

A role for leukocyte-endothelial adhesion mechanisms in epilepsy

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The mechanisms involved in the pathogenesis of epilepsy, a chronic neurological disorder that affects approximately one percent of the world population, are not well understood¹⁻³. Using a mouse model of epilepsy, we show that seizures induce elevated expression of vascular cell adhesion molecules and enhanced leukocyte rolling and arrest in brain vessels mediated by the leukocyte mucin P-selectin glycoprotein ligand-1 (PSGL-1, encoded by *Selplg*) and leukocyte integrins $\alpha_4\beta_1$ and $\alpha_L\beta_2$. Inhibition of leukocyte-vascular interactions, either with blocking antibodies or by genetically interfering with PSGL-1 function in mice, markedly reduced seizures. Treatment with blocking antibodies after acute seizures prevented the development of epilepsy. Neutrophil depletion also inhibited acute seizure induction and chronic spontaneous recurrent seizures. Blood-brain barrier (BBB) leakage, which is known to enhance neuronal excitability, was induced by acute seizure activity but was prevented by blockade of leukocyte-vascular adhesion, suggesting a pathogenetic link between leukocyte-vascular interactions, BBB damage and seizure generation. Consistent with the potential leukocyte involvement in epilepsy in humans, leukocytes were more abundant in brains of individuals with epilepsy than in controls. Our results suggest leukocyte-endothelial interaction as a potential target for the prevention and treatment of epilepsy.

Experimental data from animal models as well as human evidence indicate that seizures can lead to neuronal damage and cognitive impairment^{2,3}. However, the molecular mechanisms leading to seizures and epilepsy are not well understood. Recent data suggests that inflammation may play a role in the pathogenesis of epilepsy^{4,5}. For

instance, increases in inflammatory cytokines are seen in the central nervous system (CNS) and plasma in experimental models of seizures and in clinical cases of epilepsy^{4,5}. Moreover, CNS inflammation is associated with breakdown of the BBB, and BBB leakage has been implicated both in the induction of seizures and in the progression to epilepsy⁶⁻⁹. Leukocyte recruitment is a hallmark of and a point of therapeutic intervention in tissue inflammation^{10,11}, but a role for leukocyte-endothelial interactions in seizure pathogenesis has not been explored.

Here we have used an established mouse model of epilepsy induced by pilocarpine to ask whether leukocyte-endothelial interactions are altered by seizures and whether they can contribute to seizure pathogenesis and epilepsy. Systemic administration of pilocarpine elicits sustained seizure activity (status epilepticus), typically lasting a few hours^{12,13}. After a latent (seizure-free) phase, mice go on to develop epilepsy characterized by spontaneous recurrent seizures (SRS)^{12,13}. Chronic epilepsy in this model is associated with hippocampal as well as cortical and subcortical lesions¹⁴.

We first studied endothelial activation and found that acute seizure activity following pilocarpine administration led to enhanced vascular expression of leukocyte adhesion molecules. Vessels in control brains expressed low amounts of intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), E-selectin and P-selectin expression were below the limits of detectability (Fig. 1a). ICAM-1 and VCAM-1 were markedly upregulated after seizure induction, with the highest levels observed 24 h and 7 d after status epilepticus (Fig. 1a,b and Supplementary Table 1a online). Pharmacological suppression of status epilepticus with diazepam, a benzodiazepine with CNS depressant properties, before pilocarpine injection substantially but not completely prevented the upregulation

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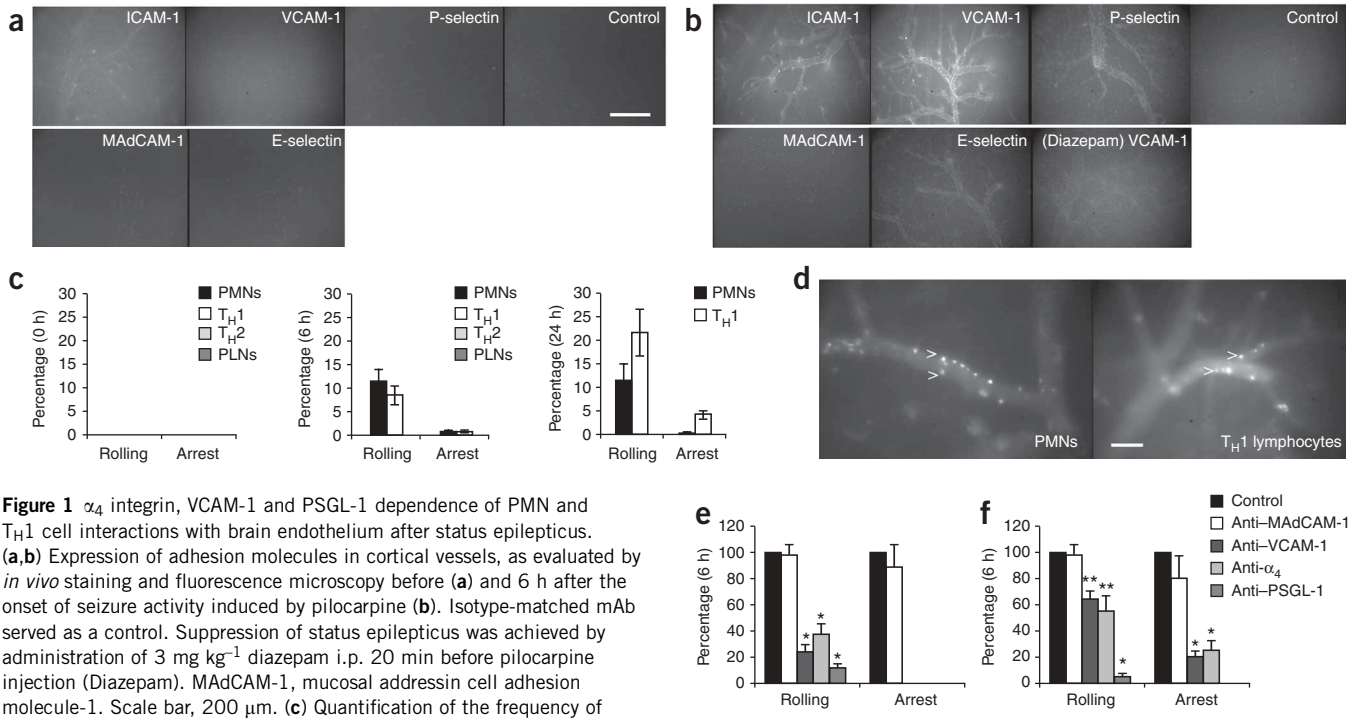


Figure 1 α_4 integrin, VCAM-1 and PSGL-1 dependence of PMN and T_H1 cell interactions with brain endothelium after status epilepticus. (a,b) Expression of adhesion molecules in cortical vessels, as evaluated by *in vivo* staining and fluorescence microscopy before (a) and 6 h after the onset of seizure activity induced by pilocarpine (b). Isotype-matched mAb served as a control. Suppression of status epilepticus was achieved by administration of 3 mg kg⁻¹ diazepam i.p. 20 min before pilocarpine injection (Diazepam). MAdCAM-1, mucosal addressin cell adhesion molecule-1. Scale bar, 200 μ m. (c) Quantification of the frequency of rolling interactions or sticking behavior of fluorescently labeled PMNs and lymphocyte subpopulations in cortical venules, studied before pilocarpine injection (0 h) and at 6 h and 24 h after status epilepticus onset, as determined by *in situ* videomicroscopy¹⁵. The percentage of total cells shown on the y axis was calculated as described in the **Supplementary Methods**. The number of venules and mice per group analyzed are provided in **Supplementary Table 1b,c**. Means \pm s.e.m. are shown. PLNs, freshly isolated peripheral lymph node cells (resting lymphocytes). (d) Intravital microscopy studies showing adherent PMNs 6 h after status epilepticus and T_H1 cells at 24 h after status epilepticus in cerebral vessels (arrows). Scale bar, 100 μ m. (e,f) mAbs specific for adhesion molecules (anti-MAdCAM-1, anti-VCAM-1, anti- α_4 and anti-PSGL-1) were used to block leukocyte or endothelial adhesion molecules at 6 h after status epilepticus as described in the **Supplementary Methods**. PMNs are shown in e and T_H1 cells are shown in f. ** $P < 0.01$ and * $P < 0.001$.

of VCAM-1 (Fig. 1b), suggesting that electrical hyperactivity contributes to high VCAM-1 expression in brain vessels, potentially in combination with a direct vascular effect of pilocarpine itself⁹. P-selectin and E-selectin were also induced on venular endothelium after seizures (Fig. 1a,b and **Supplementary Table 1a**). Thus, the elicited seizure activity is associated with inflammatory changes in the CNS vasculature that could support enhanced adhesion of circulating leukocytes and consequent amplification of vascular damage.

Direct pilocarpine treatment of leukocytes does not render them hyperadhesive (**Supplementary Fig. 1a** online), but pilocarpine induction of seizures markedly enhanced leukocyte adhesion to CNS vessels *in vivo* (Fig. 1c,d). Intravital microscopy studies performed in cortical venules¹⁵ indicate that polymorphonuclear leukocytes (PMNs) showed efficient rolling and arrest after status epilepticus onset (Fig. 1c,d). Resting lymphocytes did not interact (Fig. 1c), whereas T helper type 1 (T_H1) polarized cells rolled and stuck efficiently after seizures (Fig. 1c,d). PMN arrest was consistently more efficient at 6 h, whereas T_H1 cells interacted more efficiently at 24 h than at 6 h after seizures, consistent with progression from acute to subacute inflammation during the 24 h period after status epilepticus-induced damage (Fig. 1c). The rolling velocities (V_{roll}) and the frequency distribution of V_{roll} in velocity classes are provided in **Supplementary Figure 1b** and **Supplementary Table 1**. Notably, *in vitro* polarized T_H2 cells, known to lack functional P-selectin ligand, did not roll or stick in brain vessels (Fig. 1c).

The rolling interactions and arrest of PMNs after status epilepticus were substantially inhibited by antibody blockade of α_4 integrin or its vascular counter-ligand VCAM-1 or by blockade of PSGL-1 or its principal vascular ligand P-selectin (Fig. 1e, **Supplementary Fig. 1c,d**

and **Supplementary Discussion** online). The rolling and arrest of T_H1 cells were also reduced by α_4 integrin or VCAM-1 blockade after status epilepticus (Fig. 1f) and were almost completely blocked by antibodies to PSGL-1 and P-selectin, as well (Fig. 1f and data not shown). Monoclonal antibody (mAb) to mucosal addressin cell adhesion molecule-1 had no noteworthy effect, excluding a role for the intestinal trafficking receptor $\alpha_4\beta_7$ integrin (Fig. 1e,f and **Supplementary Fig. 1c**). These results demonstrate that the integrin $\alpha_4\beta_1$ and the selectin ligand PSGL-1 mediate vascular-leukocyte adhesive interactions in cerebral vessels after status epilepticus. We, therefore, set out to ask whether these leukocyte adhesion mechanisms might contribute to seizure induction, epileptogenesis or both in this model.

To mimic therapeutic intervention in response to severe acute seizures, we initially evaluated the effect of adhesion blockade after status epilepticus on the subsequent development of recurrent seizures. One hour of status epilepticus is sufficient to lead to chronic spontaneous seizures in the pilocarpine model in mouse and rat^{13,16,17} (**Supplementary Discussion**). Therefore, pilocarpine-injected C57BL/6 mice were allowed to convulse for 1 h (stage 5 of Racine's scale¹⁸) and were treated with α_4 integrin-specific mAb intraperitoneally (i.p.; Fig. 2), starting immediately and continuing every other day for 20 d. As expected, treatment with antibody to α_4 integrin after status epilepticus onset had no effect on the character and duration of ongoing status epilepticus, as assessed visually or by electroencephalography (EEG) analysis after pilocarpine treatment (Fig. 2g, **Supplementary Fig. 2b** online and data not shown). However, video monitoring revealed a marked reduction of spontaneous convulsions during the chronic period (from day 5 to day 20 after pilocarpine injection) in mice treated with α_4 integrin-specific antibody (Fig. 2a).

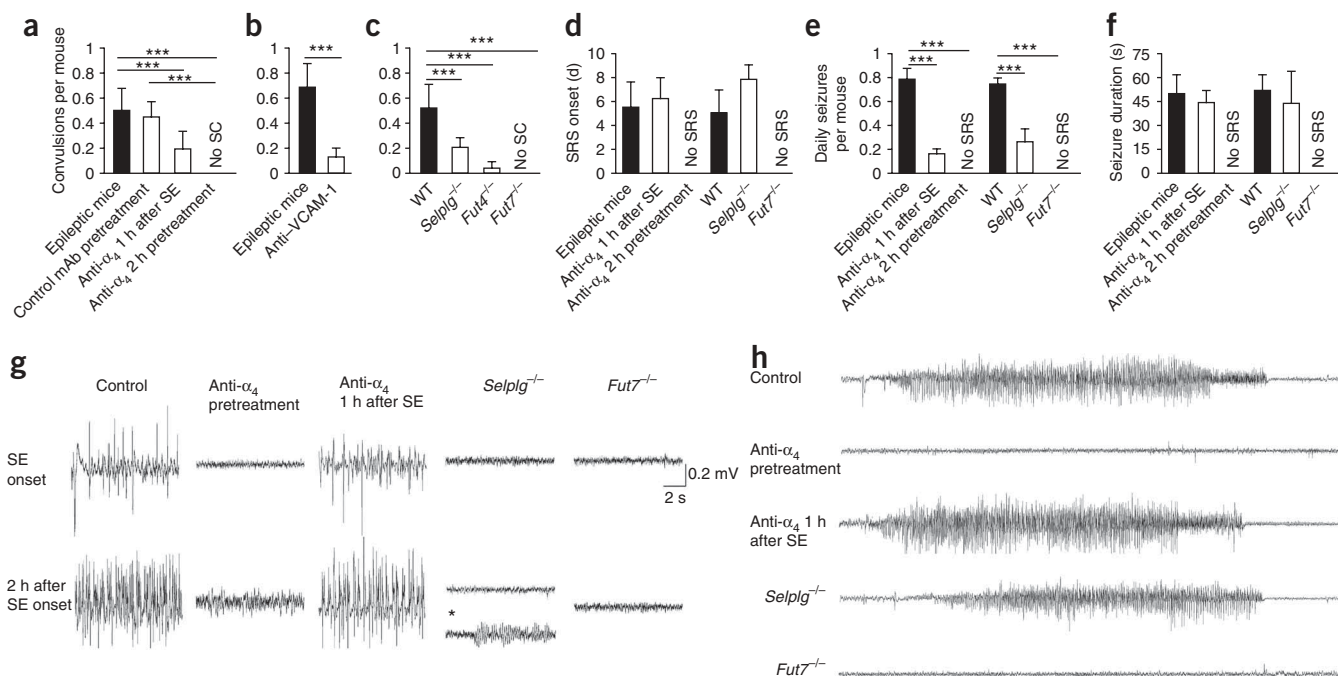


Figure 2 Effect of blockade or deficiency in leukocyte adhesion mechanisms on convulsions and seizures. (**a–c**) Quantification of the mean number of daily spontaneous convulsions (SC) per mouse, as determined by visual monitoring from days 5–20 after pilocarpine administration. Pilocarpine-treated wild-type (WT) mice that received no treatment (epileptic mice) were injected i.p. either 1 h after status epilepticus onset with 400 μ g anti- α_4 mAb or with 150 μ g anti-VCAM-1 mAb to model therapy, or were injected 2 h before pilocarpine injection with 400 μ g anti- α_4 mAb (pretreatment regimen). In both instances, treated mice also received antibody (200 μ g anti- α_4 or control mAb and 150 μ g anti-VCAM-1) every other day for 20 d. An isotype-matched control antibody was used as control (**a**). Ten to twelve mice per group were monitored for SC for 6 h d⁻¹ between days 5 and 20 after status epilepticus. One representative experiment from a series of four (**a**), two (**b**) and three (**c**) experiments with similar results is shown. (**d–f**) EEGs showing SRS onset (**d**), the mean daily seizures per mouse (**e**) and SRS duration (**f**). EEGs for each mouse were acquired 24 h d⁻¹ from days 0 to 20 after pilocarpine administration. Behaviorally, SRS was characterized in the mice by head nodding, forelimb clonus, rearing and falling. (**g**) Twenty-second EEG recordings early after initial seizures and at the end of the second hour. Theta activity was occasionally observed in *Selplg*^{-/-} mice (*). (**h**) EEG recordings of representative SRS. For **d–h**, data are representative of one experiment with three mice per group from two experiments with similar results. ****P* < 0.001.

EEG telemetry confirmed a consistent reduction in the frequency of spontaneous recurrent seizures (SRS) during the chronic phase in the treated mice (**Fig. 2e**). The latency of the first SRS and the duration of individual seizure events when they occurred were similar in the treatment and control groups (**Fig. 2d,f,h**). Neuropathological findings showed alterations after status epilepticus in both the treatment and control groups, including cerebral ventricle volume and hippocampal shrinkage, but there was a considerable decrease in neuronal cell loss in somatosensory cortex and hippocampus subfields in the treatment groups (**Fig. 3**). In addition, 30 d after status epilepticus, mice treated with antibody to α_4 integrin had a slight decrease in exploration behavior compared to normal mice but a substantial preservation of this behavior compared to epileptic mice (**Supplementary Fig. 2** and **Supplementary Table 2a** online). Treatment with antibody to the $\alpha_4\beta_1$ integrin ligand VCAM-1 after status epilepticus was also effective in preventing spontaneous convulsions during the chronic phase (**Fig. 2b**). Thus, our data suggest that α_4 integrin and its ligand VCAM-1 contribute to pathogenic events required for epileptogenesis in this model.

Notably, when antibody to α_4 integrin was given 2 h before injection of pilocarpine, it completely prevented convulsions (**Fig. 2a**). In the first hour after pilocarpine injection, mice pretreated with antibody to α_4 integrin showed sporadic tremors and oral mastication (stage 2 of Racine's scale¹⁸), but no further tremors or status epilepticus developed, and no delayed spontaneous convulsions were detected from day 5 through day 20 (mice also received 200 μ g mAb to α_4 integrin

every other day for 20 d to mimic a therapeutic regimen; **Fig. 2a**). Moreover, pretreated mice showed no EEG alterations indicative of status epilepticus or SRS after pilocarpine injection (**Fig. 2d–h** and **Supplementary Fig. 2a**). Predictably, initiation of treatment with antibody to α_4 integrin before pilocarpine led to a marked decrease in neuronal cell loss in the somatosensory cortex and hippocampus and prevented delayed cognitive impairment (**Fig. 3**, **Supplementary Fig. 2e** and **Supplementary Table 2a**). In support of these results, blockade of α_L integrin or of its endothelial ligand ICAM-1 consistently reduced leukocyte-endothelial interactions and prevented convulsions (**Supplementary Fig. 1d,e**). In contrast, control mAb to CD45 or to $\alpha_4\beta_7$ integrin or mAbs to CD4 had no effect on status epilepticus induction, chronic disease or cognitive deterioration (**Fig. 2a**, **Supplementary Fig. 2e**, **Supplementary Table 2b** and data not shown).

Intravenously administered antibody should be largely excluded from the CNS by the BBB before status epilepticus, but we could not formally exclude an effect of leaked mAbs to α_4 integrin or VCAM-1 on nervous tissue. To address this issue, as well as to assess the role of selectin-mediated interactions, we took advantage of genetically modified mice deficient in PSGL-1 (*Selplg*^{-/-} mice) or in α -1,3-fucosyltransferases (FucTs) FucT-VII and FucT-IV, enzymes required to generate functional selectin-binding carbohydrates (*Fut4*^{-/-} and *Fut7*^{-/-} mice)¹⁹. Consistent with the inhibitory effects of treatment with antibody to α_4 integrin, we observed a marked reduction in the incidence of status epilepticus and of subsequent spontaneous

convulsions and of SRS as monitored visually or electroencephalographically in *Selplg*^{-/-}, *Fut7*^{-/-} and *Fut4*^{-/-} mice (Fig. 2c–h, Supplementary Fig. 2c,d and Supplementary Movie online). *Selplg*^{-/-} and *Fut7*^{-/-} mice also showed a significant reduction in neuronal damage and a considerable preservation of open field exploration behavior compared to epileptic mice (Fig. 3, Supplementary Fig. 2e and

Supplementary Table 2c). Because PSGL-1 is selectively expressed on leukocytes and endothelium²⁰, inhibition of seizures in *Selplg*^{-/-} mice further supports a direct or indirect role for leukocyte adhesion in seizure generation in this model and rules out primary neuronal or glial effects of anti-adhesion treatments. To further confirm the role of leukocytes in seizure generation, we used a

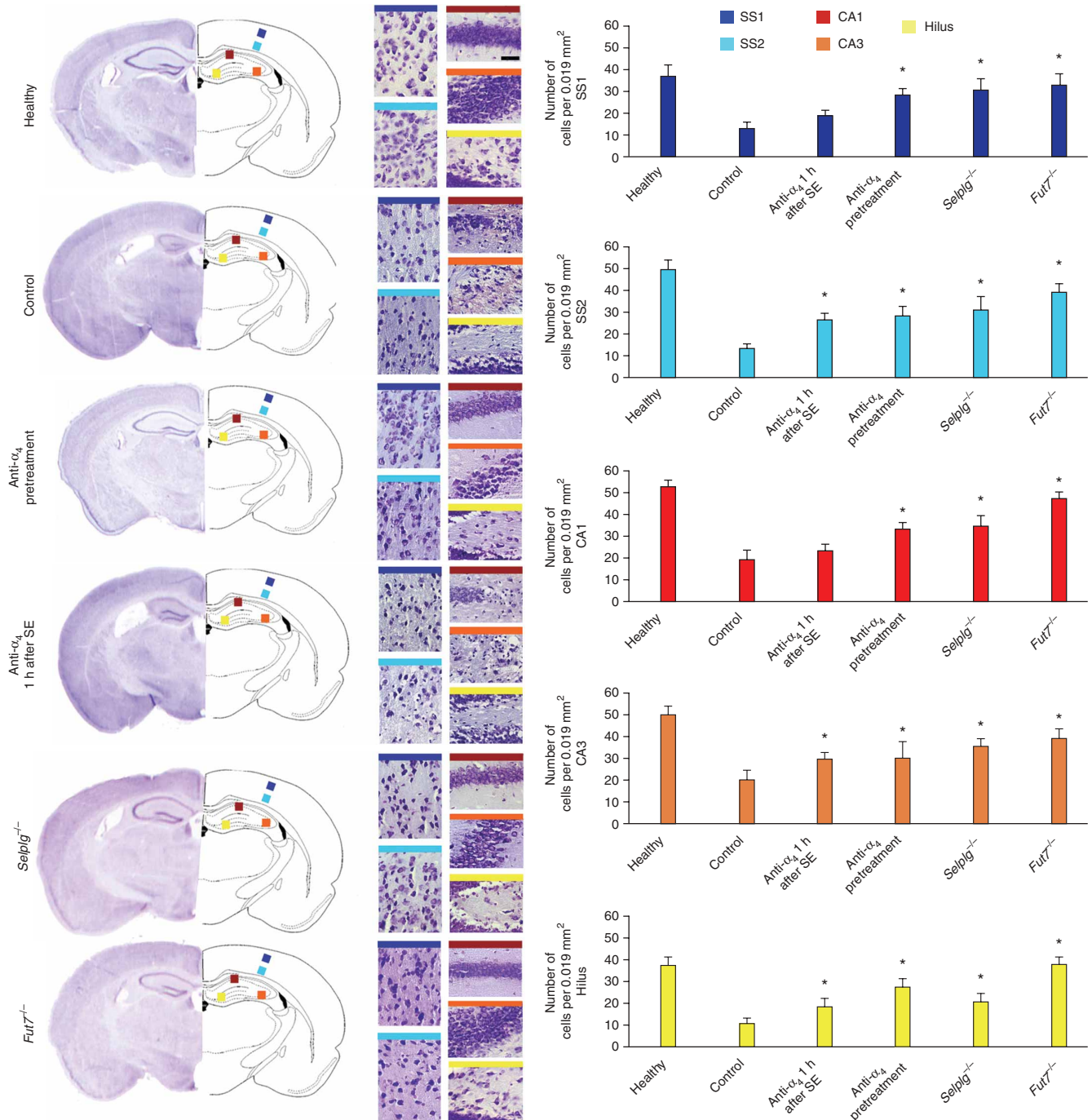


Figure 3 Effect of blockade or deficiency in leukocyte adhesion mechanisms on CNS structure and neuronal density. Left, histology images showing epilepsy-induced structural changes of the brains and effects on neuronal cell density alterations documented at 30 d after status epilepticus in healthy mice and in mice injected with pilocarpine: control (C57BL/6, WT epileptic) mice, anti- α_4 -treated mice, *Selplg*^{-/-} mice and *Fut7*^{-/-} mice. Right, quantitative evaluation of neuronal density determined by analyzing the number of Nissl-stained cell bodies in four sections per mouse (three mice per group) and using regions of interest sampling. Details are provided in the **Supplementary Methods**. Means \pm s.e.m. are shown for stereological counts. CA, corpus ammonis; SS1, somatosensory cortex layers II and III; SS2, somatosensory cortex deep layers V and VI. **P* < 0.04. Scale bar, 30 μ m.

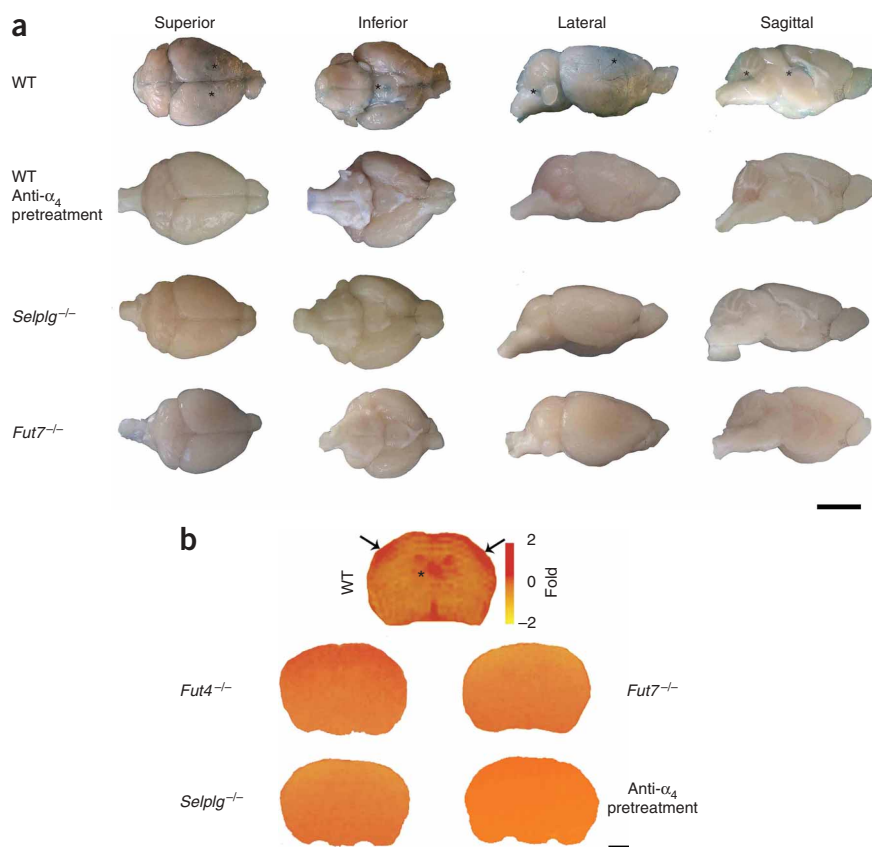


Figure 4 Inhibition or deficiency of leukocyte adhesion pathways maintains the integrity of the BBB. **(a)** Images of superior, inferior and lateral brain views and sagittal brain sections are shown to document Evans blue extravasation at 18–24 h after pilocarpine injection. In WT (control) mice, Evans blue leakage (asterisks) was observed macroscopically in brains (after removal of the meninges) in the parietal cortex and in particular in the territories of the medial cerebral artery (asterisks) in the superior and lateral views, in the pituitary gland pedunculus (where the BBB is more permeable) in the inferior view, and, finally, in the choroid plexus of the lateral ventricles and in the fourth ventricle in the sagittal view. WT mice injected only with methylscopolamine did not show any leakage (data not shown). Representative brains are shown from one of two experiments, three mice per condition, with similar results. Scale bar, 4 mm. **(b)** MRI images showing BBB alterations after status epilepticus in control (C57BL/6 WT mice) and anti- α_4 -treated mice and in *Selplg*^{-/-} and *Fut4*^{-/-} and *Fut7*^{-/-} mice at 24 h after pilocarpine injection. BBB permeability was studied by the mean of intravenously administered Magnevist, a paramagnetic iron oxide contrast agent. Pseudocolor maps evidencing contrast agent spreading in ventricles (asterisk) and in the cortical areas (arrows) near the middle cerebral artery are shown. Representative brains are shown from one of two experiments (three mice per condition) with similar results. Scale bar, 2 mm.

granulocyte-specific antibody to deplete neutrophils before pilocarpine administration. Neutrophil depletion caused a substantial reduction in status epilepticus and spontaneous recurrent convulsions (**Supplementary Fig. 2f**), confirming that leukocytes contribute to seizure pathogenesis.

Recent studies have shown that BBB breakdown induces epileptiform activity^{6–9,21}. Moreover, pilocarpine-induced seizure activity not only is associated with BBB leakage *in vivo* but may in fact require leakage of serum components—pilocarpine induces neuronal hyperactivity in *ex vivo* brain slices, but only in the context of elevated K⁺ levels mimicking plasma leakage into the CNS⁹. BBB leakage could also enhance access of pilocarpine itself to the extravascular compartment, although this is unlikely to be sufficient for epileptogenesis in the absence of plasma leakage⁹ (**Supplementary Discussion**). Therefore, we evaluated BBB leakage in our model. Pilocarpine-induced BBB opening was largely prevented in mice with leukocyte adhesion blockade, whether by antibody treatment or by genetic interference with leukocyte adhesion pathways (**Fig. 4a,b**).

Taken together, the demonstrated elicitation of neuronal hyperactivity by BBB leakage^{6–9,21} and the inhibition of BBB breakdown by blockade of leukocyte-endothelial interaction shown here suggest a mechanism for synergistic and potentially self-reinforcing interactions between endothelial activation, leukocyte adhesion, BBB opening and seizure generation. A cycle of seizure-induced inflammation and inflammation-mediated stimulation may amplify the initial effects, leading to chronic changes and recurrent seizure activity (**Supplementary Fig. 3a** and **Supplementary Discussion**). The general relevance of our findings remains to be determined in other models of epilepsy.

We evaluated human cortical CNS tissue from subjects with epilepsy and found elevated brain leukocyte numbers compared to control subjects without epilepsy, suggesting a possible role for leukocyte adhesion in humans as well (**Supplementary Fig. 4** and **Supplementary Table 3** online). Current pharmacological treatments for epilepsy inhibit neuronal excitability but do not address the inflammatory component of pathogenesis. Anti-adhesion therapies with humanized antibodies^{10,22,23} or other available approaches would allow clinical evaluation of adhesion pathways as targets for preventing epilepsy, particularly after inflammatory inciting events such as stroke or traumatic brain injury.

In conclusion, our results suggest that vascular inflammatory mechanisms and leukocyte-endothelial adhesion can contribute to the pathogenesis of seizures and epilepsy and show that inhibition of leukocyte-vascular interactions can have preventive as well as therapeutic effects in a mouse model of this debilitating disease.

METHODS

Reagents. MAbs to α_4 integrin (PS/2), ICAM-1 (YN 1.1.7.4), lymphocyte function-associated antigen-1 (TJB 213), mucosal addressin cell adhesion molecule-1 (MECA 367) were produced in our labs, and mAbs to VCAM-1 (MK 2.7), CD45 (30G12) were from American Type Culture Collection. Antibody to PSGL-1 (4RA10) was kindly provided by D. Vestweber.

Mice. We obtained C57BL/6 young male mice from Charles River. We obtained *Fut4*^{-/-} and *Fut7*^{-/-} mice as previously described^{24,25}. We prepared *Selplg*^{-/-} mice as previously described²⁶. *Selplg*^{-/-} and *Fut7*^{-/-} mice were on a C57BL/6 genetic background (backcrossed for at least nine generations). Experiments in mice were approved by the board of the Interdepartmental Center of Experimental Research Service from the University of Verona and by the Italian National Institute of Health and followed the principles of the US National Institutes of Health Guide for the Use and Care of Laboratory Animals and

the European Community Council (86/609/EEC) directive. A scheme of the experimental procedures performed in mice is provided in **Supplementary Figure 3b**.

Induction of seizures and epilepsy. Young male C57BL/6 mice (30–50 d of age, weight range: 19–23 g) were purchased from Harlan, maintained on a 12-h light-dark inverted schedule with access to food and water *ad libitum*, and habituated to the experimenters for at least 2 weeks before using them for experiments. We pretreated all mice injected with pilocarpine with methylscopolamine (1 mg kg⁻¹ i.p., Sigma) to minimize peripheral muscarinic effects. Thirty minutes later, we gave the mice 300 mg kg⁻¹ pilocarpine (Sigma) i.p. diluted in 0.01 M PBS, pH 7.4. Systemic administration of pilocarpine induced status epilepticus, with continuous seizure activity usually lasting 1–2 h. We evaluated pilocarpine-induced seizures during status epilepticus according to a modified version of the Racine scale using categories 1–5, with 5 being the most severe^{12,18}. Then, after a latent (seizure-free) phase of 1–2 weeks, the mice went on to develop chronic epilepsy characterized by spontaneous convulsions^{12,13}. All mice that developed status epilepticus also developed spontaneous seizures, as previously shown in C57BL/6 mice¹². Experiments in which more than 30% of the control mice died or did not develop status epilepticus after pilocarpine injection were not considered in this study. For *in vivo* staining studies of endothelial adhesion molecules, we injected mice with diazepam (3 mg kg⁻¹ i.p.) 20 min before pilocarpine administration to prevent status epilepticus. We injected mice with convulsions 2 h after status epilepticus onset with diazepam to achieve uniform status epilepticus duration to 2 h in all experimental groups.

Determination of blood-brain barrier permeability by MRI analysis. We performed magnetic resonance imaging (MRI) experiments with a Bruker Biospec Tomograph equipped with an Oxford 33-cm bore²⁷. We anesthetized the mice and placed them in a prone position into a 7.2-cm transmitter birdcage coil. The signal was acquired by a 1.5-cm actively decoupled surface coil. We cannulated the tail vein for the injection of 100 μmol kg⁻¹ contrast agent Magnevist (*N*-methylglucamine salt of gadolinium complex of diethylenetriamine pentaacetic acid; Schering). We acquired multislice, T1-weighted Spin Echo images before and up to 24 min after injection of Magnevist. The acquisition parameters were time of repetition, 350 ms; echo time, 14.4 ms; matrix size, 128 × 128; field of view, 3 × 3 cm². Twelve contiguous, 1-mm-thick slices were acquired to cover the whole brain.

Determination of blood-brain barrier permeability by Evans blue staining. We administered Evans blue intravenously via the tail vein (100 mg kg⁻¹, Merck) in awake, restrained mice (we avoided anesthesia to exclude artifact due to the direct effect of the anesthetic itself on the endothelium and blood flow). We performed Evans blue injection 18–24 h after pilocarpine injection. Briefly, 30 min after the intravenous Evans blue injection, we perfused the mice with a solution of mixed aldehydes containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M PBS, pH 7.4 (ref. 8). We then removed the brains, excluding the meninges, and photographed them on a stereomicroscope.

Polymorphonuclear leukocyte preparation. We isolated mouse bone marrow PMNs from femurs and tibias as previously described²⁸. Briefly, we flushed the marrow cells from the bones with HBSS without Ca²⁺ and Mg²⁺ (GIBCO). After hypotonic lysis of erythrocytes, we loaded the cells on top of a Percoll discontinuous density gradient and, after centrifugation, collected the cells and washed them before use. More than 80% of the isolated cells were Gr-1 positive PMNs as assessed by flow cytometry (data not shown).

Intravital microscopy. At 6 h and 24 h after the onset of status epilepticus, we anesthetized the C56BL/6 mice by i.p. injection of physiologic saline (10 ml kg⁻¹) containing ketamine (5 mg ml⁻¹) and xylazine (1 mg ml⁻¹)^{15,29,30}. We maintained the mice at 37 °C with a stage-mounted strip heater (Linkam CO102, Olympus). We inserted a heparinized PE-10 polyethylene catheter into the right common carotid artery toward the brain. To exclude the noncerebral vessels from the analysis, we ligated the right external carotid artery and the pterygopalatine artery (a branch from the internal carotid)^{15,29}. We reflected the scalp and bathed the skull with sterile saline, and then we applied a 24 mm × 24 mm coverslip and fixed it with silicon grease. We attached a

round chamber with an 11-mm internal diameter to the cover slip and filled it with water.

We placed the preparation on an Olympus BX50WI microscope and used a water-immersion objective with long focal distance (Olympus Achromplan; focal distance, 3.3 mm; numeric aperture, 0.5 ∞). More details regarding intravital microscopy studies are provided in the **Supplementary Methods** online.

Additional methods. *In vivo* staining of endothelial adhesion molecules, *in vitro* adhesion assays, telemetry EEG, behavioral assessment, production of mouse T_H1 and T_H2 cells, immunohistochemistry and human samples are described in the **Supplementary Methods**.

Statistical analyses. We performed statistical analysis of the data with GraphPad Prism software. We used the two-tailed Student's *t*-test for statistical comparison of two samples. We performed multiple comparisons with the Kruskal-Wallis test with the Bonferroni correction of *P* or by analysis of variance. We compared velocity histograms with the Mann-Whitney *U*-test and the Kolmogorov-Smirnov test. Differences were regarded as significant with a *P* value less than 0.05.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

G.N.M., D.B., A.C., L.Z. and E.S. performed epilepsy experiments, telemetry and open field behavior. M.M., B.R., L.O., S.B. and S.A. performed intravital microscopy, *in vivo* staining for adhesion molecules, adhesion assays and contributed to obtaining the behavioral data. A.O. provided the human samples. F.M., A.C. and E.O. performed immunohistochemistry on human and animal samples. P.M., E.N. and A.S. provided MRI expertise. J.W.H., L.X., J.B.L. and R.P.M. provided key reagents and mice. E.C.B. contributed experimental suggestions, reagents and assistance with writing. P.F.F. and G.C. designed the study, analyzed the data and wrote the paper.

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