Antimicrobial immunity impedes CNS vascular repair following brain injury

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Traumatic brain injury (TBI) and cerebrovascular injury (CVI) are the two most common causes of acquired brain injury and are major contributors to death and disability worldwide. Systemic infections often accompany these disorders and can worsen outcomes. Recovery after brain injury depends on innate immunity, but the effect of infections on this process is not well understood. Here, we demonstrate that systemically introduced microorganisms and microbial products interfered with meningeal vascular repair after TBI in a type I interferon (IFN-I)-dependent manner, with sequential infections promoting chronic disrepair. Mechanistically, we discovered that MDA5-dependent detection of an arenavirus encountered after TBI disrupted pro-angiogenic myeloid cell programming via induction of IFN-I signaling. Systemic viral infection similarly blocked restorative angiogenesis in the brain parenchyma after intracranial hemorrhage, leading to chronic IFN-I signaling, blood–brain barrier leakage and a failure to restore cognitive–motor function. Our findings reveal a common immunological mechanism by which systemic infections deviate reparative programming after central nervous system injury and offer a new therapeutic target to improve recovery.
Fig. 1 | Systemic infections and PAMPs impede meningeal vascular repair following mild traumatic brain injury. a, c, e, g Representative confocal images from meningeal whole-mounts show vascular damage and repair in mice after mTBI. Blood vessels are labeled with anti-laminin staining (red) and i.v. fluorescent Tomato lectin (green). The white dashed lines delineate the area of injury and vascular repair. Scale bars, 200 μm. b, d, f, h, i Dot plots show quantification of the percentage of lesion repair (mean ± s.d.). Each symbol represents an individual mouse, and asterisks denote statistical significance (**P ≤ 0.001, ****P ≤ 0.0001); b, d, one-way analysis of variance (ANOVA)/Tukey test; f, i, two-tailed Student's t-test; h, two-tailed Mann-Whitney U test). Data are representative of two independent experiments. a, b, Area of injury and repair on days 1, 3, 5 and 7 after mTBI (n = 4 mice per group). c, d, Area of injury and repair 7 d after mTBI relative to unchallenged controls (Ctrl); decreased repair was observed following injection of LCMV i.v., VSV intranasally or polyI:C i.v. (Ctrl and LCMV n = 7, VSV n = 6, polyI:C n = 8 mice). e, f, Images captured on day 7 after TBI show decreased repair in mice injected i.v. with C. albicans relative to Ctrl (Ctrl n = 6, C. Alb. n = 5 mice). g, h, Meningeal images on day 7 after TBI depict decreased repair in mice injected i.v. with LPS relative to Ctrl (Ctrl n = 6, LPS n = 7 mice per group). i, Quantification of repair demonstrates complete repair of LCMV carrier mice relative to Ctrl (B6 n = 9, carrier n = 7 mice).

j, k Quantification of angiogenesis-related gene expression by qPCR in the injured meninges and superficial neocortex from uninfected and day 4 LCMV-infected mice on day 5 after injury. Data are a compilation of two independent experiments (TBI n = 11, TBI LCMV n = 12 mice). Volcano plot showing differential expression (double delta Ct analysis, ΔΔCt) of 88 genes related to angiogenesis. Statistical analysis was performed using multiple two-tailed t-tests and the Benjamini, Krieger and Yekutieli method to correct for the false discovery rate (FDR), with a desired Q value of 5% (dotted line); j, Bar graph showing relative gene expression (ΔΔCt) with Q value of less than 5% (mean ± s.d.; k). Each symbol represents an individual mouse, and asterisks denote statistical significance (**P ≤ 0.05, ***P ≤ 0.01, ****P ≤ 0.001, *****P ≤ 0.0001; two-way ANOVA/Holm-Sidak test). Statistical analysis is available in Supplementary Table 5.

whole-mounts immunohistochemically to visualize laminin. Tomato lectin labels healthy vascular endothelium and serves as an indicator of functional blood vessels, whereas laminin staining identifies both functional and nonfunctional vascular structures. Following initial mTBI-induced meningeal damage at day 1, the lesion steadily revascularized over the ensuing week (Fig. 1a,b). As
described previously, the new vascular network consisted of small loops that are easily distinguished from surrounding uninjured vasculature (Fig. 1a).

Having established repair kinetics, we next determined how different systemic infections influenced the meningeal repair trajectory. As a representative, we administered LCMV Armstrong, a non-cytopathic RNA virus, i.v. 4 d after mTBI. LCMV infection significantly impaired meningeal vascular repair, resulting in revascularization of only 23.1% ± 6.6% of the lesion at day 7 after mTBI relative to near complete repair observed in uninfected mice (Fig. 1c,d). This impairment was not observed in LCMV carrier mice persistently infected from birth with the virus (Fig. 1i). Carrier mice have LCMV distributed throughout every tissue and are immunologically tolerant to the virus. The normal meningeal repair observed in carrier mice indicates that LCMV itself is not responsible for the disruption that occurs following acute LCMV infection.

To evaluate another viral paradigm, we infected mice intranasally with vesicular stomatitis virus (VSV) Indiana 1 d before mTBI. VSV administered intranasally is known to infect nasal neuroepithelium and then travel caudally into the olfactory bulb via olfactory sensory neuron projections. Intranasal VSV infection reduced repair to 37.7% ± 8.8% of the lesion over 7 d. To determine if decreased meningeal repair was due to viral replication or recognition of PAMPs, we administered polyinosinic:polycytidylic acid (polyI:C) systemically on day 4 after injury. PolyI:C is a synthetic analog of PAMPs, we administered polyinosinic:polycytidylic acid (polyI:C) systemically on day 4 after injury. PolyI:C impeded repair of meningeal vasculature after mTBI and then travel caudally into the olfactory bulb via olfactory sensory neuron projections. Intranasal VSV infection reduced repair to 37.7% ± 8.8% of the lesion over 7 d. To determine if decreased meningeal repair was due to viral replication or recognition of PAMPs, we administered polyinosinic:polycytidylic acid (polyI:C) systemically on day 4 after injury. PolyI:C is a synthetic analog of double-stranded RNA that activates antiviral PAMP receptors. PolyI:C impeded repair of meningeal vasculature after mTBI but not as much as intact virus (Fig. 1c,d). Collectively, these data demonstrate that both viruses and viral PAMPs are detrimental to the meningeal repair process.

To assess nonviral infections and PAMPs, we systemically administered the pathogenic yeast Candida albicans (a representative fungal infection) or lipopolysaccharides (LPS) from Escherichia coli O55:B5 (a surrogate for bacterial infection) on day 4 after mTBI. C. albicans infection and LPS administration both reduced repair to 66.3% ± 4.1% (Fig. 1e,f) and 66.3% ± 4.1% of the lesion (Fig. 1g,h), respectively. Our results indicate that a broad range of systemic pathogens/PAMPs can impair meningeal revascularization following mTBI.

**Viral infection alters angiogenic programming.** We next sought to determine how a systemic viral infection influenced these aspects of lesion repair. Angiogenic programming was assessed by quantifying expression of 90 angiogenesis-related genes in small punch biopsies containing lesioned meninges and superficial neocortex (see Supplementary Table 1 for a complete list of genes). These biopsies were obtained from uninfected and LCMV-infected mice 5 d after mTBI. LCMV was introduced i.v. into the infected mouse group on day 4. These data revealed that LCMV infection altered an angiogenic pathways, markedly decreasing expression of seven angiogenesis-related genes (Cxc12, Ccn1, Fn1, Thbs1, Vegfa, Pdgfrb and Angpt2) and increasing expression of three genes (Ang, Tnfrsf12a and Epha2; Fig. 1j,k and Supplementary Table 1).

**Viral infection alters the reparative macrophage distribution.** We showed previously that angiogenesis is induced in the meninges following mTBI and that the lesion distribution of macrophages...
derived from classical versus nonclassical monocytes is important for the reparative process\(^9\). We examined whether LCMV influenced the distribution of mTBI lesion macrophages known to facilitate angiogenesis. By first conducting a time-course experiment in uninfected mice, we confirmed that CD11b\(^+\)CD206\(^+\) inflammatory macrophages gradually accumulate in the meningeal lesion core within 5 d of mTBI, whereas CD11b\(^+\)CD206\(^-\) wound-healing macrophages localize more so to the lesion perimeter (Fig. 2a). LCMV infection significantly altered this macrophage distribution pattern on day 5, impeding the accumulation of CD11b\(^+\)CD206\(^+\) macrophages within the mTBI lesion core (Fig. 2b–d). These cells were instead retained in the lesion perimeter where they conformed with an elevated number of CD11b\(^+\)CD206\(^-\) macrophages. Collectively, these data demonstrate that a systemic viral infection can disrupt the meningeal lesion macrophage distribution and angiogenic programming within 24 h of inoculation.

Secondary infection promotes a prolonged state of disrepair. We demonstrated that systemic viral infection was able to disrupt a meningeal repair process, but it was unclear whether this was a permanent block or a delay in repair. To address this question, we infected mTBI mice with LCMV on day 4 and then assessed meningeal repair on days 7 and 30 after injury. Relative to uninfected controls, LCMV-infected mice had 39% \(\pm\) 2.4% repair at day 7, but this progressed to 92.3% \(\pm\) 0.9% repair by day 30 (Fig. 3a,b), indicating that infected mice can eventually repair over time. Given the slower repair kinetics in injured mice following acute infection, we next evaluated the impact of a secondary infection. We infected mTBI mice with LCMV on day 4 and then introduced a homologous (i.e., LCMV) or heterologous (intranasal VSV) challenge on day 10. As expected, due to preexisting immunity, infection with LCMV on day 4 and then again on day 10 had no additional effect on the meningeal repair process relative to a single infection on day 4 (Fig. 3c–e). These mice showed near complete meningeal revascularization on days 14 and 30 after injury. By contrast, mice exposed to intranasal VSV on day 4 followed by intranasal VSV on day 10 had only 50% \(\pm\) 6.8% lesion repair at day 14 and 60.4% \(\pm\) 4.7% lesion repair at day 30 (Fig. 3c–e). These data demonstrate that exposure to a secondary infection can further impede the meningeal repair process following mTBI.

Viral infection induces an IFN-I response. We next explored the antiviral immune response that developed in the context of mTBI and how this might influence reparative programming. The IFN-I family is known to facilitate innate and adaptive immune reactions against diverse microorganisms, including LCMV\(^7\). We used real-time quantitative PCR (qPCR) to quantify expression of known IFN-Is and downstream interferon-stimulated genes (ISGs) in meningeal/superficial neocortex biopsies acquired from the lesions of uninfected and infected mTBI mice (see Supplementary Table 1 for a complete list of genes). Mice that received a mTBI were infected i.v. on day 4 with LCMV and then analyzed on day 5 (TBI d5 + LCMV). IFN-I and ISG expression levels were compared to uninfected/unjured mice (Ctrl), uninfected mice on day 1 (TBI d1) and day 5 (TBI d5) after mTBI, as well as LCMV-infected mice on day 1 (LCMV d1) and day 5 (LCMV d5) after infection. We quantified the expression of 14 different IFN-Is and detected a marked increase in all species only in mTBI mice infected with LCMV (Fig. 3f and Supplementary Table 1). Quantification of 79 ISGs in the same groups of mice uncovered two distinct expression patterns (Fig. 3g and Supplementary Table 1). Five days following mTBI, the ISG expression pattern was defined by a cluster of genes that included Jak2, Tyk2, Irf1, Stat3 and Thr3, among others. By contrast, LCMV-infected mTBI mice at day 5 expressed a different and broader pattern of ISGs that included high levels of Stat1, Stat2, Ift1, Ifit3, and Oas1b. These data indicate that LCMV induces a potent IFN-I response in injured mice that dramatically shifts the ISG expression pattern relative to their uninfected counterparts.

Interferon signaling in myelomonocytic cells impedes repair. Given the differential IFN-I response in uninfected versus infected mTBI mice, we evaluated the impact of this signaling on meningeal repair. This was first accomplished by studying IFN-I receptor (IFNAR)-deficient mice. Uninfected wild-type and Ifnar1\(^-/-\) mice were able to similarly repair meningeal vasculature 7 d following mTBI (Fig. 4a), indicating that IFNAR-mediated signaling is not required for the normal repair process. However, IFNAR deficiency completely rescued meningeal repair in LCMV-infected mTBI mice (Fig. 4a). These mice showed a repair level comparable to that observed in uninfected controls. A similar result was obtained when mice were injected intraperitoneally with an anti-IFNAR-blocking antibody on day 3 after mTBI, 1 d before LCMV infection on day 4 (Fig. 4b). Treatment with anti-IFNAR-blocking antibody also improved repair in mice exposed to LPS or C. albicans (Extended Data Fig. 1). Collectively, these data demonstrate the importance of IFNAR signaling in blocking meningeal repair after infection.

We next sought insights into the relevant cell population(s) modulated by infection-induced IFNAR signaling. We demonstrated previously that peripheral myeloid cells are required for meningeal repair following mTBI\(^8\). We therefore generated floxed IFNAR mice expressing Cre recombinase under control of the lysozyme M promoter (LysM\(^{-/-}\)/Ifnar\(^{1\alpha/\alpha}\)). These mice allowed us to assess whether IFNAR signaling in peripheral myelomonocytic cells was responsible for faulty meningeal repair. Importantly, deletion of IFNAR from myelomonocytic cells resulted in complete restoration of meningeal repair on day 7 in LCMV-infected mTBI mice (Fig. 4c). These data support the conclusion that IFN-I signaling in myelomonocytic cells is responsible for the reparative defect seen in infected mTBI mice.

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**Fig. 3** Infections induce a prolonged state of disrepair and interferon signaling in the meninges following mTBI. a–c. Confocal microscopy images from meningeal whole-mounts show laminin staining in red, and functional vessels visualized with i.v. fluorescent tomato lectin in green. White dashed lines denote areas of injury and vascular repair. Scale bars, 200 \(\mu m\). b, e. dot plots show quantification of the percentage of lesion repair (mean \(\pm\) s.d.). Each symbol represents an individual mouse. Data are representative of two independent experiments a, b. Areas of injury and repair day 7 and 30 after mTBI, with or without i.v. LCMV. (d7 \(n=6\), Ctrl d30 \(n=8\), LCMV d30 \(n=10\), ****\(P<1\times10^{-5}\); two-way ANOVA/Holm–Sidak test). c–e. Areas of injury and repair on days 14 and 30 after mTBI. Uninfected (Ctrl) mice and mice infected i.v. with LCMV on day 4, or days 5 and 10 (LCMV \(x=2\)), demonstrate near complete lesion repair on days 14 and 30 after mTBI. Mice infected with LCMV i.v. on day 4 and intranasally with VSV on day 10 showed incomplete repair on days 14 and 30 (Ctrl, LCMV \(n=8\), other groups \(n=6\), ****\(P<0.0001\) compared to Ctrl; one-way ANOVA/Tukey test). f. Dot plot depicting qPCR analysis of relative IFN-I gene expression (\(\Delta\DeltaCT\)) in punch biopsies of meninges and superficial neocortex. Mice underwent mTBI following i.v. LCMV infection on day 4 and quantification of gene expression on day 5 (TBI d5/LCMV d1, \(n=5\)). Gene expression was compared to uninfected/unjured mice (Ctrl, \(n=6\)), uninfected mice day 1 (TBI d1, \(n=6\)) and day 5 (TBI d5, \(n=4\)) after mTBI as well as i.v. LCMV mice on day 1 (LCMV d1, \(n=6\)) and day 5 (LCMV d5, \(n=5\)) after infection. Each symbol represents an individual mouse. Data are represented as the mean \(\pm\) s.d. Data represent two independent experiments. (\(**P<0.01\), ****\(P<0.0001\); two-way ANOVA/Holm–Sidak test). g. Heat map depicting qPCR analysis of IFN-I signaling–related genes that were significantly increased (\(P<0.05\)) based on two-way ANOVA/Holm–Sidak multiple-comparison method. Groups are the same as those shown in f. Data are representative of two independent experiments (\(n=4\) mice per group, Ctrl \(n=8\)). Statistical analysis is available in Supplementary Table 5.
MDA5-mediated sensing is required to block meningeal repair. To further explore the mechanism by which viral infection impeded repair, we evaluated a step preceding IFN-I and ISG induction. We specifically addressed whether innate detection of LCMV was required for the meningeal repair impediment. Melanoma-differentiation-associated gene 5 (MDA5) is a
RIG-I-like receptor double-strand RNA helicase enzyme that functions as a pattern-recognition receptor and can detect viruses like LCMV\(^{35}\). We infected MDA5-deficient mice at day 5 after mTBI and evaluated meningeal repair on day 7. Relative to wild-type LCMV-infected controls, repair of meningeal vasculature was almost fully restored at day 7 after mTBI in Mda5\(^{-/-}\) mice (Fig. 4d,e). We then assessed whether the pattern of IFN-I and ISG expression observed in LCMV-infected mTBI mice was influenced by MDA5 deficiency. IFN-I and ISG expression in the damaged meninges and neocortex was quantified on day 5 after mTBI in wild-type and Mda5\(^{-/-}\) mice—1 d following i.v. infection with LCMV on day 4 (Fig. 4f,g). Uninfected mTBI mice were also processed as controls for this experiment. Importantly, MDA5 deficiency reduced expression of all LCMV-induced IFN-Is to levels observed in uninfected mTBI mice (Fig. 4f). Moreover, ISG expression was markedly reduced relative to wild-type LCMV-infected mice and to levels often below those observed in uninfected controls (Fig. 4g). The few upregulated genes (Cd2l2, Ccl4, Grp and H2-M10.1) detected in Mda5\(^{-/-}\) mice appeared to have no impact on meningeal repair. These data demonstrate that innate recognition of LCMV via MDA5 is required to initiate IFN-I-dependent disruption of meningeal repair.

Transcranial IFN-\(\beta\) administration impairs meningeal vascular repair. Given the role of MDA5 and IFN-I in disrupting meningeal repair, we next focused on whether IFN-I alone could mediate this effect and whether signaling needed to occur locally or systemically. This was accomplished by administering IFN-I-\(\beta\) locally or systemically to mTBI mice on days 5 and 6 after injury. For systemic administration, IFN-I-\(\beta\) was injected i.v., whereas it was applied transcranially through the thinned skull bone for the local application. Quantification of meningeal repair on day 7 after mTBI revealed that i.v. and transcranial administration of IFN-I-\(\beta\) reduced repair to 63.2\%±1.9\% and 74.6\%±7.3\%, respectively (Fig. 5a,b). LCMV-infected controls in this experiment showed 43.6\%±3.7\% repair, whereas uninfected mTBI mice had near complete meningeal repair (99.9\%±0.9\%). To further interrogate whether systemic or local IFN-I was required to impede repair, we created a model of bilateral mTBI lesions and administered IFN-I-\(\beta\) transcranially to only one of the two lesions (ipsilateral). In this experiment, the ipsilateral IFN-I-\(\beta\)-treated lesion demonstrated only 59.4\%±4.3\% repair, whereas the vehicle control-treated contralateral lesion had 92.2\%±4.1\% repair (Fig. 5c,d). From these data, we conclude that IFN-I-\(\beta\) acting locally within the mTBI lesion phenocopies the detrimental effect that systemic LCMV infection has on meningeal repair.

To determine how IFN-I-\(\beta\) disrupted the reparative process, we conducted an intravital two-photon imaging study in Cx3cr1\(^{GFP^{+}/+}\). Ccr2\(^{RFP^{+}/+}\) mice to examine peripheral myeloid cell lesion dynamics following transcranial application of IFN-I-\(\beta\). Cx3cr1\(^{GFP^{+}/+}\), Ccr2\(^{RFP^{+}/+}\) mice received a mTBI and were then incubated transcranially with IFN-I-\(\beta\) or vehicle for 1 h. Myeloid cell dynamics in the lesion and peri-lesion area were filmed for an additional hour by intravitral two-photon microscopy. Transcranial IFN-I-\(\beta\) markedly enhanced recruitment of CCR2\(^{+}\) monocytes relative to the vehicle control group and promoted their movement into the peri-lesion area (Fig. 5e,f and Supplementary Video 1). These data demonstrate that IFN-I-\(\beta\) alone (without infection) can alter the local distribution of peri-lesion myeloid cells following mTBI.

IFN-I impairs brain parenchymal angiogenesis. Because IFN-I triggered by viral infection was able to disrupt meningeal angiogenesis following mTBI, we became interested in whether repair of other CNS vasculature was similarly disrupted by an infection. For example, vasculature within the brain parenchyma can be damaged by stroke, TBI or intracranial hemorrhage, requiring immune-mediated angiogenesis to restore function\(^{36,37}\). We recently developed a model of isolated CVI that involves application of low-intensity pulsed ultrasound through a thinned skull window of mice injected i.v. with microbubbles\(^{38}\). This approach causes immediate cerebrovascular damage and hemorrhage in the parenchyma beneath the thinned skull window, which is followed by immune-mediated vascular remodeling. We evaluated the impact of viral infection on vascular repair in the brain parenchyma by challenging mice i.v. on day 4 after CVI with LCMV. We showed previously in this model that cerebrovascularisation is rebuilt by day 10 after injury\(^{39}\). We therefore identified cerebrovascularisation with fluorescent tomato lectin or laminin staining and quantified the extent of parenchymal vascular coverage in uninfected versus infected mice at day 10 after CVI. At this time point, we observed development of new vessels in uninfected CVI mice. These vessels had an irregular distribution, increased diameter and increased density as described previously (Fig. 6a)\(^{39}\). However, neovascular coverage was markedly reduced 25.1-fold in LCMV-infected mice relative to controls (Fig. 6a,b), demonstrating that viral infection can impede angiogenesis in the parenchyma similarly to the meninges.

To determine if the IFN-I system was involved in blocking parenchymal repair following CVI, we compared vascular coverage in LCMV-infected wild-type versus Mda5\(^{-/-}\) mice (Fig. 6c). Complete restoration of vascular repair was observed in infected Mda5\(^{-/-}\) mice on day 10 after CVI. In fact, the repair level was comparable to that seen in uninfected wild-type controls (Fig. 6c). Similarly, full restoration of vascular repair was observed in LCMV-infected Iftarn1\(^{-/-}\) mice; however, deletion of IFNAR from myelomonocytic cells in LysM\(^{cre}\). Ifnar1\(^{-/-}\) mice only achieved partial restoration of repair (Fig. 6d,e), suggesting disruption of another cell population by IFN-I signaling. Collectively, these data indicate that LCMV infection impedes cerebrovascular repair in an MDA5, IFN-I-dependent manner, but that interferon signaling is required in cells other than just myelomonocytic cells to achieve the full defect.

Fig. 4 | Deletion of pathogen sensing or interferon signaling reconstitutes meningeal repair after mTBI. a, Dot plot shows quantification of the percentage of lesion repair (mean±s.d.) 7 d after mTBI in uninfected B6 (\(n=10\)) and Iftarn1\(^{-/-}\) (\(n=8\)) mice versus i.v. LCMV-infected B6 (\(n=9\)) and Iftarn1\(^{-/-}\) (\(n=9\)) mice. b, Dot plot shows the percentage of lesion repair 7 d after mTBI in uninfected mice (\(n=10\)), LCMV-infected mice (\(n=10\)) and LCMV-infected mice + antiIFN antibody (\(n=9\)). c, Dot plot shows the percentage of lesion repair 7 d after mTBI in LCMV-infected B6 mice, as well as uninfected and infected LysM\(^{cre}\). Ifnar1\(^{-/-}\) mice (\(n=8\) per group). d, Confocal images show vascular damage and repair in uninfected B6 mice, as well as LCMV-infected B6 and Mda5\(^{-/-}\) mice at day 7 after mTBI. The white dashed lines delineate areas of injury and repair. Fluorescent tomato lectin is shown in green and laminin is shown in red. Scale bars, 200\(\mu\)m. e, Dot plot shows quantification of the percentage of lesion repair (mean±s.d.) for the groups in d. (Ctrl, B6 LCMV \(n=6\), MDA5\(^{-/-}\) LCMV \(n=9\) mice). f, Dot plot depicting qPCR analysis of relative IFN-I gene expression (ΔΔCt) in punch biopsies of meninges and superficial neocortex at day 7 after mTBI for uninfected B6 mice, as well as day 4 LCMV-infected B6 and Mda5\(^{-/-}\) mice. Data are representative of two independent experiments from four and five mice per group. g, Heat map depicting qPCR analysis of IFN-I signaling-related genes that were significantly increased (\(P<0.005\)) based on two-way ANOVA followed by the Holm–Sidak multiple-comparison method. Groups are the same as those shown in f. Data are representative of two independent experiments with four and five mice per group. a, c and e, one-way ANOVA/Tukey test; and f and g, two-way ANOVA/Holm–Sidak test. Statistical analysis is available in Supplementary Table 5.
Viral infection following CVI promotes neuronal loss. To define other pathological consequences (if any) associated with failed revascularization after LCMV infection, we examined neocortical anatomy in uninfected versus infected mice at day 10 after CVI. Confocal imaging of the brain parenchyma in LCMV-infected mice at this time point revealed a notable lack of Tomato lectin-positive...
blood vessels as well as some abnormal vascular structures (Fig. 6f). These regions of damaged neocortex also showed a considerable loss of NeuN-positive neurons (5.9-fold when compared to uninfected controls) and an increase in Iba1+ myeloid cells (0.6-fold increase; Fig. 6f–h). These results indicate that viral infection following CVI not only impedes angiogenesis but also promotes neuronal loss and myeloid cell accumulation.

Viral infection following CVI promotes sustained blood–brain barrier breakdown. We also assessed the impact of infection on blood–brain barrier (BBB) breakdown and recovery after CVI. Analysis of axial sections from the neocortex of mice at day 10 after CVI revealed that LCMV infection on day 4 significantly enhanced Evans blue leakage and the extent of glial fibrillary acidic protein (GFAP)-positive gliosis relative to uninfected controls (Fig. 7a–c).
Further analysis of BBB leakage kinetics by fluorometry uncovered that the BBB resealed in uninfected mice by day 10 after CVI (Fig. 7d). By contrast, enhanced BBB leakage was observed on day 5 (1 d following LCMV infection), and this leakage persisted until day 20 when compared to uninfected controls. At this time point, a reduction in vascular tight junction proteins (claudin-5 and ZO-1) was observed in previously infected mice relative to controls (Extended Data Fig. 2). These data demonstrate that viral infection during the CVI-induced repair process promotes a persistent state of BBB leakage and increased GFAP-positive astrocytes.

Infection promotes IFN-I signaling and cognitive dysfunction after CVI. Repair of damaged CNS tissue after injury represents an attempt to reestablish steady-state functionality and gene expression. To better understand how an infection encountered early during the reparative process might deviate the return to homeostasis, we analyzed gene expression in the neocortex by RNA-sequencing (RNA-seq) analysis at day 20 after CVI (uninfected versus infected) relative to uninjured controls. We selected this time point because our previous study showed that CVI mice regain neurological function and relatively normal gene expression in the neocortex by

**Fig. 6** Viral infection and subsequent interferon signaling blocks angiogenesis after cerebrovascular injury. a–e, Intravital two-photon microscopy of cerebral cortical vasculature and image-based quantification of vascular coverage at day 10 after CVI. Fluorescent tomato lectin (green) and Evans blue (red; EB) were injected i.v. before imaging. Scale bars, 25 μm. a, Representative images show parenchymal vasculature in the neocortex of uninfected and LCMV-infected B6 mice at day 10 after CVI. b, Dot plot shows quantification of vascular coverage for the groups in a (Ctrl n = 9, LCMV n = 10). c, Dot plot depicts the quantification of parenchymal vascular coverage at day 10 after CVI in uninfected B6 mice (Ctrl) as well as LCMV-infected B6 and Mda5−/− mice (Ctrl n = 8, LCMV n = 10, Mda5−/− LCMV n = 16). d, Representative two-photon images show vascular coverage at day 10 after CVI in day 4 LCMV-infected Ifnar1−/− and LysM−/−-Ifnar1−/− mice. e, Dot plot shows the quantification of vascular coverage at day 10 after CVI for uninfected B6 mice (Ctrl), as well as day 4 LCMV-infected B6, Ifnar1−/− and LysM−/−-Ifnar1−/− mice (Ctrl n = 8, LCMV n = 9, Ifnar1−/− LCMV n = 17, LysM−/−-Ifnar1−/− LCMV n = 14). f, Representative confocal microscopy images of neocortex at day 10 after CVI show NeuN+ neurons (green), Iba1+ myeloid cells (red), and fluorescent tomato lectin-labeled blood vessels (white) for uninfected (Ctrl) and day 4 LCMV-infected B6 mice. Scale bars, 100 μm. g, h, Dot plots demonstrate image-based quantification of Iba1+ myeloid cells (g) and NeuN+ neurons (h; Ctrl n = 7, LCMV n = 8). Data in b, c, e, g, and h are represented as the mean ± s.d. and are a compilation of two independent experiments. Each symbol represents an individual mouse, and asterisks denote statistical significance (***P < 0.001, ****P < 0.0001; c and e, one-way ANOVA/Tukey test; b, g and h, two-tailed Student’s t-test). Statistical analysis is available in Supplementary Table 5.
day 20 (ref. 27). Comparison of gene expression patterns using a Pearson correlation-based clustered heat map revealed clustering of samples into three distinct groups with clear differences in expression profiles (Fig. 8a). Principal-component analysis (PCA) based on 94.2% of the detected genes confirmed differential clustering of the three groups (Fig. 8b). The most notable separation among these groups was evident along the PCA2 axis, representing only 12.3% of the genes. It was along this axis that the LCMV-infected CVI mice separated more distinctly from the other two groups.

We next used ingenuity pathway analysis (IPA) to delve more deeply into the concordant dysregulated gene expression in uninjured versus previously infected mice at day 20 after CVI relative to uninjured controls (Fig. 8c,d and Supplementary Tables 2–4). Following CVI, uninjured mice expressed genes associated with acute-phase response signaling ($P = 1.26 \times 10^{-16}$), granulocyte adhesion and diapedesis ($P = 1.58 \times 10^{-15}$) and neuroinflammation signaling ($P = 7.94 \times 10^{-14}$), among other pathways (Fig. 8c and Supplementary Table 2). By contrast, CVI mice that were previously infected with LCMV on day 4 showed a different gene expression signature in the neocortex on day 20. Upregulated genes were associated with interferon signaling ($P = 3.16 \times 10^{-14}$), acute-phase response signaling ($P = 1.58 \times 10^{-15}$) and the complement system ($P = 3.98 \times 10^{-11}$) when compared to uninjured controls (Fig. 8d and Supplementary Table 3). Among canonical interferon pathway genes, 19% were upregulated in uninjured CVI mice ($P = 1.41 \times 10^{-7}$) relative to 36% in the LCMV-infected group ($P = 3.16 \times 10^{-14}$; Fig. 8e,f and Supplementary Table 4). Top transcriptional regulators including Stat1 ($z$-score = 5.031), Stat2 ($z$-score = 2.24), Ifn7 ($z$-score = 6.738) and Ifn9 ($z$-score = 2.088) were activated by previous infection. Comparison of a more extensive list of genes directly or indirectly linked to interferons revealed substantial dysregulation in the neocortex of day 4 infected CVI mice relative to uninfected mice (Fig. 8g and Supplementary Table 4). These results demonstrate that systemic infection following CVI leads to long-term activation of the IFN-I pathway.

To determine the impact of this differential programming on functional recovery, we evaluated cognitive–motor function using a Y maze in uninjured control mice as well as uninjured mice...
and day 4 LCMV-infected CVI mice at day 20. We demonstrated previously that CVI mice (relative to uninjured controls) lose cognitive–motor function as assessed by Y maze at day 10, which is followed by functional recovery at day 20 (ref. 23). We confirmed this result by showing that uninfected CVI mice at day 20 performed similarly to uninjured controls in the Y-maze test (Fig. 8h,i).
By contrast, LCMV-infected mice did not recover function at day 20 after CVI, demonstrating a sizable reduction relative to their uninjured counterparts (Fig. 8h,i). To determine if IFN-I signaling impeded functional recovery in infected mice, we also evaluated Y-maze performance in Ifnar1−/− CVI mice that were either uninjured or infected at day 4 with LCMV. Importantly, infected Ifnar1−/− mice demonstrated improved functional recovery relative to infected C57BL/6 (B6) controls (Fig. 8h,i). The functional recovery in Ifnar1−/− mice consisted of a partial improvement in the number of gates entered (Fig. 8h), as well as a complete recovery of triptic ratio performance (Fig. 8i). Collectively, these data indicate that systemic infection interferes with recovery of neurological function after CVI via a mechanism that depends in part on IFN-I signaling.

Discussion

Our study provides important insights into how systemic infections interfere with the recovery process after brain injury. We demonstrate that a broad range of infections and PAMPs disrupt vascular repair following TBI and CVI. In fact, sequential infections encountered after mTBI induce a chronic state of disrepair. At least one mechanism by which infectious agents disrupt repair is via IFN-I induction. These cytokines deviate reparative programming in myeloid cells and alter their spatial distribution in damaged CNS tissue. This disruptive process is induced by the innate viral sensor, MDA5, after LCMV infection. However, it is expected that other innate sensors triggered by different pathogen types will have a similar negative impact on CNS repair. It is nevertheless clear that one disruptive thread common to many infections is IFN-I production, which we show interferes with both meningeal and parenchymal vascular remodeling. Especially devastating is the effect of infection on CVI—a scenario that not only impedes angiogenesis but also promotes chronic IFN-I signaling, downregulation of tight junction proteins, BBB leakage and a failure to recover neurological function.

Our data show that different classes of infectious agents and PAMPs encountered after mTBI impede meningeal vascular repair. Previous rodent studies demonstrated that systemic LPS administration 1 month following TBI induces CNS inflammation that leads to progressive cognitive decline. Streptococcal pneumonia in mice also promoted poor motor recovery and mortality after TBI. In the periphery, wound healing is severely inhibited by bacteremia, sepsis or distal inflammation, which is attributed to dysregulation of the early innate immune response crucial for wound healing. Approximately 20–50% of individuals hospitalized with TBI have some type of infection. These infections are associated with poor acute and long-term outcomes. Multiple infections are also common in patients with moderate-to-severe TBI due to post-injury immunosuppression, as well as an increased frequency of sepsis that renders the host susceptible to opportunistic secondary infections. The association between sepsis and mortality in TBI patients extends up to 1 year after injury, and pneumonia is independently associated with poor outcomes in patients with severe TBI, extending to 5 years after injury. Based on our findings, it is important to consider aggressive approaches to prevent and/or treat infections following brain injury.

While many different inflammatory mediators are generated in response to infection, we show that IFN-I is the major disruptor of repair in injured mice challenged with LCMV, LPS or C. albicans. All known IFN-Is were detected in LCMV-infected mTBI mice, and knockout or inhibition of IFNAR restored meningeal vascular repair. Moreover, transcranial IFN-β administration (without infection) alone was able to impede meningeal repair. It is well known that IFN-Is orchestrate innate and adaptive immunity against viruses; however, bacteria, parasites and fungi can also elicit IFN-I responses. IFN-I is produced after pathogens are recognized by many different PAMP receptors. For example, LCMV is detected by retinoic acid-inducible gene I and MDA5 (two cytosolic pathogen recognition receptors). We observed reduced IFN-I expression and normal meningeal repair in Mda5−/− mice, suggesting that the detection and response to viral infection is what inhibits repair after mTBI. Brain injury even without infection can elicit some detrimental IFN-I production. Rodent studies demonstrated that release of cytosolic and mitochondrial nucleic acids by injured cells activates the cGAS–STING pathway, resulting in IFN-I production. This IFN-I enhances secondary neuroinflammation and neurodegeneration. We observed that viral infection elevates expression of IFN-I after mTBI, which likely amplifies the already deleterious effects of these cytokines.

Myelomonoctytic cells play a central role in orchestrating repair after injury. While the acute response to infection bears similarities to wound-healing responses, distal viral infections can nevertheless dampen the innate inflammatory response to peripheral wounds, which delays healing. Meningeal repair following mTBI requires a precise arrangement of different macrophage subsets in the lesion core and perimeter to scavenge dead cells and promote angiogenesis, respectively. Our studies demonstrate that viral infection fundamentally alters this spatial distribution of myeloid cells. IFNAR signaling causes pro-inflammatory myelomonocytic cells to decrease in the mTBI lesion core after infection and redistribute to the lesion perimeter where they comingle with wound-healing macrophages. Transcranial administration of IFN-β to a mTBI lesion promoted myeloid cell recruitment within hours, and deletion of IFNAR specifically from LysM+ myelomonocytic cells completely restored meningeal repair. In addition, deletion of MDA5 eliminated the ISG response to LCMV while allowing upregulation of myeloid-cell-recruiting chemokines like CCL2 and CCL4 in response to injury. These data demonstrate that a single signaling pathway (IFNAR) is responsible for redirection of the CNS immune-mediated wound-healing program.

We also observed faulty repair in the brain parenchyma following systemic LCMV infection of mice with ultrasound-induced CVI (a model of hemorrhagic stroke). One-third of all acute stroke hospitalizations are complicated by systemic infections, which are linked to poor outcomes, in both the short term and the long term. Infections may also increase the risk of recurrent stroke. Prophylactic antibiotics have failed to prevent infection rates or affect associated outcomes, due to stroke-induced systemic immunosuppression. In rodent studies of ischemic stroke, acute LPS administration exacerbated brain damage and neurological deficit via interleukin-1-induced potentiation of neutrophil mobilization. While the immunology and vasculature of the meninges and brain parenchyma are different, our data indicate that infection-induced IFN-I impedes reparative angiogenesis similarly in both compartments. Angiogenesis is critical for repair of injured brain tissue, and vascular density is associated with improved outcomes following CVI. Circulating inflammatory monocytes were shown to help mature neo-vessels and promote functional recovery following stroke. Specific deletion of IFNAR from LysM+ myelomonocytic cells resulted in complete restoration of meningeal vascular repair following mTBI, but only a partial improvement in parenchymal repair after CVI. This finding is likely due to the detrimental effects that IFN-I has on other parenchymal residents, such as microgila, astrocytes and pericytes.

Systemic viral infection after CVI not only impeded angiogenesis but also led to chronic IFN-I signaling, decreased tight junction proteins, persistent BBB leakage, gliosis, neuronal loss and a failure to recover neurological function. Importantly, genetic deletion of IFNAR markedly improved functional recovery in virally infected CVI mice. Cerebrovascular disease has been associated with an increased risk of developing neurodegenerative dementia, and imaging studies suggest that cerebral ischemia may initiate a long-term neurodegenerative process. One-third of patients...
with stroke have cognitive impairment within the first months following their ischemic event\(^1\). Persistent BBB leak is a possible driver of dementia in the setting of cerebrovascular disease\(^2\), and systemic inflammatory markers in stroke survivors are associated with progressive degeneration\(^3,4\). In rodents, LPS administration during reperfusion of mice that underwent middle cerebral artery occlusion increased brain atrophy 1 month after injury\(^5\). Based on our data in CVI mice, we postulate that acute infections following CVI in humans can induce a chronic state of disrepair, BBB leakage and inflammation, leading to neurodegeneration.

In conclusion, our study provides fundamental insights into how systemic infections impede recovery following TBI and CVI. CNS tissue repair requires a spatially, temporally and functionally coordinated innate immune response\(^6,7\). Single or recurrent infections encountered during the recovery period can deviate or halt this reparative response. In patients with TBI and CVI, infections are common and result in poor outcomes, but treatment options are not currently available\(^8,9\). We believe that new therapeutic opportunities exist to improve outcomes in patients with TBI and CVI via modulation of antimicrobial immunity and that a search for associated pathogens should be initiated in patients experiencing a chronic state of disrepair following CNS injury.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-021-01012-1.

Received: 15 December 2020; Accepted: 27 July 2021; Published online: 23 September 2021

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Methods

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Acute LCMV infection was administered in mice following unilateral mTBI and bilateral mTBI. For the bilateral mTBI paradigm, IFN-β1 was applied to one injury and aCSF to the other in the same mouse.

Antibody treatment. To block IFNAR, we administered a single dose of 500 µg of IFNAR antibody (MAR1-5A3; BioXcell) in PBS intraperitoneally on day 3 after mTBI or CVI, 1 d before LCMV, LPS or C. albicans administration. Animals were evaluated on day 7 after mTBI or day 10 after CVI.

Immunochemistry. Meninges were collected on days 1, 3, 5 and 7 after mTBI injury. Vasculature was labeled by i.v. injection of 70 µl DyLight 649-labeled Lycopersicon Esculentum (Tomato) Lectin (Vector Labs) 5–10 min before euthanasia. A 5 × 5 mm area of the meninges was carefully dissected with scissors and placed in PBS containing Background Buster (Innovex Biosciences), as well as a 1:500 dilution of purified rat anti-mouse CD16/CD32 (Fc receptor blocker; clone 93, BioLegend; blocking buffer) for 30 min at room temperature (20–25 °C). Primary antibodies were added directly to the blocking buffer and incubated at 4 °C overnight. After primary staining, slices were washed three times in staining buffer (PBS containing 1% fetal bovine serum). Secondary antibodies were added and incubated for 4 h at room temperature (20–25 °C). Meninges were again washed three times in staining buffer and placed in 4% parafomaldehyde overnight. Meninges were removed from the skull by careful peeling using fine-tipped forceps. The free-floating meninges were placed flat in 50 µl of FluorSave Reagent (Millipore Sigma) on a slide and imaged using a 20× objective. The meninges were stained with the following primary antibodies: anti-CD11b Pacific blue (1:300 dilution; MA170; BioLegend), anti-CD206 Alexa Fluor 488 (1:500 dilution; C06826; BioLegend) and polyclonal anti-laminin (1:500 dilution; ab11575; Abcam). The following secondary antibody was used: rhodamine red-X conjugated donkey anti-rabbit (1:1,000 dilution; Jackson ImmunoResearch).

Brains were collected 10 d after injury. For vascular staining, mice were injected i.v. with 70 µl fluorescence-conjugated tomato lectin DyLight 649 or fluorescence-conjugated tomato lectin DyLight 488 (Vector Labs) 5–10 min before euthanasia. To evaluate BBB leakage, mice were injected with 100 µl of 0.1 mg ml⁻¹ Evans blue (Sigma) i.v. 1 h before euthanasia. Mice then received an intracranial perfusion with 4% parafomaldehyde. Afterward, brains were collected and placed in 4% parafomaldehyde overnight. Fixed brains were sectioned axially or coronally using a Compressoste Tissue Slicer (Precisionary). For staining, tissues were initially blocked and permeabilized by incubating with PBS containing 0.5% Triton-X, Background Buster (Innovex Biosciences) and FcR block (blocking buffer) for 30 min at room temperature (20–25 °C). Primary antibodies were added directly to the blocking buffer and incubated at 4 °C overnight. After primary staining, slices were washed three times in staining buffer (PBS containing 2% fetal bovine serum). Secondary antibodies were added and incubated for 4 h at room temperature (20–25 °C). After secondary staining, slices were washed three times in staining buffer. The free-floating slices were carefully mounted on the slide and covered with one drop of FluorSave Reagent (Millipore Sigma) and a coverslip was added. The tissues were stained with the following primary antibodies: rabbit anti-Ifb1 (1:500 dilution; 019-19741; Wako), chicken anti-GFAP (1:1,000 dilution; ab4674; Abcam), guinea pig polyclonal anti-NeuN (1:1,500 dilution; ABN09P; Millipore), rabbit anti-claudin-5 (1:250 dilution; 341760; Thermofisher), rabbit anti-ZO-1 (1:250 dilution; ab96587) conjugated to Alexa fluor 647 using antibody labeling kit (Invitrogen). Secondary antibodies (1:1,000 dilution) included: donkey anti-rabbit IgG (H + L) Alexa Fluor 488 (1:1,000 dilution; A-21206; Thermofisher), donkey anti-rabbit IgG (H + L) Alexa Fluor 594 (A-21207; Thermofisher), goat anti-guinea pig IgG (H+L) DyLight 488 (AB_215105; Thermofisher) and donkey anti-chicken IgY (Gy; H + L) DyLight 405 (AB_234373; Jackson ImmunoResearch).

Confocal imaging and analysis. Confocal images were obtained using an Olympus FV2000 laser-scanning confocal microscope equipped with four detectors, six laser lines (405, 458, 488, 515, 559 and 635 nm) and five objectives (×4/0.16 NA, ×10/0.4 NA, ×20/0.75 NA and ×40/0.95 NA, and chromatic aberration-corrected ×60/1.4 NA). For imaging acquisition, the Leica Application Suite X 3.3.5.19976 was used. All confocal images were subsequently imported into Imaris version 9.3 software (Bitplane) for additional analyses performed by a blinded investigator. mTBI lesions were quantified in meningeal tissue collected 7 d after mTBI injury in uninjected versus LCMV-infected B6, Ifn1−/−, LysMCre Ifn1−/− and MDA5−/− mice. Lesions were also quantified 7d after injury in mice treated with i.v. LPS, i.v. polyIC, i.v. or transcranial IFN-β1, as well as LCMV-infected versus uninfected mice treated with IFNAR antibody. The lesions of infected B6 mice were also evaluated 1, 3, 5 and 7 d after mTBI injury. To quantify meningeal vascularization,
we used the surface function in Imaris as described previously\(^1\). At different time points after injury, the entire meningeal lesion induced by mTBI (both repaired and non-repaired) was identified and circumscribed using Imaris. The total lesion area was identified based on regions containing small laminin-positive, Tomato lectin-positive neovascular loops (that is, repaired vasculature), as well as those containing laminin-positive, Tomato lectin-negative vessels (that is, unrepaired/damaged blood vessels)\(^2\). The area of unrepaired meninges was also determined, and the final percentage of repaired meningeal tissue was calculated using the following equation:

\[
\% \text{ repair} = \frac{\text{total lesion area} - \text{unrepaired area}}{\text{total lesion area}} \times 100
\]

Cell number quantification was completed using meningeal samples collected 5 d after mTBI from uninfected and LCMV-infected mice. We generated ‘spots’ in Imaris for all immunohistochemically labeled CD11b\(^+\) and CD206\(^+\) cells within the lesion core as well as within the peri-lesion area. For this quantification, a 1.5 mm × 1.5 mm region of interest was centered on the middle of the lesion. We next identified CD11b\(^+\)CD206\(^+\) cells using the ‘spots’ colorization function. We then calculated the total number of individual CD11b\(^+\) cells, as well as the number of CD11b\(^+\)CD206\(^+\) and CD11b\(^+\)CD206\(^+\) cells in the lesion core and peri-lesion area.

To quantify BBB leakage, mice were injected with 100 μl of 0.1 mg ml\(^−1\) Evans blue (Sigma) i.v. 10 d after injury and 1 h before euthanasia as described above. Following processing and confocal imaging of the brain slices, images were imported into Imaris version 9.3 software. For quantification of Evans blue extravasation and GFAP-positive gliosis, a 100-μm axial section encompassing the entire surface of the CVI-damaged hemisphere was cut and a ×4 confocal tile scan was acquired. Resulting tile scans were imported into Imaris and a ‘surface’ corresponding to Evans blue or GFAP signal was generated and used to calculate sum fluorescence intensities. Control axial brain hemispheres stained only with secondary antibody was used to determine the background signal and set the absolute intensity thresholds to create ‘surfaces’ for Evans blue and GFAP signal. We also quantified the number of NeuN\(^+\) neurons and Iba1\(^+\) microglia/macrophages in similar axial brain slices collected 10 d after injury using the ‘spots’ function in Imaris and divided by the three-dimensional (3D) volume of the acquired image to obtain the cell density (that is, number of cells per mm\(^3\)).

For right junction analysis, areas of injury were identified in coronal mouse brain sections from uninfected and day 4 LCMV-infected B6 mice at day 20 after CVI. For vascular staining, mice were injected i.v. with fluorescently conjugated tomato lectin Dylight 488 (Vector Labs) 5–10 min before euthanasia. Sections were subsequently stained with antibodies to claudin-5 and ZO-1. After processing and confocal imaging of the brain slices, images were imported into Imaris version 9.3. Using Imaris, ‘contours’ were generated around all individual blood vessels in each mouse brain section based on tomato lectin Dylight 488 signal and used to create a 3D surface. The total volume of this surface was calculated using the ‘statistics’ function (vascular volume). Afterward, the claudin-5 and ZO-1 sum fluorescence intensities (total number of voxels × mean fluorescence intensity) within this surface were measured using the ‘statistics’ function and the intensity per unit area was calculated as follows:

\[
\text{Fluorescence intensity per vascular volume} = \frac{\text{sum fluorescence intensity}}{\text{vascular volume}}
\]

Intravital two-photon imagine and analysis. Ten days following CVI, the original incision was re-accessed and a metal bracket was glued onto the skull leaving exposed a circular area of the skull that was thinned previously to 20–25 μm. Images were obtained using a Leica SP8 two-photon microscope with an 8,000-Hz resonant scanner, a ×20 water-corrected water-dipping objective (1.0 NA) or a ×20 water-dipping objective (1.0 NA), a quad HDY external detector array, a Mai Tai 20 25 collar-corrected water-dipping objective (1.0 NA) or a time-lapse images of the injured meninges were obtained before transcranial

IFN-β1 administration as well as for 2 to 3 h following administration. To generate time-lapse images, 100-μm z-stacks (3-μm step size) were captured at a 5-min time interval. Signal contrast was enhanced by averaging six frames per plane in resonance scanning mode. Time-lapse imaging data were then imported into Imaris 9.0, and CCR2\(^+\) cells were subsequently manually counted at 90 min following transcranial incubation with IFN-β1. At the 90-min time point, CCR2\(^+\) monocytes were identified as 20–30-μm RFP\(^+\) cells whose movement could be followed over at least three frames. Videos were processed using Adobe Premiere Pro 14.0.

Blood-brain barrier integrity assay. BBB integrity was assessed at 1 h, 1 d, 3 d, 5 d, 7 d, 10 d and 20 d after CVI. At the denoted time points, we administered 1 mg of sodium-fluorescein (Na-Fl; 100 μl of 1% wt/vol Na-Fl in PBS; Sigma) i.v. 10 min before euthanasia. Mice were perfused with PBS before collecting the injured hemisphere. Tissues were homogenized in 1 ml of 0.5% tritonx-100 acid and centrifuged at 10,000 g at 4°C. Following centrifugation, supernatants were collected and 200 μl of 5N sodium hydroxide was added. Fluorescence was quantified using a fluorometer (Varioskan Flash; Thermo Fisher) at an excitation at 485 nm, emission at 530 nm and a gain of 50. We generated a standard curve by plotting mean fluorescence intensity versus Na-Fl concentration (mg ml\(^−1\)) for known standards (ranging from 2×10\(^−2\) to 8×10\(^−1\) mg ml\(^−1\)) diluted in brain tissue lysate. This standard curve was used to calculate the concentration of Na-Fl in our samples. The total amount of Na-Fl in each sample was expressed as a percentage of the initial injected dose (1 mg).

Real-time PCR analysis. mTBI and control mice received an intracardiac perfusion with saline. Afterward, a 2 × 2 × 2 mm cube of superficial cortical tissue and meninges was removed. This included the mTBI lesion as well as some surrounding brain tissue. The tissue was snap frozen using dry ice. Total RNA was extracted with a Micro RNA kit (Qiagen) following the manufacturer’s protocol. RNA quantity and integrity were assessed using a spectrophotometer (Nanodrop One, Thermo Scientific). CDNA was generated using an iScript cDNA Synthesis kit (Bio-Rad). Pre-made commercial and custom-made PrimePCR plates were used for qPCR experiments (Angiogenesis M96, IFN-I response M96, IFN-I custom-made plate; Bio-Rad; see Supplementary Table 1 for individual genes). qPCR was performed using universal SYBR Green Supermix (Bio-Rad) and CDNA template or water (non-template negative control) at an annealing temperature of 60 °C with a CFX96 Real-Time PCR machine (Bio-Rad). PCR products were subjected to melt analysis to confirm purity after DNA amplification. For each gene, expression values were normalized to the Gapdh housekeeping gene. The resulting relative gene expression was then expressed as a fold-change from uninjured control samples (ΔΔCT).

Bulk RNA sequencing. For RNA-seq, B6 mice were divided into three groups: uninjured (control), injured (CVI), and injured and LCMV-infected (CVI LCMV) with four mice per group. At day 20 following CVI, mice were perfused with normal saline, and a 3 × 3 × 3 mm cube of injured tissue was collected and snap frozen using dry ice. Total RNA was extracted with a Qiagen Micro RNA kit following the manufacturer’s protocol. RNA quantity and integrity were assessed using a Bioanalyzer (Agilent). Next, 500 ng of total RNA was used in conjunction with the TruSeq Stranded Total RNA Library Prep kit (Illumina). Library quantification was checked with a Bioanalyzer and quantified by Qubit (Thermo Scientific). Equimolar quantities from each sample library were pooled and run on a high-output NextSeq 550 kit (Illumina). Sequencer output files were deposited in the NCBI Short Read Archive via the Gene Expression Omnibus under access GSE172102.

RNA-sequencing data analysis. Paired-end sequence files (fastq) per sample were quality checked using the FastQC tool 0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) then adaptor clipped (TruSeq3-PE.2-2:30:10) and trimmed to remove 5′ nucleotide bias (HEADCROP:11) and low-quality calls (TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:15) using the Trimomatic tool 0.39 (https://www.usadellab.org/cms/page/trimomatic). Surviving input pairs of reads per sample were then imported into the CLC Bio Genomics Workbench v11 (https://www.qiagenbioinformatics.com/), down-sampled to 40 million read pairs per sample, then reference mapped by sample in stranded fashion against the current instance of the mouse genome (GRCm38) using the RNA-seq analysis tool supported therein under default parameters. Expression per known annotated gene (Mus_musculus.GRCm38.83.chr.gtf) in transcripts per kilobase million units was then exported from the Workbench and imported into R (https://cran.r-project.org/). In R (v3.6.2), transcripts per kilobase million expression per sample was pedestalled by 2 then log transformed. Genes lacking an expression value of >1 after transformation for at least one sample were discarded, while expression across samples for genes not discarded were quantile normalized. To assure quality of the data after normalization and absence of sample-level outliers, exploratory inspection was performed using a Tukey box plot, covariance-based PCA scatterplot and correlation-based heat map. To remove noise-bias expression values, locally weighted scatterplot smoothing was applied across normalized expression for all genes by sample class (coefficient
of Variation – mean expression). Locally weighted scatterplot smoothing fits were then over-plotted and inspected to identify the common low-end expression value where the relationship between mean expression (that is, ‘signal’) and coefficient of variation (that is, ‘noise’) grossly deviated from linearity. Expression values were then floored to equal this value if less, while expression for genes not observed greater than this value for at least one sample were discarded as noise-biased values. For genes not discarded, expression differences across sample classes were tested for using the one-factor ANOVA test under Benjamini–Hochberg FDR multiple-comparison correction condition using sample class as the factor. Genes having a type III corrected $P<0.05$ by this test were then subset and the TukeyHSD post hoc test was used to generate mean differences and $P$ values for each possible pairwise comparison of classes. Genes having a post hoc $P<0.05$ for a specific comparison and a linear difference of means $\geq 1.5x$ for the same comparison were deemed to have expression significantly different between the compared groups. After testing, sample-to-sample relationships were investigated via covariance-based PCA scatterplot and Pearson correlation-based clustered heat map using the unique union of genes deemed to have a significant difference of expression between at least two classes. Enriched pathways, functions and top-scoring networks for the same union set of genes were obtained using the IPA tool (https://www.qiagenbioinformatics.com/).

Cognitive–motor function test. The cognitive–motor function of B6 mice following sham surgery, as well as injured B6 and C57BL/6 mice with or without LCMV infection was evaluated at day 20 following injury using a Y maze with three white, opaque plastic arms at 120° angles from one another. Mice were placed in the center of the maze and allowed to freely explore for 5 min per session. An entry was defined when all four limbs were within the arm. We recorded the total number of arm entries to assess the overall movement in the maze. We also recorded the number of times the mouse sequentially entered all three arms (for example A → B → C not A → B → A) as a measure of exploration and cognitive function. The number of triplicate A → B → C entries was divided by total number of gates entered to obtain the ‘triplicate ratio’. Each mouse was evaluated at one time point.

Statistical analysis. Statistical analysis and graph design were performed using Prism 8.4 (GraphPad software) except for bulk RNA-seq analysis, described in detail above. Distribution normality was assessed using the Shapiro–Wilk normality test. Experiments containing two groups were analyzed using a two-tailed Student’s $t$-test for normally distributed data or the Mann–Whitney $U$ test for non-normally distributed data. Experiments involving more than two groups were analyzed by one-Way ANOVA followed by Tukey’s multiple-comparison test for normally distributed data or Kruskal–Wallis test followed by Dunn’s multiple-comparison test for non-normally distributed data. Data grouped based on more than one nominal variable were analyzed using a two-way ANOVA followed by the Holm–Sidak multiple-comparison method. Groups were considered statistically different at $P<0.05$. All data are displayed as the mean $\pm$ s.d. For the qPCR data analysis, we used two-way ANOVA followed by the Holm–Sidak multiple-comparison method or multiple $t$-tests using the Benjamini, Krieger and Yekutieli method, to correct for the FDR, with a desired $Q$ value of 1% or 5%. Statistical analyses for each graph are provided in Supplementary Table 5. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications$^{66,67}$. Animal littermates were randomly assigned to each group, and samples were randomly selected for data acquisition and analysis; no active randomization protocol was used.

Ethics statement. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH. The protocol was approved by the NINDS Animal Care and Use Committee (protocol no. 1295–20).

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

Data availability
The data that support the findings of this study are available from the corresponding author upon request. There are no restrictions on data availability.

Bulk RNA-seq data are available in the NCBI Gene Expression Omnibus under accession code GSE172102. The mouse genome database used in our RNA-seq analysis was GRCm38 (https://www.ncbi.nlm.nih.gov/assembly/GCF_00001635.20/). Source data are provided with this paper.

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Acknowledgements
This research was supported by the intramural program at the NINDS, NIH. We thank A. Elkahlon and W. Wu in the National Human Genome Research Institute Microarray core for their assistance with the RNA-seq experiment.

Author contributions
P.M. and D.B.M. conceived and designed the study, performed the data acquisition and analysis. P.M. and D.B.M. wrote and edited the manuscript. B.E.C., Z.P., H.W. and D.B.M. performed the experiments. J.R.P., T.P., J.S., K.M. and L.G. performed the computation analyses of RNA-seq data. P.M. and D.B.M. wrote and edited the manuscript. D.B.M. supervised and directed the project and participated in experimental design, data acquisition and analysis.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41590-021-01012-1.
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41590-021-01012-1.
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Peer review information Nature Immunology thanks Dennis Simon and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Editor recognition statement: L. A. Dempsey was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Inhibition of interferon signaling improves meningeal repair after mTBI. The dot plot depicts the percent of meningeal lesion repair 7 days after mTBI in uninfected mice (Ctrl) as well as mice challenged with LCMV, LPS, or Candida albicans (C. Alb) on day 4 post-injury, with or without αIFNAR antibody treatment. Cumulative data from two independent experiments. Each symbol represents an individual mouse, and asterisks denote statistical significance. Data are represented as mean ± SD. (Ctrl n=6, LCMV n=8, LPS n=15, C. Alb n=15; ****P<0.0001; Two-way ANOVA/ Holm-Sidak test). Representative confocal images from meningeal wholmounts show laminin staining in red, and functional vessels visualized with i.v. fluorescent tomato lectin in green. White dotted lines denote areas of injury and vascular repair. Scale bar, 200 μm. Source data in Source Data Extended Data Fig. 1.
Extended Data Fig. 2 | Viral infection after CVI reduces tight junction protein expression. a. Axial confocal images show i.v. injected tomato lectin (green), claudin-5 (red) and ZO-1 (white) in the superficial neocortex of uninfected (Ctrl) and d4 LCMV-infected B6 mice at day 20 post-CVI. Scale bar, 50 μm. b, c. Dot plots show image-based quantification of claudin-5 (b) and ZO-1 (c) sum intensity per vascular volume. Data represent a compilation of two independent experiments. Each symbol represents an individual mouse and asterisks denote statistical significance. Data are represented as mean ± SD. (Ctrl n=6, LCMV n=8; **P<0.01, ***P<0.0001; Two-tailed Student’s t-test). Source data for b,c in Source Data Extended Data Fig. 2.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- Two-photon acquisition: Leica Application Suite X (LAS X) 3.5.5.19976
- Confocal image acquisition: Olympus Fluoview 4.2a

Data analysis

- GraphPad/Statistics: GraphPad Prism 8.4
- Confocal/Two-photon image and video analysis: Bitplane Imaris 9.3
- Video Analysis: Adobe Premiere Pro 14.0
- RNA sequencing data analysis: FastQC tool 0.11.8, Trimmomatic tool 0.39, CLCbio Genomics Workbench 11, R 3.6.2, Ingenuity Pathway Analysis (IPA) tool

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The data that support the findings of this study are available from the corresponding author upon request. There are no restrictions on data availability. Bulk RNA-seq data are available in the NCBI Gene Expression Omnibus under accession code GSE172102. The mouse genome database used in our RNA sequencing analysis
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Life sciences study design

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

**Sample size**

For all studies, 3-8 mice per experimental group were used and all experiments were repeated 2 or 3 times. The bulk sequencing experiment was performed once. Sample sizes were determined based on our experience in previous studies using similar models and feasibility of surgical procedures. We site previous manuscripts were similar experimental setting were used: Roth, T. L., et al. Transcranial amelioration of inflammation and cell death after brain injury. Nature 505, 223-228 (2014).

**Data exclusions**

No data points were excluded after processing.

**Replication**

All experiments were repeated 2 or 3 times. The bulk sequencing experiment was performed once. Results were reliably reproduced for each experiment. Attempts at replication were successful once the conditions of the experiment were carefully optimized with pilot experiments.

**Randomization**

For all experiments involving mice were age matched and randomly assigned to each group.

**Blinding**

For survival studies, behavioral studies, imaging analysis and quantification, the investigator was blinded to the groups.

Reporting for specific materials, systems and methods

We require information from authors about some specific types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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| ☒ Human research participants | ☐ |
| ☒ Clinical data | ☐ |
| ☐ Dual use research of concern | ☐ |

**Antibodies**

The meninges were stained with the following primary antibodies: anti-CD11b Pacific blue (M1/70; BioLegend), anti-CD206 AlexaFluor 488 (CD68; Abcam), polyclonal anti-laminin (catalog# ab11575; Abcam). The following secondary antibody was used: rhodamine red-X conjugated donkey anti-rabbit (Jackson ImmunoResearch).

The brain tissues were stained with the following primary antibodies: rabbit anti-Iba1 (catalog# 019-19741; Wako, Richmond, VA), chicken anti-GFAP (catalog# ab4674; Abcam, Cambridge, UK), guinea pig polyclonal anti-NeuN (catalog# ABN09P; Millipore, Burlington, MA), rabbit anti-claudin-5 (catalog# 341600; ThermoFisher, Waltham, MA), rabbit anti-ZO-1 (catalog# ab36587) conjugated to Alexa Fluor 647 using antibody labeling kit (Invitrogen, Carlsbad, CA). Secondary antibodies included: donkey anti-rabbit IgG (H+L) Alexa Fluor 594 (A-21207; ThermoFisher), donkey anti-rabbit IgG (H+L) Alexa Fluor 488 (A-21206; ThermoFisher, Waltham, MA), goat anti-guinea pig IgG (H+L) Alexa Fluor 647 (A-21450, ThermoFisher) and donkey anti-chicken IgY (IgG) (H+L) DyLight 405 (AB_2340373; Jackson ImmunoResearch, West Grove, PA).

For tissue blocking purified rat anti-mouse CD16/CD32 (Fc receptor block; clone 93, BioLegend) was used.

**Validation**

All reagents are commercially available and have been validated by the respective companies as well as in previously published studies. We also evaluated the antibodies used in this study for the degree of non-specific binding (as measured in samples that do not express the epitope or by using isotype controls for comparison). The protocol used for staining of the meninges was previously published (Russo M, et al. 2018. Nat. Immunol. 19(5):442-452; Anti-CD11b Pacific blue (M1/70; BioLegend) was quality control tested by immunofluorescent staining with flow cytometric analysis and verified for immunocytochemistry (ICC) and frozen immunohistochemistry (IHC-F) by the manufacturer and used in Hata H, et al. 2004. J. Clin. Invest. 114:582, Zhang Y, et al. 2002. J. Immunol. 168:3088; and Russo M, et al. 2018. Nat. Immunol. 19(5):442-452 for
IHC. Anti-CD206 AlexaFluor 488 (C068C2; BioLegend) was quality control tested by intracellular immunofluorescent staining with flow cytometric analysis and verified for immunohistochemistry (IHC) by the manufacturer and used in Itó H. et al. 2012. J Am Soc Nephrol. 23:1797; Yang X. et al. 2015. PNAS. 112:2920; and Russo M. et al. 2018. Nat. Immunol. 19(5):442-452 for IHC. Polyclonal anti-laminin (catalog# ab11575; Abcam) was tested by the manufacturer for IHC and used in Marsala M. et al. 2020 Stem Cells Transl Med. 9:177-188; Molé MA. et al. 2020 Dev Cell. 52:321-334; and Russo M. et al. 2018. Nat. Immunol. 19(5):442-452 for IHC. The protocol used for staining of the meninges was previously published (Mastorakos P. et al. 2021. Nat. Neuroscience 24:245-258). Rabbit anti-ide-1 [catalog# 019-19741; Wako, Richmond, VA] has been previously used for IHC by Ohsawa K. et al. 2000. J Cell Sci. 113:3073; Sasaki Y. et al. 2001. Biochem. Biophys. Res. Commun. 286:292; Kanazawa H. et al. 2002. J. Biol. Chem. 277:20026 and Mastorakos P. et al. 2021. Nat. Neuroscience 24:245-258. Chicken anti-GEFAP (catalog# ab4674; Abcam, Cambridge, UK) was tested by the manufacturer for IHC use and has been used in Urker KE. et al. 2020. Nat. Commun. 11:306 and Skorkowska A. et al. 2020. Neuronot Res. 37:683 and Mastorakos P. et al. 2021. Nat. Neuroscience 24:245-258. Guinea pig polyclonal anti-NeuN (catalog# ABNO99; Millipore) was evaluated by the manufacturer for use in Western Blotting, ICC, IHC/P for the detection of NeuN purified and used in Lentz MR et al. 2014. PloS one. 9:105752 and Mastorakos P. et al. 2021. Nat. Neuroscience 24:245-258. Rabbit anti-claudin-5 [catalog# 341600; ThermoFisher, Waltham, MA] was validated for IHC by the manufacturer and used by Park H. et al. 2019. Nat. Commun. 10(1):5243; Mahamad MR. et. al. 2019. Development. 146(21):dev284218; and Hudson N. et al. 2019 JCI Insight. 4(15):e130273 for IHC. Rabbit anti-ZO-1 [catalog# ab96587] was validated for IHC by the manufacturer and used by Bi J. et al. 2020. J Cell Mol Med 24:996-1009; Ma X. et al. 2020 Bioact Mater 5:124-132; and Qin W. et al. 2019. Aging 11:11391-11415 for IHC. Purified rat anti-mouse CD16/CD32 (Fc receptor block; clone 93, BioLegend) were quality control tested by immunofluorescent staining with flow cytometric analysis by the manufacturer and are recommended to be used for blocking of CD16/CD32 interactions with the Fc domain of immunoglobulins.

### Animals and other organisms

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**Laboratory animals**

| Name                  | Genotype/Strain                                                                 |
|-----------------------|--------------------------------------------------------------------------------|
| CS7BL/6J             | B6.129P2-Lyztm1[cre]hop/J (Ly5McCre/Cre); IoxP-flanked ifnar1 [B6(Cg)-lnarf1tm1.1(Eos); I FNARII/l]; B6.Cg-Ifnar1tm1.1Cl/J (MDAS/-); B6.129P-CX3CR1tm1Litt/J (Cx3cr1gfp/gfp); and B6.129(Cg)-Ccr2tm2.1Icf/J (Ccr2rfp/rfp) mice were purchased from Jackson Laboratories and were then bred and maintained under specific pathogen-free conditions at the National Institute of Health (NIH). Type 1 interferon-α/β receptor 1-deficient mice on B6 background (IFNAR/-/) were provided by Jonathan Sprent (Scripps Research Institute, La Jolla, CA) and then bred and maintained under specific pathogen-free conditions at the National Institute of Health. LysMCre-IFNARII/l mice were obtained in the F2 generation by originally crossing LysMCre/Cre and IFNARII/l mice and were screened using PCR. CX3CR1gfp/wt CCR2rfp/wt double reporter mice were generated from an F1 cross of CX3CR1gfp/gfp and CCR2rfp/rfp mice. All mice in this study were handled in accordance with the guidelines set forth by the NIH Animal Care and Use Committee and the recommendations in the AAALAC Guide for the Care and Use of Laboratory Animals. The protocol was approved by the NINDS Animal Care and Use Committee. Male and female mice in this study were used at 8-12 weeks of age with age matched control groups. Mice were bred and maintained under specific pathogen-free conditions at the National Institute of Health. Housing conditions included temperature 72 F, humidity 50% and light/dark cycle 12h each. |

**Wild animals**

| | This study did not involve wild animals. |

**Field-collected samples**

| | This study did not involved samples collected from the field. |

**Ethics oversight**

| | All mice in this study were handled in accordance with the guidelines set forth by the NIH Animal Care and Use Committee and the recommendations in the AAALAC Guide for the Care and Use of Laboratory Animals. The protocol was approved by the NINDS Animal Care and Use Committee. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.