced EAE in Cx3cr1<sup>GFP</sup>/+ Ccr2<sup>RFP</sup>/+ mice (in which chemokine receptor CX3CR1<sup>+</sup> microglia express green fluorescent protein (GFP), and chemokine receptor CCR2<sup>+</sup> macrophages express red fluorescent protein (RFP)) and then...
treated these mice with 5B8. We found that 5B8 inhibited both the accumulation of CX3CR1+ microglia and the infiltration of CCR2+ monocytes into the CNS in these mice (Fig. 5). Increased NADPH oxidase activity and excessive ROS production have been linked to MS28, and ROS generated by invading and resident CNS macrophages mediate demyelination and axonal damage in EAE. 5B8 markedly diminished axonal damage, the generation of ROS, inflammation and demyelination in EAE (Fig. 5 and Supplementary Fig. 5a,b). In accordance with published studies7,12, 5B8 inhibited microglial activation, monocyte recruitment and axonal damage in EAE. Microscopy (top) of spinal cord sections from mice with PLP139–151 EAE, treated with 5B8 or IgG2b (left margin), showing SMI-32 immunoreactivity (indicative of axonal damage); dashed lines demarcate white matter of the spinal cord dorsal column. Bottom, quantification of results in mice as above (horizontal axis), presented as the proportion of the area with SMI-32 immunoreactivity. Scale bar (top), 200 μm. Data are from n = 8 mice per group. ***P = 0.0002 (two-tailed Mann-Whitney test).

To determine whether blocking fibrin–CD11b interactions protected mice against amyloid-related neurodegeneration, we treated 5XFAD mice with 5B8 for 2 months, starting at 3.5 months of age (1.5 months after the appearance of Aβ plaques and microglial activation) (Fig. 7a). Treatment with 5B8 reduced the loss of cholinergic neurons and microglial activation around plaques in 5XFAD mice (Fig. 7b,c) without significantly affecting Aβ plaques or the number of MAC-2+ macrophages around plaques (P = 0.1142 (two-tailed Mann-Whitney test); Supplementary Fig. 8a,b). Whole-genome microarray and GO analysis of cortical gene expression in 5XFAD mice showed that 5B8 suppressed five key pathways: the complement pathway, antigen presentation, cytokine response, lysozyme generation and reduced the expression of genes encoding molecules that promote inflammation and oxidative stress.
Fibrin-targeting immunotherapy suppresses genes of the TYROBP network. Gene-network analysis has broadened the understanding of AD pathogenesis by revealing immunological gene networks as probable causal contributors to AD pathology. Targeting these networks could culminate in the development of novel therapeutics. To identify gene networks that might be regulated by 5B8, we investigated our transcriptomics data by biological network analysis. First, we performed genetic network modeling...
of genes downregulated by 5B8. Co-expression analysis revealed 5B8-downregulated genes to be densely interconnecting encoded molecules mainly of the complement pathway, including C4b and C1q, with Tyrobp (which encodes the adapter DAP12) forming a major hub (Fig. 8a). The cluster of co-expressed genes downregulated by 5B8 showed a striking overlap with networks of genes encoding molecules linked to the pathogenesis of human AD, including those encoding the TYROBP-related microglial module. Indeed, Tyrobp, which encodes a co-receptor for CD11b and the human AD–associated receptor Trem2, was one of the genes with the greatest degree of downregulation by 5B8 (Figs. 7d and 8a). Overlay of gene-expression data obtained from 5B8-treated 5XFAD mice with networks of genes encoding inflammatory molecules from brains of humans with AD showed a reduction in the expression of genes in the human AD–related inflammatory networks by 5B8. By overlaying our data onto a mouse version of the human AD TYROBP module, we found that 5B8 downregulated 65% of the Tyrobp–related network at various levels of significance, including Tyrobp at the nexus of the network (Fig. 8b). Several components of the TYROBP signaling pathway were also downregulated (Supplementary Fig. 8c). This bioinformatics approach allowed us to gain insight into the mechanisms of action of treatment with 5B8, as we identified downregulation mainly of co-expressed genes encoding components of the complement pathway that were relevant to the pathogenesis of AD. Integrated gene network analysis might be a useful tool with which to better understand the mechanism of action of new drugs and assess their efficacy in suppressing inflammatory gene networks relevant to human disease. Thus, the inhibition of fibrin by 5B8 might suppress the pathogenic activation of innate immunological pathways that mediate amyloid-related neurodegeneration.

**Discussion**

Our study has revealed a previously unknown neurodegenerative pathway in which fibrin activated NADPH oxidase in innate immune cells to promote ROS production and neurotoxicity. By developing an antibody-based approach to selectively target a key inflammatory fibrin domain, we showed here that fibrin-targeting immunotherapy can be selective and efficacious in suppressing neuroinflammation and neurodegeneration. Consequently, preventing fibrin from engaging this mechanism might lead to the development of therapeutic strategies for the treatment of neurodegenerative diseases with neurovascular dysfunction. This discovery might have implications beyond the CNS, since fibrin deposition is a common thread in inflammatory pathologies, such as rheumatoid arthritis, colitis and Duchenne muscular dystrophy (reviewed in refs. [16])

Fibrin–CD11b signaling induces chemokine release and macrophage recruitment and increased activation of NADPH oxidase, which culminates in the release of ROS and oxidative stress (as we have shown here). Therefore, inhibitors of the fibrin–CD11b interaction, such as 5B8, have the potential to affect both inflammatory processes and oxidative stress. Inhibiting the formation of fibrin with anticoagulants can cause adverse effects by increasing the risk of bleeding. Epitope-selective targeting of fibrin might overcome that challenge through selective suppression of its damaging functions without adverse anticoagulant effects. Overall, fibrin-targeting immunotherapy might represent a selective multi-indication therapy for the suppression of fibrin-induced chronic inflammation.
through inhibition of the release of proinflammatory factors and oxidative stress at sites of increased vascular permeability in neurological and other inflammatory diseases.

Our study has revealed a previously unknown function for fibrin–CD11b signaling as an activator of NADPH oxidase–dependent production of ROS. CD11b–dependent respiratory bursts require signaling via DAP12. CD11b and DAP12 are co-expressed in microglia and act synergistically to promote the production of superoxide ions and induce neuronal death. Intriguingly, DAP12 in microglia and act synergistically to promote the production of ROS. CD11b–dependent respiratory bursts (fibrin–CD11b signaling as an activator of NADPH oxidase–dependent signaling complexes) might increase DAP12 expression and promote oxidative damage dependent on CD11b–DAP12 by CD11b–CD18 signaling. Other modulators of innate immunity, such as complement, CR3, TNF, NF-κB, PI3K and TLR4, are ubiquitously expressed in the brain and the periphery and have reported roles in regulating physiological CNS functions. In contrast, fibrin is not present in the normal brain but is abundantly deposited in the CNS after disruption of the BBB. 5B8 was selective in inhibiting the activation of innate immunity via targeting fibrin and did not affect the activation of Aβ after 2 months of administration, which would suggest that its neuroprotective effects occur in the presence of Aβ. Future studies should determine whether 5B8 also affects cognitive performance in mice with AD.

Fibrin-targeting immunotherapy represents a novel approach with which to selectively suppress pathogenic innate immune responses with potential clinical implications. In MS, anti-inflammatory medications have little effect during the progressive phase of disease, which is mediated by innate immunological mechanisms. Other modulators of innate immunity, such as complement, CR3, TNF, NF-κB, PI3K and TLR4, are ubiquitously expressed in the brain and the periphery and have reported roles in regulating physiological CNS functions. In contrast, fibrin is not present in the normal brain but is abundantly deposited in the CNS after disruption of the BBB. 5B8 was selective in inhibiting the activation of innate immunity via targeting fibrin and did not affect the activation of macrophages by other ligands, such as LPS, which suggested that the activation of innate immunity by other ligand–receptor interactions could proceed normally. Phagocytic pathways in innate immunity are independent and additive predictors of age-related cognitive decline in the elderly, which suggests that they might need to be targeted independently for maximal therapeutic benefit. Fibrin-targeting immunotherapy did not reduce the deposition of Aβ after 2 months of administration, which would suggest that its neuroprotective effects occur in the presence of Aβ. Future studies should determine whether 5B8 also affects cognitive performance in mice with AD.

Fibrin-targeting immunotherapy represents a novel approach with which to selectively suppress pathogenic innate immune responses with potential clinical implications. In MS, anti-inflammatory medications have little effect during the progressive phase of disease, which is mediated by innate immunological mechanisms. Other modulators of innate immunity, such as complement, CR3, TNF, NF-κB, PI3K and TLR4, are ubiquitously expressed in the brain and the periphery and have reported roles in regulating physiological CNS functions. In contrast, fibrin is not present in the normal brain but is abundantly deposited in the CNS after disruption of the BBB. 5B8 was selective in inhibiting the activation of innate immunity via targeting fibrin and did not affect the activation of macrophages by other ligands, such as LPS, which suggested that the activation of innate immunity by other ligand–receptor interactions could proceed normally. Phagocytic pathways in innate immunity are independent and additive predictors of age-related cognitive decline in the elderly, which suggests that they might need to be targeted independently for maximal therapeutic benefit. Fibrin-targeting immunotherapy did not reduce the deposition of Aβ after 2 months of administration, which would suggest that its neuroprotective effects occur in the presence of Aβ. Future studies should determine whether 5B8 also affects cognitive performance in mice with AD.

Fibrin-targeting immunotherapy represents a novel approach with which to selectively suppress pathogenic innate immune responses with potential clinical implications. In MS, anti-inflammatory medications have little effect during the progressive phase of disease, which is mediated by innate immunological mechanisms. Other modulators of innate immunity, such as complement, CR3, TNF, NF-κB, PI3K and TLR4, are ubiquitously expressed in the brain and the periphery and have reported roles in regulating physiological CNS functions. In contrast, fibrin is not present in the normal brain but is abundantly deposited in the CNS after disruption of the BBB. 5B8 was selective in inhibiting the activation of innate immunity via targeting fibrin and did not affect the activation of macrophages by other ligands, such as LPS, which suggested that the activation of innate immunity by other ligand–receptor interactions could proceed normally. Phagocytic pathways in innate immunity are independent and additive predictors of age-related cognitive decline in the elderly, which suggests that they might need to be targeted independently for maximal therapeutic benefit. Fibrin-targeting immunotherapy did not reduce the deposition of Aβ after 2 months of administration, which would suggest that its neuroprotective effects occur in the presence of Aβ. Future studies should determine whether 5B8 also affects cognitive performance in mice with AD.

Fibrin-targeting immunotherapy represents a novel approach with which to selectively suppress pathogenic innate immune responses with potential clinical implications. In MS, anti-inflammatory medications have little effect during the progressive phase of disease, which is mediated by innate immunological mechanisms. Other modulators of innate immunity, such as complement, CR3, TNF, NF-κB, PI3K and TLR4, are ubiquitously expressed in the brain and the periphery and have reported roles in regulating physiological CNS functions. In contrast, fibrin is not present in the normal brain but is abundantly deposited in the CNS after disruption of the BBB. 5B8 was selective in inhibiting the activation of innate immunity via targeting fibrin and did not affect the activation of macrophages by other ligands, such as LPS, which suggested that the activation of innate immunity by other ligand–receptor interactions could proceed normally. Phagocytic pathways in innate immunity are independent and additive predictors of age-related cognitive decline in the elderly, which suggests that they might need to be targeted independently for maximal therapeutic benefit. Fibrin-targeting immunotherapy did not reduce the deposition of Aβ after 2 months of administration, which would suggest that its neuroprotective effects occur in the presence of Aβ. Future studies should determine whether 5B8 also affects cognitive performance in mice with AD.
immune cells are required for the clearance of debris and remyelination40. Fibrin-primed macrophages inhibit the differentiation of oligodendrocyte precursor cells41, which are susceptible to oxidative stress42. Fibrin-targeting immunotherapy could suppress fibrin-induced chronic inflammation and potentially promote repair by inhibiting oxidative stress and the release of proinflammatory factors. Microglia and macrophages are functionally distinct populations with diverse functions in neurodegenerative diseases43–46. Studies using mass cytometry and large transcriptomics profiling of microglia and macrophages will be needed to determine the potential differential effects of fibrin on innate immune cell populations. The inflammatory epitope γ377–395 of fibrin has been confirmed genetically in inflammatory models in the brain and periphery with findings replicated in numerous laboratories (reviewed in refs 11,12). It is possible that pharmacological targeting of fibrin might represent a selective treatment to add to the toolbox of therapies directed against innate immunity that might be particularly efficacious for suppressing neuroinflammation at sites of BBB leakage and vascular damage with fibrin deposition.

The genetic and pharmacological tools available for studying the epitope γ377–395 (Fgg390–396A mice, which express a γ390–396A mutant fibrinogen γ-chain; the γ377–395 peptide; and antibody 5B8 in our study here) have revealed mainly pathogenic functions for the epitope γ377–395 in inflammation. Fgg390–396A mice, which lack fibrinogen, and Fgg390–396A mice47,48, as well as mice treated with 5B8 (as in our study here), with γ377–395 peptide49 or with anticoagulants50,51, are not immunocompromised and can be housed in conventional animal facilities without opportunistic infections. In humans, congenital afibrinogenemia, a genetic disorder characterized by a complete absence of fibrinogen, is associated with excessive bleeding but no increase in opportunistic infections50. Fibrin inhibits repair, as persistent deposition of fibrin inhibits wound healing52, while depletion of fibrin increases remyelination53–55. Such studies do not exclude the possibility that the fibrinogen epitope γ377–395 might have physiological functions that have not yet become apparent through the use of the genetic and pharmacologic tools available. Collectively, prior genetic studies of the γ377–395 epitope and our findings here support the proposal that fibrin induces, in a ligand-selective manner, a specific gene signature in immune cells that produces mainly pathogenic functions. Consequently, targeting fibrin-induced activation of innate immunity has the potential to suppress proinflammatory and neurotoxic pathways without substantial interference with protective functions of the innate immune system. Immunotoxicology studies will also be needed in the future to fully test potential immunological dysfunction in the context of fibrin-targeting immunotherapy.

Fibrinogen is a coagulation factor essential for blood clotting. Studies of Fgg390–396A mice have shown that the epitope γ377–395 is not required for fibrin polymerization or platelet aggregation and does not increase bleeding risk40. Fgg390–396A mice have normal concentrations of fibrinogen, fibrin polymerization and platelet aggregation, normal thrombus formation in FeCl3-injured carotid arteries, and normal pregnancy and birth rates. Furthermore, they do not develop spontaneous hemorrhagic events and tolerate major surgical procedures (such as abdominal surgeries) without inordinate bleeding40. In contrast to Fgg390–396A mice, Fgg4– mice, which lack fibrinogen exhibit broad hemostatic abnormalities secondary to the lack of clotting function and failure of fibrinogen-supported platelet aggregation and uniformly cannot sustain pregnancy due to the hemostatic requirements at the placental–maternal interface40, a phenotype also observed in mice that lack the transglutaminase IXIIIA54. Consistent with experimental findings for Fgg390–396A mice, the fibrin peptide γ377–395 does not inhibit normal clotting time in vivo or fibrin polymerization in vitro55. Our study has shown that 5B8 did not affect normal clotting time in vivo, fibrin polymerization in vitro or sPTT in human plasma. Similar to Fgg390–396A mice, mice treated with 5B8 even for prolonged periods of up to ~2 months did not develop any spontaneous bleeding. The γ377–395 epitope targeted by 5B8 does not overlap with the γ398–406 residues known to be enzymatically cross-linked by FIXIIIA11. Nevertheless, comparative studies of FIXIIIA-mediated crosslinking in vitro and in vivo will be of interest in future studies of 5B8. This could be of particular interest in light of studies of Fgg390–396A mice in a model of venous thrombosis41. In those studies, Fgg390–396A mice were shown to maintain FIXIIIA-mediated cross-linking of the γ-chain but at a reduced rate, and this resulted in smaller venous thrombi. Overall, studies of Fgg390–396A mice40, data obtained with the fibrin peptide γ377–395 (ref. 11) and our results in this study suggest that the epitope γ377–395 and 5B8 do not interfere with fibrin clotting and present a limited risk of spontaneous hemorrhagic events. Additional toxicology studies will be needed to determine whether 5B8 interferes with other features of hemostasis and thrombosis.

Together, data from human pathology, genetic and pharmacological studies of mice, transcriptomics and signal-transduction studies have established fibrin as a dual inflammatory and oxidative-stress signal in the CNS and suggest that fibrin-targeting immunotherapy might prevent neurodegeneration and other complications in conditions associated with fibrin deposits at sites of disruption of the BBB. Fibrin-targeting immunotherapy might hold promise as a new therapeutic strategy for neurological and inflammatory disorders with vascular damage.

Online content
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-018-0232-x.

Received: 17 June 2017; Accepted: 7 September 2018; Published online: 15 October 2018

References
1. Rivest, S. Regulation of innate immune responses in the brain. Nat. Rev. Immunol. 9, 429–439 (2009).
2. Ransohoff, R. M. & Brown, M. A. Innate immunity in the central nervous system. J. Clin. Invest. 122, 1164–1171 (2012).
3. Lassmann, H., van Horssen, J. & Mahad, D. Progressive multiple sclerosis: pathology and pathogenesis. Nat. Rev. Neurol. 8, 647–656 (2012).
4. Schuh, C. et al. Oxidative tissue injury in multiple sclerosis is only partly reflected in experimental disease models. Acta Neuropathol. 128, 247–266 (2014).
5. Heppner, F. L., Ransohoff, R. M. & Becher, B. Immune attack: the role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. 16, 358–372 (2015).
6. Lassmann, H. Mechanisms of neurodegeneration shared between multiple sclerosis and Alzheimer’s disease. J. Neural Transm. (Vienna) 118, 747–752 (2011).
7. Nikic, I. et al. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat. Med. 17, 495–499 (2011).
8. Chitnis, T. & Weiner, H. L. CNS inflammation and neurodegeneration. J. Clin. Invest. 127, 3577–3587 (2017).
9. Frischer, J. M. et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132, 1175–1189 (2009).
10. Zhao, Z., Nelson, A. R., Betsholtz, C. & Zlokovic, B. V. Establishment and dysfunction of the blood-brain barrier. Cell 163, 1064–1078 (2015).
11. Petersen, M. A., Ryu, I. K. & Akassoglou, K. Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. Nat. Rev. Neurosci. 19, 283–301 (2018).
12. Davalos, D. & Akassoglou, K. Fibrinogen as a key regulator of inflammation in disease. Semin. Immunopathol. 34, 43–62 (2012).
13. Adams, R. A. et al. The fibrin-derived γ377-395 peptide inhibits microglia activation and suppresses relapsing paralysis in central nervous system autoimmune disease. J. Exp. Med. 204, 571–582 (2007).
14. Ugarova, T. P. et al. Sequence γ377-395(P2), but not γ190-202(P1), is the binding site for the alpha MI-domain of integrin αMβ2 and γC-domain of fibrinogen. Biochemistry 42, 9365–9373 (2003).
15. Davalos, D. et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat. Commun. 3, 1227 (2012).
16. Ryu, J. K. et al. Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. Nat. Commun. 6, 8164 (2015).
17. Yates, R. L. et al. Fibrinogen and neurodegeneration in the progressive multiple sclerosis cortex. Ann. Neurol. 82, 259–270 (2017).
18. Marik, C., Feltz, P. A., Bauer, J., Lassmann, H. & Smith, K. J. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain 130, 2800–2815 (2007).
19. Lee, N. J. et al. Spatiotemporal distribution of fibrinogen in marmoset and human inflammatory demyelination. Brain 141, 1637–1649 (2018).
20. Strickland, S. Blood will out: vascular contributions to Alzheimer’s disease. J. Clin. Invest. 128, 556–563 (2018).
21. Nathan, C. & Cunningham-Bussel, A. Beyond oxidative stress: an immunologist’s guide to reactive oxygen species. Nat. Rev. Immunol. 13, 349–361 (2013).
22. Fischer, M. T. et al. NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. Brain 135, 486–499 (2012).
23. Shimohama, S. et al. Activation of NADPH oxidase in Alzheimer’s disease brains. Biochem. Biophys. Res. Commun. 273, 5–9 (2000).
24. Ma, M. W. et al. NADPH oxidase in blood vessels and neurodegenerative disorders. Mol. Neurodegener. 12, 7 (2017).
25. Haslund-Vinding, J., McBeane, G., Jaquet, V. & Vilhardt, F. NADPH oxidases in the development of rheumatoid arthritis: activating receptors, pharmacology and association with disease. Br. J. Pharmacol. 174, 1733–1749 (2017).
26. Han, M. H. et al. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 451, 1076–1081 (2008).
27. Davalos, D. et al. Early detection of thrombin activity in neuroinflammatory disease. Ann. Neurol. 75, 303–308 (2014).
28. Haider, I. et al. Oxidative damage in multiple sclerosis lesions. Brain 134, 1914–1924 (2011).
29. Cortes-Canteli, M., Mattei, L., Richards, A.T., Norris, E.H. & Strickland, S. NADPH oxidase in brain injury and neurodegenerative disease. Ann. Neurol. 71, 303–308 (2014).
30. Anna, V. M., S.H.Z. were employees of Lundbeck during the time the work was performed. Correspondence and requests for materials is available at www.nature.com/reprints. Additional information is available for this paper at https://doi.org/10.1038/s41590-018-0232-x. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to K.A. © The Author(s), under exclusive licence to Springer Nature America, Inc. 2018

Acknowledgements We thank I.F. Charo (Gladstone Institutes) for Coc2203Tm mice on a C57Bl/6 background; J.L. Degen for advice and critical reading of the manuscript; S. Pintchovski, I. Kadiu, J. Palop and J. Igebeg for discussions; B. Cabrera, R. Meza Acedo, L. Ta and A. Williams for technical assistance; G. Maki for graphics; and G. Howard and K. Claiborne for editorial assistance. The Gladstone Center for In vivo Imaging Research was supported in part by grants from H. Lundbeck A/S; the S.D. Bechtel Jr. Foundation, and the Conrad N. Hilton Foundation (17348 to K.A.). The microscopy studies were carried out in part at facilities adapted for this project at the National Center for Microscopy and Imaging Research, which is supported by grant P41 GM10341 (awarded to M.H.E.). Gladstone Institutes was supported by NIH/NCCR grant RR18928. The Mouse Pathology Core of the UCSF Helen Diller Family Comprehensive Cancer Center was supported by CA082013, J.K.R., D.D. and A.S.M. were supported by National Multiple Sclerosis Society (NMSS) Postdoctoral Fellowships; K.J.R. and D.D. were supported by Race to Erase MS Young Investigator Awards and American Heart Association (AHA) Scientist Development Grants; V.A.R. was supported by postdoctoral fellowships from AHA and NIH/NINDS F32 NS096920; A.S.M. was supported by NIAID T32AI033429 and NMSS FG-1708-2892; K.K.H. was supported by NSF pre-doctoral fellowship DGE-0648991/114427; M.A.P. was supported by a NIH/NICHD R21 HD072222; I.M.W. was supported by a gift from the Dolly Family; S.S.Z. was supported by NIH RO1 NS092835; R1N1 NS081589, NMSS R17G1-26628, BG 5179A10/2, the Weill Institute and the Maisin Foundation; and R.A.S was supported by NIH RO1 NS081149. This work was also supported by grants from K.A. to NMSS (RG7382), H. Lundbeck A/S, the Conrad N. Hilton Foundation (17348), a gift from the Levine Family; and NIH/NINDS (R01 NS051828, R21 NS082976 and R35 NS09976).
Mouse monoclonal antibody production. The synthesis of human fibrinogen peptides γ190–202 and γ377–395, and production of mouse monoclonal antibodies were performed by A&B Biologicals. Supernatants were performed by ELISA against peptides or the carrier protein. Positive clones were expanded and re-tested to confirm peptide epitope reactivity to either γ190–202 or γ377–395. Target candidates were identified as outlined in Supplementary Fig. 1a. The structural map of the γc domain of fibrinogen was generated using the PyMOL Molecular Graphics System, version 1.6 (Schrodinger) with PDB accession code 1HFC.

Peptide-binding assays. Human peptide γc377–395 (Genscript) in carbonate buffer (0.1 M NaHCO3 and 0.15 M NaCl) was coated onto Maxisorp ELISA plates (Thermo Fisher Scientific) overnight at 4°C. Wells were incubated with blocking buffer (2% bovine serum albumin (BSA) and 0.1% NP-40 in PBS) for 2 h at 37°C in washing with 0.05% Tween-20 in PBS. Antibodies SBB, 4F1, 4E11 and 1E3 were diluted in blocking buffer and were added to the wells for 2 h at 25°C, followed by secondary polyclonal goat anti-mouse IgG(HRP) (#P0447, DAKO) in blocking buffer for 2 h at 25°C and results were developed with TMB substrate (Sigma-Aldrich) with absorbance at 450 nm measured with an Envision Microplate reader (Perkin Elmer). For competition ELISA, antibody SBB was diluted to 2 nM in blocking buffer and was pre-incubated with increasing concentrations of human or mouse peptide γc377–395 and γc190–202, for 3 h at 25°C. Competition of SBB with γc377–395 or γc190–202 was assessed by incubation on plates coated with human peptide γc377–395.

Fibrin or fibrinogen binding. Human plasminogen-free fibrinogen (#341578, EMD Millipore) was depleted of IgG with a Pierce albumin/IgG removal kit (Thermo Fisher Scientific). IgG-depleted human plasminogen-free fibrinogen (25 µg/ml) in 20 mM HEPES buffer, pH 7.4, was treated with 0.3 U/ml bovine thrombin (Sigma-Aldrich) and 7 mM CaCl2 in Maxisorp ELISA plates (Thermo Fisher Scientific) for 1.5 h at 37°C. Formed fibrin was dried onto the wells at 37°C overnight. IgG-depleted human fibrinogen (25 µg/ml) was coated onto the wells at 37°C for 2.5 h. Plates were washed with binding buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA and 0.05% Tween-20) and were incubated with blocking buffer (binding buffer containing 3% BSA) for 1 h at 25°C. Monoclonal antibodies in blocking buffer were incubated for 1 h at 25°C. Following washing with blocking buffer, polyclonal goat anti-mouse IgG/HRP (#P0447, DAKO) in blocking buffer was added for 1 h at 25°C, and the signal was developed with Lumi-Phos HRP (Lumigen) and measured with an Envision Microplate reader. Experiments of antibody binding to peptides, fibrin and fibrinogen performed at the Gladstone Institutes were independently reproduced at the UCSF Small Molecule Discovery Center and at Lundbeck, US with similar results.

CD11b 1-domain production. Plasmid pcET5-His-TEV-CD11b was constructed by cloning of the cd11b-I domain (residues Q310-S326) into a pcET15b-derived plasmid at the NdeI and XhoI sites, preceded by an N-terminal 6xHis tag and followed by a TEV protease cleavage site (pcET5-His-TEV-CD11b), then transformed into Rosetta(DE3) cells. Protein was expressed and purified as described67, with the exception that cells were lysed by a pressure-driven microfluidizer, and His-containing proteins were trapped using TALON cobalt affinity resin. Protein purity was assessed to be >90% by LC-MS and gel electrophoresis.

CD11b 1-domain ELISA. 96-well ELISA plates (Greiner) were coated with 25 µg/ml fibrin or fibrinogen and were incubated in blocking buffer as indicated for binding assays for 1 h before the addition of 50 µl per well of biotinylated CD11b 1-domain in PBS with 0.5% BSA and 0.05% Tween-20 for 2 h at 37°C, followed by incubation with 1 µg/ml HRP-coupled streptavidin (BD Pharmingen, 1:10 000) for 1 h at 25°C and were developed by incubation with TMB/ε-aminophenazone (Cellmation-Millipore), and absorbance was measured at 450 nm with a Synergy H4 plate reader (BioTek). For SBB competition ELISA, after incubation in blocking buffer, SBB was diluted at twofold concentrations from 0.02 µM to 10 µM and was incubated for 2 h at 37°C, followed by incubation of 8.8 µg/ml CD11b I-domain for 2 h at 25°C.

Cell culture. Primary microglia and BMDMs were isolated, cultured and treated with fibrin or LPS as previously described18. BMDMs (AlcEus Cells) (2 × 106 cells/ml) were plated in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin and 50 µg/ml human M-CSF (300–25, Peprotech) in tissue-culture-treated dishes (Corning). After 24 h, non-adherent cells were removed, and adherent cells were cultured for an additional 7–8 d at 37°C in 5% CO2.

Morphometry. Primary microglia were stimulated with fibrin for 48 h as described67. Microglia were fixed with 4% PFA, permeabilized with 0.1% Triton X-100 and immunostained with FITC-labeled Isolecton B4 (1:300; Sigma-Aldrich) and/or CellMask Red Stain (Thermo Scientific). Blocking involved pre-incubation with 80 µg/ml antibodies or mouse IgG2b (clone M-11 BioXCell BE0086, distributed by the UCSF Monoclonal Antibody Core, endotoxin concentrations <0.002 EU/µg or IgG2b) (eBioscience, endotoxin level <0.01 EU/µg) for 2 h at 37°C before plating of cells for a final concentration of antibodies at 40 µg/ml. For manual analysis, images were collected using an Axioplan 2 Zeiss microscope with an AxioCam HRc camera and analyzed using ImageJ. 250 cells per condition were counted for each experiment. Activated microglia were classified by the border of a surface area of >2,000 µm2. For automated image acquisition and analysis, images of microglia cells were acquired with the GEHC In-Cell Analyzer 2000, using a 10X lens and excitation/emission filter pairs of 350 nm/455 nm (CellMask Red Stain) and 579 nm/624 nm (Hoechst dye). Images were analyzed with the GEHC In-Cell Dye Detection software. The box version 1.7.3 was used. Activated microglia were segmented using a ‘nuclear’ segmentation type, with a set minimum target area of 30 µm2 and a set sensitivity of 75%. To minimize artifacts, segmentation with less than 120 intensity units or area greater than 1,000 µm2 were excluded. The CellMask Red–stained whole microglia were segmented using an intensity threshold and a set minimum area units and 4095 intensity units. The borders of adjacent contacting cells were resolved using the clump breaking’ segmentation process-post-processing, which utilizes discrete nuclei as seeds. For segmentation by size, cells >800 µm2 were classified as activated microglia. Only cells containing a nucleus within the cell body area were accepted. All segmented nuclei and cells were then recorded as individual counts.

Fibrin polymerization assay. Human fibrinogen was diluted to 0.15 mg/ml in 20 mM HEPES (pH 7.4) containing 0.15 M NaCl and 5 mM ε-aminocaproic acid and was combined with 0.3 U/ml thrombin (Sigma-Aldrich) and 10 mM CaCl2. Antibodies (50 µg/ml) or GPRP peptide (Bachem) were incubated with fibrinogen for 2 h at 37°C before mixture with CaCl2–thrombin. Absorbance at 350 nm over intervals of 60 s was measured using a SpectraMax M5 microplate reader (Molecular Devices) with SoftMax Pro 5.2 software (Phoenix Technologies).

Real time qPCR, multi-plex qPCR and microarray analysis. RNA isolation, real-time PCR and microarray analysis were performed as described. Primers are listed in Supplementary Table 3. Microarray data from GEO accession code GSE100000 were used to select ensembles of genes with significant expression (log, values) to generate heat maps for primary microglia and BMDMs. For multiplex qPCR, primary microglia were plated in 96-well black μ-clear-bottomed microtiter plates (Greiner Bio-One) in DMEM, 10% FBS, 1% penicillin-streptomycin and were allowed to adhere for 24 h before being treated for 4 h with 1 µM fibrin d-dimer (HyTest) as described9. SBB or IgG2b (20 µg/ml) was pre-incubated with fibrin d-dimer in DMEM for 3 h at 37°C before cell treatment. Gene expression was assessed using the RT2 Profiler PCR Array (Ray Immunoflaryngology & Autoimmunity; Qiagen), and only differentially expressed genes (a difference in expression of over twofold; P < 0.05) were reported as the ‘mean fold change’. Experiments analyzing the inhibition of gene expression by antibodies performed at the Gladstone Institutes were independently reproduced at Lundbeck, US with similar results.

Oxidant detection with DHE. BMDMs or PBMCs were incubated in RPMI containing 5 µM DHE (Invitrogen) for 30 min. 100,000 cells/well were plated on 96-well black μ-clear-bottomed microtiter plates (Greiner Bio-One) pre-coated with 25 µg/ml fibrin. SBB or IgG2b (each 20 µg/ml) (UCSF Monoclonal Antibody Core clone LTF-2) was added in fibrin-coated wells 2 h before plating of cells. BMDMs were incubated with 300 µM acrylamid (Calbiochem) for 1 h or 5 µg/ml of anti-CD11b (M1/70, eBioscience) for 30 min before plating. Cells were incubated on fibrin for 24–48 h and were fixed with 4% PFA for 10 min, and DHE fluorescence was detected with a Leica SP8 microscope (358 nm/605 nm) using a SpectraMax M5 microplate reader. Cells were quantified by DAPI counterstaining. In mice, in vivo administration of DHE and detection were performed as described11.

NADPH-oxidase activity. BMDMs were plated on 25 µg/ml fibrin-coated six-well plates (Greiner Bio-One). SBB or IgG2b (each 20 µg/ml) was added to the
fibrin plates 2 h before plating cells. NADPH-oxidase activity was assayed by the lucigenin-enhanced chemiluminescence method. Cells were collected with a cell scraper and were homogenized in ice-cold Krebs buffer, pH 7.4 (119 mM NaCl, 2.5 mM KCl, 1 mM NaH2PO4, 1.3 mM MgCl2, 2.5 mM CaCl2, 11 mM glucose and 20 mM HEPES, pH 7.4). The cell homogenate was centrifuged at 1,000g, and the pellet was resuspended with luminescence buffer (Krebs buffer containing 10 µM lucigenin; Cayman Chemicals), before the addition of 100 µM NADPH substrate (Sigma-Aldrich). Luminescence was detected by an EnSpire microplate reader.

ImmunobLOTS. BMDMs cultured on plates coated with 25 µg/ml fibrin were lysed in RIPA lysis buffer (EMD Millipore) with protease-phosphatase inhibitor cocktail (EMD Millipore). Protein extracts (20 µg) were separated by electrophoresis on NuPAGE 4–12% Bis-Tris Gel (Life Technologies) and were transferred onto nitrocellulose. Blots were blocked in 5% milk in TBST and were incubated with antibodies against OPN (1:1,000; clone EP6991; Abcam), phospho-p44/42 MAPK (1:1,000; Th154, Cell Signaling Technology) or GAPDH (1:5,000; clone 14C10, Cell Signaling Technology), followed by horseradish peroxidase-conjugated secondary antibody (1:5,000; Cell Signaling Technology #7074S) and an enhanced chemiluminescence (ECL) kit (GE Healthcare) for detection. Densitometry analysis was performed using ImageJ.

Cortical neuron–macrophage co-culture. Cortices from P0 rats were finely minced and were digested for 30 min at 37 °C in DPBS (Gibco) containing papain (Worthington Biochemicals) and DNase (Sigma-Aldrich). Papain was inhibited by the addition of CuSO4 and EDTA (Worthington Biochemicals). Neurons were isolated at a density of 60,000 cells/cm² on poly-a-lysine-pre-coated eight-well Permanox chambers (Sigma-Aldrich) in Neurobasal/B27 medium (Invitrogen) and were cultured for 10 d. Rat BMDMs were cultured in RPMI-1640 with 10% heat-inactivated FBS (Invitrogen), 1% penicillin-streptomycin (Corning), and 10 ng/ml rat M-CSF (#400–28, Peprotec). BMDMs were plated on 25 µg/ml fibrin-coated plates at 20,000 cells/well on day 0 (day of transduction), and transferred with PBS-EDTA as described, then were added to cortical neuron cultures for 2 d, fixed with 4% PFA and immunostained with anti-MAP-2 (1:1,000; clone AP20, EMD Millipore); thresholded images were quantified with the Neurolucida software plug-in in ImageJ. 3.5 x 10⁶ g/ml of AAV1.hSyn.TurboRFP (University of Pennsylvania Vector Core) was used to transduce primary cortical neurons for 8 d before the addition of fibrin-stimulated BMDMs for 12 h. RFP images were thresholded and the neurite fragments were analyzed using the ImageJ plugin 'Analyse Particles'. Quantification was performed by an observer blinded to experimental treatment.

Pharmacokinetics. C57BL/6 mice were given intraperitoneal injection of 400 µg s88. Blood samples were collected by micro-accusampler and were centrifuged at 2,500 r.p.m. for 15 min. Plasma was stored at −80 °C until analysis. Plasma was diluted 1:1,000 in blocking buffer (3% BSA and 0.1% NP-40 in PBS). 5B8 was diluted 1:1,000 in blocking buffer and were added to wells coated with human peptide (MEVGWYRSPFSRVVHLYRDGK; Auspep) in complete Freund’s adjuvant. 5B8 was added to wells coated with human peptide and brains were processed as described. Antibodies used were as follows: mouse specific data models using WikiPathways and HomoloGene. The co-expression network was constructed by extraction of the dominant cluster of downregulated genes of interest (selected from those with change in expression of −0.5-fold or less (log, values) and mean P value of <0.05) on the basis of co-expression interaction data provided by GeneMANIA. Subsequent network visualization, layout, data overlays and subnet extraction were performed in Cytoscape.

Flow cytometry. Primary mouse splenocytes were isolated from s88- or IgG2b-treated mice 10 d after immunization with PLP191–211. Cells were stained with antibodies to non-phosphorylated neurofilament H (1:100; BioLegend) and brains were processed as described. Antibodies used were as follows: 3.5 x 10⁶ g/ml of AAV1.hSyn.TurboRFP (University of Pennsylvania Vector Core) was used to transduce primary cortical neurons for 8 d before the addition of fibrin-stimulated BMDMs for 12 h. RFP images were thresholded and the neurite fragments were analyzed using the ImageJ plugin 'Analyse Particles'. Quantification was performed by an observer blinded to experimental treatment.

Stereotactic fibrinogen injection and drug treatment. Stereotactic injection of fibrinogen into the corpus callosum of Cx3cr1Δ/Δ or C57BL/6 mice and analysis were performed as described. Mice were given intraperitoneal injection of 800 µg/mouse of biotinylated s88 every 2 d (three total doses). Mice were perfused with saline, and the spinal cord or brain was processed for fresh frozen sections as described. Sections were fixed with 4% PFA for 10 min, and biotinylated s88 was detected using Cy3-conjugated streptavidin (1:100; Invitrogen) for 30 min at 25 °C. Sections were incubated for 1 h with antibody to fibrinogen (1:2,000), followed by FITC donkey anti-rabbit (1:500; Jackson ImmunoResearch) for 30 min at 25 °C. For antibody–plaque staining, sections were counterstained with Methoxy-X04 (Tocris; 4% v/v of 10 mg/ml).

Two-photon in vivo imaging. Thy1-YFP and Thy1-YFP:5XFAD mice were given intravenous injection of Alexa594-conjugated fibrinogen (Invitrogen) daily for 3 d as described. Mice were also given intraperitoneal injection of Methoxy-X04 24 h before imaging. Methoxy-X04 was solubilized with DMso–glycerol–PBS pH 7.5 (ratio, 2:9:9) at 5 mg/ml. On the day of imaging, a small cranioectomy was made, and a custom-made metal plate was affixed to a stage to stabilize the skull. Alexa594–fibrinogen solution was injected retro-orbitally before imaging. The anesthetized animal was placed on a heated pad under an Ultima-Iv multiphoton microscope (Prairie) equipped with MaïTai DeepSee-eHP lasers (Spectra Physics). The excitation wavelength was 820 nm to simultaneously visualize fibrinogen, methoxy-X04 and YFP dendra. Imaging was performed from 20 to 150 µm below the dura, using a Nikon 40x and 0.8 NA immersion lenses with a 1.0µm z-step. z-stacks of images were projected along the z-axis to recreate two-dimensional representations of the 3D structures within the imaged volumes. Images were adjusted for brightness, contrast and background noise with ImageJ. Spectral unmixing plugin in ImageJ was used to separate overlapping signals.

Network analyses. The TYROB network and the Microglia Pathogen Phagocytosis Pathway were ‘translated’ from human-specific, static figures to mouse-specific data models using WikiPathways and HomoloGene. The co-expression network was constructed by extraction of the dominant cluster of downregulated genes of interest (selected from those with change in expression of −0.5-fold or less (log, values) and mean P value of <0.05) on the basis of co-expression interaction data provided by GeneMANIA. Subsequent network visualization, layout, data overlays and subnet extraction were performed in Cytoscape.
Statistical analyses. Data are presented as mean ± s.e.m. Statistical calculations were performed with GraphPad Prism (Version 6.03). Sample sizes were based on previous experiments and were not predetermined by statistical methods. Statistical significance was determined with a non-parametric two-sided Mann-Whitney test, Kruskal-Wallis test, Welch's two-sample t-test, or one-way or two-way analysis of variance followed by Bonferroni or Tukey's post-test (multiple comparisons). Linear models were fitted for each gene using the Bioconductor limma package in R<sup>66</sup>. Moderated t-statistics, change in expression ('fold' values) and the associated P values were calculated for each gene. Mice were randomly assigned to experimental groups for EAE and 5XFAD experiments. The assignment of scores for EAE in mice was performed in a blinded fashion. All animals survived to the end of the study, and all data points were included in analysis. All histopathological analysis and quantification was performed in a blinded way.

To compare clinical scores for EAE, the linear mixed-effects models were performed using the R statistical package. Means of maximum clinical scores were compared using a two-sample t-test as implemented in R using the Welch's t-test and the Wilcoxon rank sum test. P values were corrected for multiple testing using the Holm procedure. The statistical significance of the changes in the mean clinical score for each day of the experiment was estimated using permutation tests. The corresponding P values were estimated using 1,000 permutations in all prophylactic antibody treatment and 10,000 permutations for therapeutic antibody treatment. In each permutation, mice were randomly permuted. To compare the first day of onset, the log-rank test was used as implemented by the ‘survdiff’ function in the ‘survival’ package in R.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
GEO data supporting the findings of this study have been deposited in the GEO depository under accession numbers GSE118920 and GSE118921. Networks are permanently referenced in two Wikipathways entries: TYROBP Causal Network (Mus musculus) ([http://wikipathways.org/index.php?title=Pathway:WP3625&oldid=85694](http://wikipathways.org/index.php?title=Pathway:WP3625&oldid=85694)) and Microglia Pathogen Phagocytosis Pathway (Mus musculus) ([http://wikipathways.org/index.php?title=Pathway:WP3626&oldid=85691](http://wikipathways.org/index.php?title=Pathway:WP3626&oldid=85691)). The authors declare that all other data supporting the findings of this study are available within the paper. Any additional data can be made available from the corresponding author upon request.

References
55. Jung, S. et al. Analysis of fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* **20**, 4106–4114 (2000). Medline.
56. Feng, G. et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**, 41–51 (2000).
57. Oakley, H. et al. Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer’s disease mutations: potential factors in amyloid plaque formation. *J. Neurosci.* **26**, 10129–10140 (2006).
58. Jackson, S. H., Gallin, J. I. & Holland, S. M. The p47phox mouse knock-out model of chronic granulomatous disease. *J. Exp. Med.* **182**, 751–758 (1995).
59. Saeedup, N. et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS One* **5**, e13693 (2010).
60. Jensen, M. R. et al. Structural basis for simvastatin competitive antagonism of complement receptor 3. *J. Biol. Chem.* **291**, 16963–16976 (2016).
61. Abid, M. R., Spokes, K. C., Shih, S. C. & Aird, W. C. NADPH oxidase activity selectively modulates vascular endothelial growth factor signaling pathways. *J. Biol. Chem.* **282**, 35373–35385 (2007).
62. Klunk, W. E. et al. Imaging Abeta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. *J. Neuropathol. Exp. Neurol.* **61**, 797–805 (2002).
63. Kutmon, M. et al. WikiPathways: capturing the full diversity of pathway knowledge. *Nucleic Acids Res.* **44**, D488–494 (2016).
64. Warde-Farley, D. et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* **38**, W214–220 (2010).
65. Shannon, P. et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504 (2003).
66. Gentleman, R. C. et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* **5**, R80 (2004).
Corresponding author(s): Katerina Akassoglou

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
- Give $P$ values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s $d$, Pearson’s $r$), indicating how they were calculated
- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Software and code

Policy information about availability of computer code

Data collection

- Fibrin clotting was traced by measuring absorbance at 350 nm over intervals of 60 s using a SpectraMax M5 microplate reader (Molecular Devices) with SoftMax Pro 5.2 software (Phoenix Technologies Ltd.).
- qPCR data were collected on the StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific).
- Flow cytometry data were acquired on a BD LSRII (BD Bioscienves) with BD FACSDiva™ software.
- Confocal microscopy images were taken using a Fluoview FV1000 (Olympus) confocal microscope and FluoView Software.
- Immunohistochemical microscopy images were collected on an Axioplan II epifluorescence microscope (Zeiss).
- Two-photon in vivo imaging data were acquired on an Ultima-IV multiphoton microscope (Prairie) equipped with MaiTai DeeSee-eHP lasers (Spectra Physics).
- The PyMOL Molecular Graphics System, Version 1.6 Schrödinger, LLC was used for the structural map of the γC-domain of fibrinogen.

Data analysis

- GraphPad Prism software v7.03 (GraphPad Software, Inc.) was used to prepare graphs and to perform statistical analyses.
- Automated Image acquisition and Analysis: Images of microglia cells were acquired with the GEHC IN-Cell Analyzer 2000™ and analyzed with the GEHC IN-Cell Developer Toolbox version 1.9. Multiplex qPCR for primary rat microglia: Array analysis was performed with the online RT2 Profiler Data Analysis Software following manufactures default setting (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).
- Fold change was calculated based on the comparative delta delta Ct method and statistical significance was determined by Student’s t-test. Differentially expressed genes were deemed when fold change was >2 and $p<0.05$. Flow cytometry data were analyzed using FlowJo software (Tree Star, Inc.).
- MAP-2+ neurite area was quantified with the NeurphologyJ software plug-in to skeletonize the entire well images. Resulting images were thresholded and area was measured with NIH ImageJ analysis software (v1.50). Fluorescent images of primary neurons expressing RFP were thresholded and the degree of fragmentation (number of fragments) was analyzed using the ImageJ plug-in ‘Analyze Particles.’ The statistical test for the differential expression that informed the network figure data overlays: Linear
models were fitted for each gene using the Bioconductor ‘limma’ package in R. Moderated t-statistics, fold change and the associated P values were calculated for each gene. The coexpression network was constructed by extracting the dominant cluster of downregulated genes of interest (selected from those with log2 fold change of -0.5 or less and raw p-value < 0.05) based on coexpression interaction data provided by GeneMANIA. Subsequent network visualization, layout, data overlays and subnetwork extraction were performed in Cytoscape. Two classes of statistical tests were used to test for differences in the variation of clinical scores and time of onset in the four EAE models between treatment groups. To compare EAE clinical scores, the linear mixed effects models were first performed using the R statistical package. The comparison of means of maximum clinical scores was performed using a two-sample t-test as implemented in R using the Welch’s t-test and the Wilcoxon rank sum test. P-values were corrected for multiple testing using the Holm procedure. The statistical significance of the changes in the mean clinical score for each day of the experiment was estimated using permutation tests. The corresponding p-values were estimated using 10000 permutations in all prophylactic antibody treatment and 10000 permutations for therapeutic antibody treatment. In each permutation, mice were randomly permuted. To compare the first day of onset, the log-rank test was used as implemented by the survdiff function in the survival package in R.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data
Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

GEO data supporting the findings of this study have been deposited in the GEO depository under accession numbers GSE118920 and GSE118921. The TYROBP network and the Microglia Pathogen Phagocytosis Pathway were translated from human-specific, static figures to mouse-specific data models using WikiPathways and HomoloGene, and are permanently referenced by these links, respectively: http://wikipathways.org/index.php?title=Pathway:WP3625&oldid=85694, http://wikipathways.org/index.php?title=Pathway:WP3626&oldid=85691. The authors declare that all other data supporting the findings of this study are available within the paper. Any additional data can be made available from the corresponding author upon request.

Field-specific reporting
Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical methods were used to predetermine sample size, but our sample sizes are similar to those reported previously.

Data exclusions
No samples or animals were excluded from the analysis.
For the microglia morphology assay, an exclusion criterion applied was fibrin-induced activation < 20 % compared to control. Experiments with activation > 20% were used for analysis.

Replication
Experiments were replicated several times with reproducible results, as indicated in each figure legend.
Fibrin antibody binding to peptides, fibrin, and fibrinogen and gene expression antibody inhibition experiments performed at the Gladstone Institutes were all independently validated at Lundbeck, US with similar results.
Fibrin antibody binding studies performed at the Gladstone Institutes were also independently validated at the University of California, San Francisco Small Molecule Discovery Center with similar results.

Randomization
Animals were randomly assigned to experimental groups at the beginning of experiments. The randomization of animal studies is stated in the Methods section.

Blinding
For EAE studies and histopathological experiments, the researchers were blinded as to the mouse treatment conditions. Mice were scored by blinded observers. Mice were divided into experimental groups in an unbiased manner. The mice were randomized and coded to assign groups or collect data for animal experiments.

A set of the binding, morphology and fibrin polymerization assays of the monoclonal fibrin antibodies were performed in a blinded manner and the code was unblinded at the end of all three experimental assays.

All imaging of immunohistology and quantification experiments were performed in a blinded manner.

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf
### Materials & experimental systems

| Method | Involved in the study |
|--------|-----------------------|
| n/a    |                       |
| -      | Unique biological materials |
| -      | Antibodies |
| -      | Eukaryotic cell lines |
| -      | Palaeontology |
| -      | Animals and other organisms |
| -      | Human research participants |

### Methods

| Method | Involved in the study |
|--------|-----------------------|
| n/a    |                       |
| -      | ChIP-seq |
| -      | Flow cytometry |
| -      | MRI-based neuroimaging |

### Unique biological materials

**Policy information about availability of materials**

**Obtaining unique materials**

Distribution of antibodies to non-profit investigators will be under a Material Transfer Agreement (MTA) where applicable. A request for reimbursement of expenses for shipping and handling, and other costs associated with production and delivering materials to the requesting investigator where applicable.

**Antibodies**

- gp91phox (clone EPR6991, Abcam), Phospho-p40phox (clone Thr154, Cell Signaling Technology), GAPDH (clone 14C10, Cell Signaling Technology), MAP-2 (clone AP20, EMD Millipore), neurofilament H non-phosphorylated (clone SMI-32, Biolegend), Iba-1 (catalog# 019-19741, Wako), myelin basic protein (clone SMI-99, Biolegend), choline acetyltransferase (catalog# AB144P, EMD Millipore), CD11b (clone M1/70, eBioscience), MAP-2 (clone M3/38, Cedarlane), fibrinogen (kind gift of Dr. Jay Degen), human amyloid beta (catalog# 18558, IBL-America), CD45 (clone 30-F11, Biolegend), anti-IFN-γ (clone XMG1.2, Biolegend) and anti-IL-17-FITC (clone TC11-18H10.1, Biolegend).
- polyclonal goat anti-mouse IgG-HRP (catalog# P0447, DAKO), Purified mouse IgG2b isotype control (clone MPC-11, UCSF Monoclonal Antibody Core), and Purified Rat IgG2b isotype control (clone LTF-2, UCSF Monoclonal Antibody Core).

**Validation**

All antibodies used in this study are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer’s website. Appropriate antibody dilutions were performed based on preliminary experiments and intensity of fluorescent signals.

### Animals and other organisms

**Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research**

**Laboratory animals**

- SJL/J, C57BL/6, Cx3cr1GFP/+,
- Thy1-YFP (Tg(Thy1-YFP)HJrs), 5XFAD (B6SJL-Tg(APPswFlLon,PSEN1*M146L*L286V) 6799 Vas/Mmjax), and p47phox/- mice were purchased from The Jackson Laboratory. 5XFAD were crossed with Thy1-YFP to generate 5XFAD:Thy1-YFP mice. Mothers and their newborn Sprague-Dawley rats (PO) were purchased from Charles River Laboratories. Ccr2RFP/RFP on a C57BL/6 background were bred to generate Cx3cr1GFP/+Ccr2RFP/+ mice. Microglia were prepared from neonatal rat pups at postnatal day (P) 2–3. Bone marrow cells were isolated from tibia and femur of 10-week-old C57BL/6 mice. Active experimental autoimmune encephalomyelitis (EAE) was induced in 8–9-week-old female SJL/J, C57BL/6, or Cx3cr1GFP/+Ccr2RFP/+ mice. Both male and female 5XFAD mice were used.

**Wild animals**

The study did not involve wild animals.

**Field-collected samples**

The study did not involve samples collected from the field.
Flow Cytometry

Plots

- Confirm that:
  - The labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Primary splenocytes were harvested and processed for flow cytometric analysis as described in Methods. Supplementary Fig 6. Cells were harvested, stained for surface antigens, fixed and then immediately analyzed by LSR II (BD Biosciences). Supplementary Fig 6b. For cytokine recall analysis, cells were incubated for 4h with Cell Activation Cocktail (Biolegend) in vitro prior to surface and intracellular antigen staining, and then analyzed via LSR II (BD Biosciences).

Instrument

LSR II (BD)

Software

BD FACSDiva and FlowJo software v10

Cell population abundance

When cells were sorted or enriched, the purity was confirmed by flow cytometry and routinely >95%.

Gating strategy

Lymphocytes were gated by FSC-A/SSC-A and then doublet discrimination was performed first by FSC-H/FSC-W followed by SSC-H/SSC-W. Next, CD45-negative cells were gated out and then gating strategy focused on quantifying cell specific populations based on the following definitions: CD45+CD3+CD8+ T cells; CD45+CD3+CD4+ T cells; CD45+CD3+CD4+IL17+ and CD45+CD3+CD4+IFNγ+ T cells; CD45+CD3+B220+ B cells; CD45+CD11b+Ly6G+ neutrophils; CD45+CD11b+CD11c+ dendritic cells; CD45+CD11b+CD11b-Ly6g- myeloid cells

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.