



HAL
open science

Loss of USP18 in microglia induces white matter pathology

Marius Schwabenland, Omar Mossad, Adam G Peres, Franziska Kessler, Feres Jose Mocayar Maron, Laura-Adela Harsan, Thomas Bienert, Dominik von Elverfeldt, Klaus-Peter Knobloch, Ori Staszewski, et al.

► **To cite this version:**

Marius Schwabenland, Omar Mossad, Adam G Peres, Franziska Kessler, Feres Jose Mocayar Maron, et al.. Loss of USP18 in microglia induces white matter pathology. *Acta Neuropathologica Communications*, 2019, 7 (1), pp.106. 10.1186/s40478-019-0757-8 . hal-04883969

HAL Id: hal-04883969

<https://hal.science/hal-04883969v1>

Submitted on 13 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.




Distributed under a Creative Commons Attribution 4.0 International License

LETTER TO THE EDITOR

Open Access

Loss of USP18 in microglia induces white matter pathology



Marius Schwabenland¹, Omar Mossad^{1,2}, Adam G. Peres¹, Franziska Kessler¹, Feres Jose Mocayar Maron¹, Laura-Adela Harsan^{3,4}, Thomas Bienert³, Dominik von Elverfeldt³, Klaus-Peter Knobloch¹, Ori Staszewski¹, Frank L. Heppner^{5,9,10}, Marije E. C. Meuwissen⁶, Grazia M. S. Mancini⁶, Marco Prinz^{1,7,8} and Thomas Blank^{1*} 

Keywords: Microglia, Type I interferon, *Usp18*, White matter, Phagocytosis, Corpus callosum, Behavior, Magnet resonance spectroscopy, Microgliosis

Main text

Ubiquitin specific protease 18 (USP18) is a major negative regulator of the type 1 interferon (IFN) pathway. In a recent publication we showed that USP18 is a key molecule imposing microglial quiescence specifically in the white matter [7]. USP18 is a negative regulator of the type 1 interferon (IFN) pathway [9]. Microglia lacking *Usp18* exhibited constitutive activation of type I IFN signaling pathways resulting in markedly elevated expression of multiple interferon-stimulated genes (ISGs) [7]. Additionally, *Usp18*-deficient brains exhibited clusters of microglia in the white matter that strongly resembled the neuropathological state in several human microgliopathies. Human diseases in which microgliopathies play a primary role comprise Nasu-Hakola disease [14], hereditary diffuse leukoencephalopathy with spheroids (HDLS) [15] and Pseudo-TORCH syndrome (PTS), including Aicardi–Goutières syndrome [12]. One might speculate that activated microglia in the white matter induce white matter abnormalities with functional consequences. However, there were no cells which had taken up myelin in young adult mice as seen by luxol fast blue–PAS (LFB–PAS) histology (unpublished data). Myelin uptake by other cells, like macrophages, would have been indicative of myelin damage. That is why we now characterized conditional myeloid-specific *Usp18* deficient mice in more detail.

We know that *Usp18* transcripts are highly expressed in unstimulated white matter microglia with only negligible expression levels in other CNS cells [7]. In a

previous study, we have confirmed by PCR analysis that *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice have an *Usp18* deletion in microglia but not in neuroectodermal cells of the CNS. These mice displayed a significant increase of Iba1⁺ microglia cell numbers in several white matter regions including the corpus callosum as young adult mice [7]. This microgliosis persisted with increasing age and was detectable even in 4- and 8-month old mice (Fig. 1a, b). *Usp18*-deficient microglia exhibit constitutive expression of IFN target genes and fail to downregulate IFN-induced genes because the termination of type I IFN signaling is severely impaired. This became evident by the increase in ISG15 positive cells in the corpus callosum (Fig. 1a, b) and the elevated phosphorylation of STAT1 in *Usp18*-deficient microglia when compared to *Usp18^{fl/fl}* mice (Fig. 1c). We next investigated animals at later ages than before by immunostainings against lysosome-associated membrane protein-2 (LAMP2) as a marker of phagocytosis [4]. We found increased LAMP2 positive signals in microglia, which were localized in the corpus callosum of *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice at an age of 4 months (Fig. 2a, b) and 8 months (Fig. 2c, d). To analyze white matter integrity, we performed high-resolution (11.7 T) diffusion tensor imaging (DTI). We calculated the fractional anisotropy (FA) values, permitting an exploration of the orientation coherence of axons in this fiber bundle. We found that the FA values were reduced in the corpus callosum, the internal and external capsule of *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice (cf. *Usp18^{fl/fl}* controls), suggesting diminished structural integrity of the white matter in 4- and 8-month old animals (Fig. 2e). Additionally, we found increased numbers of cells that had incorporated myelin

* Correspondence: thomas.blank@uniklinik-freiburg.de

¹Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany

Full list of author information is available at the end of the article



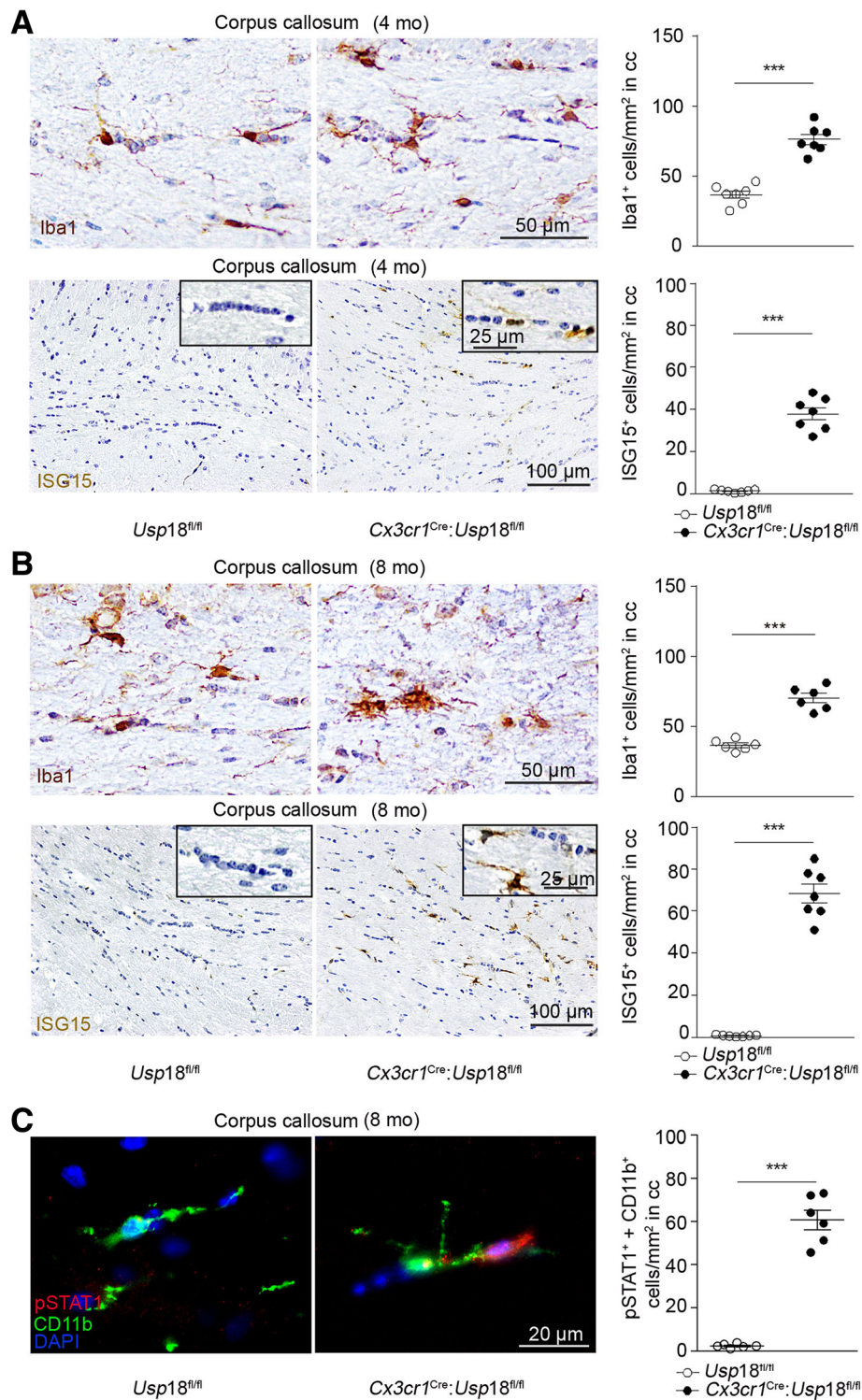


Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Microgliosis in corpus callosum of *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice. **a, b** Histology of corpus callosum in the cerebrum of adult *Usp18^{fl/fl}* and *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice at 4 (**a**) and 8 months of age (**b**). Primary antibodies against Iba1 and ISG15 were used. To quantify the number of Iba1⁺ or ISG15⁺ cells at least six mice per genotype and 5 sections per mouse from two independent experiments were counted. Quantification of cells is shown next to the respective histological images. Significant differences were determined by an unpaired *t*-test or Mann-Whitney *U*-test and marked with asterisks (***) $P < 0.001$ versus control littermates). Bars represent means \pm S.E.M. Scale bars = 25 μ m, 50 μ m, 100 μ m. **c** Immunohistochemistry for phosphorylated STAT1 (pSTAT1, red), CD11b (green) and DAPI (blue) in the corpus callosum of 8-month old *Usp18^{fl/fl}* and *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice. Scale bar: 20 μ m. Quantification of pSTAT1⁺CD11b⁺ cells is shown next to the respective histological images. Each symbol represents one mouse. Error bars represent S.E.M. Significant differences are determined by an unpaired *t*-test and marked with asterisks (***) $P < 0.001$)

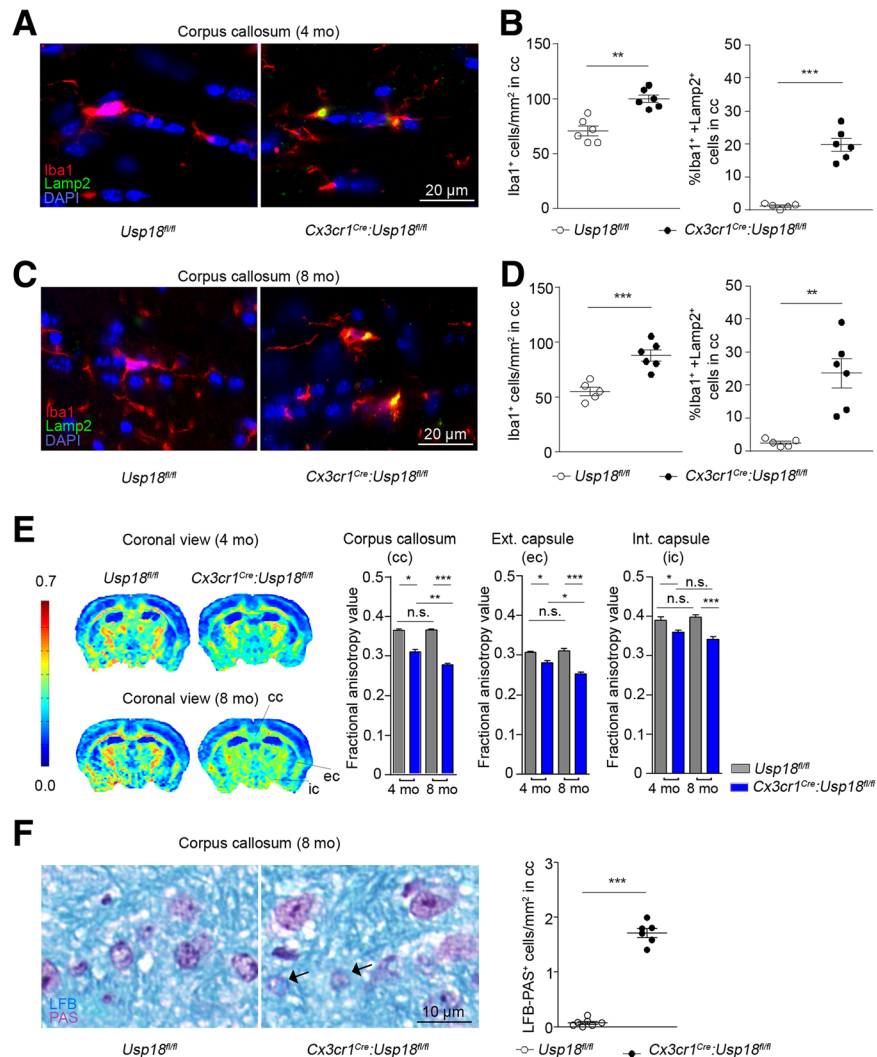


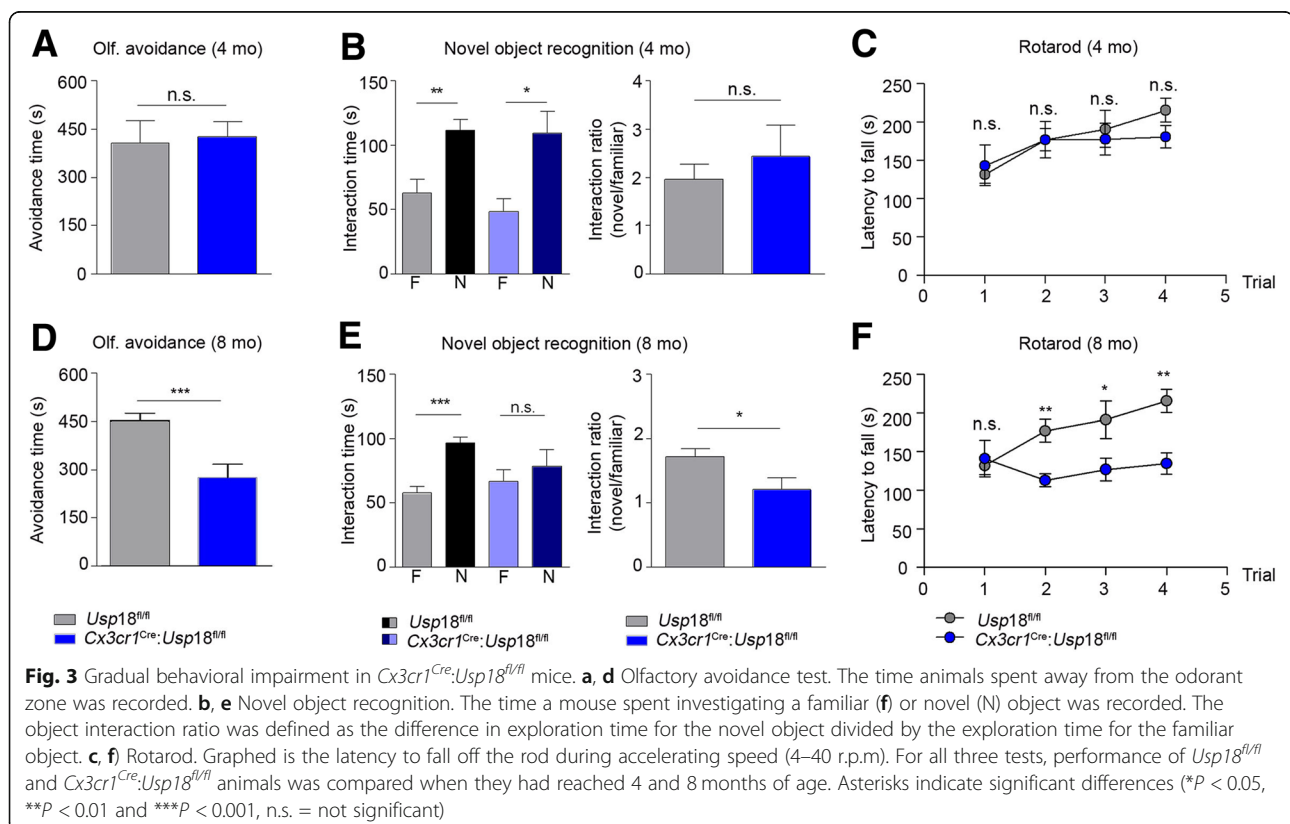
Fig. 2 USP18-deficient microglia reduces structural integrity in corpus callosum. **a** Immunofluorescent histochemistry for Iba1 (red), Lamp2 (green) and DAPI (blue) in the corpus callosum of 4 months and 8 months (**c**) old *Usp18^{fl/fl}* and *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice. Scale bar: 20 μ m. Quantification of Iba1⁺ and percentage of Iba1⁺Lamp2⁺ cells is shown next to the respective histological images (**b, d**). Each symbol represents one mouse. Error bars represent s.e.m. Significant differences are determined by an unpaired *t*-test and marked with asterisks (***) $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. = non-significant). **e** DTI was performed on 4 and 8 months old *Usp18^{fl/fl}* and *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice to measure the FA of the corpus callosum. Tensor images were collectively acquired in several horizontal planes from +2.0 to -4.0 mm from the bregma, with an interplane distance of 0.5 mm (*Usp18^{fl/fl}*, $n = 6$; *Cx3cr1^{Cre}:Usp18^{fl/fl}*, $n = 4$). Heat maps of the FA values showing the average (of all *Usp18^{fl/fl}* and *Cx3cr1^{Cre}:Usp18^{fl/fl}* animals) of one plane from each group (from anterior to posterior). Warm colors indicate fiber tracts with strong diffusion coherence. For both age groups the FA values were significantly reduced in *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice in comparison to *Usp18^{fl/fl}* mice. Approximate locations of the regions of interest (ROIs) are indicated. Data are means \pm SEM. (** $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. = non-significant). Statistical significance was determined using multiple *t* tests corrected for multiple comparisons using the Holm-Sidak method with $\alpha = 0.05$. **f** Histological analysis by luxol fast blue-PAS (LFB-PAS) in 8-month-old *Usp18^{fl/fl}* mice and *Cx3cr1^{Cre}:Usp18^{fl/fl}* littermates. Representative of $n = 6$ *Usp18^{fl/fl}* and $n = 7$ *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice. Circles represent individual mice. Unpaired two-tailed *t*-test

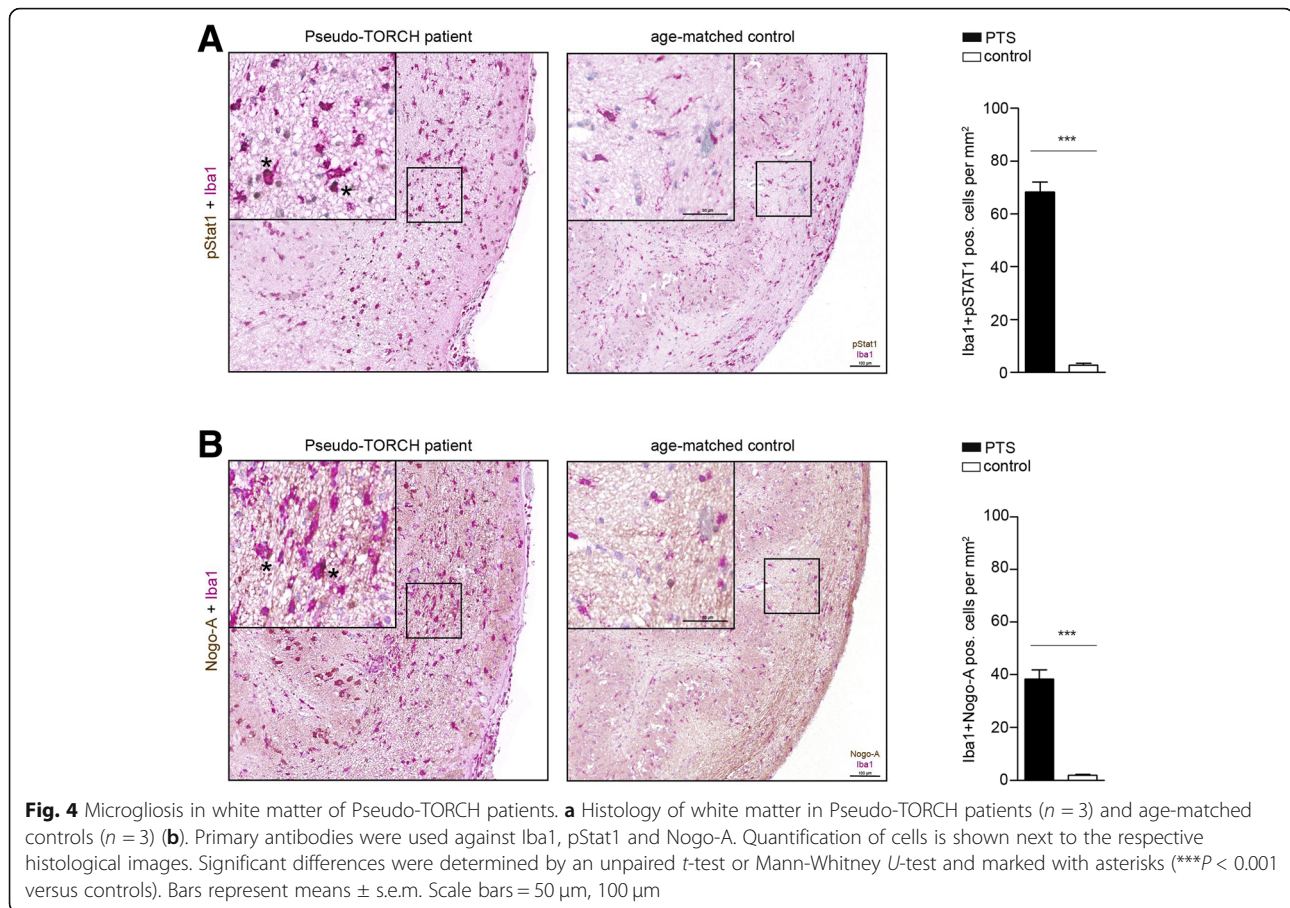
and thereby indicate damage to the myelin sheaths (Fig. 2f). Together, these findings point to a reduction in myelination or even to a loss of fibers in *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice [2, 17].

Deterioration of white matter tracts, affecting brain structural (SC) and functional connectivity (FC) is often paralleled by behavioral declines [3, 6, 8]. We therefore tested *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice and *Usp18^{fl/fl}* littermate controls in different behavioral paradigms. While mice lacking *Usp18* in microglia performed normal in the odor avoidance test at 4 months of age (Fig. 3a), 8-month old *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice showed severely impaired olfaction (Fig. 3d). Similarly, learning and recognition memory was fully intact at 4 months of age (Fig. 3b) but decreased when *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice were 8-month old compared to age-matched *Usp18^{fl/fl}* control mice (Fig. 3e). Rotarod performance, which measures motor coordination and motor learning, was also significantly impaired in 8-month old *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice (Fig. 3f) with no deficits in 4 months old mice (Fig. 3c). In addition to the indicated mouse model we investigated brainstem tissue samples from three PTS patients with loss-of-function recessive mutations of *USP18* [12]. Immunohistochemistry showed increased STAT1 phosphorylation in microglia of PTS patients when compared to age-matched control tissue (Fig. 4a). In patients' material there were also more

microglial cells, which engulfed cells positive for Nogo-A (Fig. 4b), which represents an oligodendroglial marker [11].

The data presented here indicate that in myeloid-specific *Usp18* knockout animals, microglia in the white matter were not only activated, but also caused advancing damage to this structure with subsequent behavioral impairment of the animals. *USP18*-deficiency in humans belongs to a group of genetic disorders that are collectively termed type I interferonopathies. These disorders are first characterized by the persistent up-regulation of type I interferon signaling [16]. There have been at least seven possible cellular mechanisms described, which result in sustained activation of interferon signaling [16]. One of them, PTS, is a group of not so well-defined genetic diseases, which can originate from *USP18* deficiency. We found that microglia in PTS patients displayed not only enhanced type I IFN signaling, but also close contact to oligodendroglia. A direct interaction might indicate that activated microglia, as suggested by their focally elevated cell density together with altered morphological properties inflict damage to oligodendroglia. This strongly resembles the white matter damage observed in *Cx3cr1^{Cre}:Usp18^{fl/fl}* mice. Type I interferon can be regarded as a neurotoxin if its levels are not tightly controlled. Accordingly, experiments undertaken in mice demonstrate that overexpression of





interferon in the CNS results in neuropathology reminiscent of that seen in certain type I interferonopathies [1, 10]. In the case of PTS, but also in the case of type I IFN overexpression, damage to the white matter seems to be prevalent [5, 12]. It is still unclear what the type I IFN source is in the context of interferonopathies. Likewise it is enigmatic which signals are responsible for microglia activation in the white matter. The escalating spiral of white matter damage might be initiated by type I IFN that is induced in microglia via stimulator of interferon genes (STING), and this IFN likely influences the microglial phenotype in an autocrine and paracrine fashion [13].

The white matter specificity of the USP18 effect on microglia is of particular interest and further developments in this area may have implications for an entire range of neurological disorders in which there is a preponderance of white matter pathology.

Abbreviations

DTI: Diffusion tensor imaging; IFN: Interferon; ISG: Interferon-stimulated gene; MRI: Magnetic resonance imaging; NOR: Novel Object Recognition; PTS: Pseudo-TORCH syndrome; RT: Room temperature; STAT1: Signal transducer and activator of transcription 1; STING: Stimulator of interferon genes; USP: Ubiquitin-specific protease

Acknowledgements

The authors are thankful to Margarethe Ditter for excellent technical assistance.

Authors' contributions

TB, KPK, MECM, GMSM, OS, FLH and MP were responsible for the conception and design of experiments; Tbi, LAH and DvE were responsible for MRI measurements; MS, OM, FK, AP and FJMM performed experiments, analysed and interpreted the data, they drafted the paper and revised and edited the final article. All authors read and approved the final manuscript.

Funding

TB was supported by the DFG (BL 1153/1–2). TB and MP are supported by the DFG (SFB/TRR167 "NeuroMac").

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal experiments were approved by the Federal Ministry for Nature, Environment and Consumers' Protection of the state of Baden-Württemberg (G12/71; G16/107) and were performed in accordance with the respective national, federal and institutional regulations. For patients' samples written parental consent was obtained. Genetic tests were performed according to The Erasmus University Medical Center's local ethics board approved protocol MEC-2012387.

Consent for publication

All the authors have approved publication.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany. ²Faculty of Biology, University of Freiburg, Freiburg, Germany. ³Department of Radiology, Medical Physics, University Medical Center Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany. ⁴Engineering Science, Computer Science and Imaging Laboratory (iCube), Integrative Multimodal Imaging in Healthcare, CNRS, University of Strasbourg, Strasbourg, France. ⁵Department of Neuropathology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1 (Virchowweg 15), 10117 Berlin, Germany. ⁶Department of Clinical Genetics, Erasmus University Medical Center, 3015, GD, Rotterdam, the Netherlands. ⁷Signalling Research Centres BIOS and CIBSS, University of Freiburg, Freiburg, Germany. ⁸Center for NeuroModulation, Faculty of Medicine, University of Freiburg, Freiburg, Germany. ⁹Cluster of Excellence, NeuroCure, Charitéplatz 1, 10117 Berlin, Germany. ¹⁰German Center for Neurodegenerative Diseases (DZNE) Berlin, 10117 Berlin, Germany.

Received: 16 May 2019 Accepted: 20 June 2019

Published online: 04 July 2019

References

- Akwa Y, Hassett DE, Eloranta ML, Sandberg K, Masliah E, Powell H, Whittton JL, Bloom FE, Campbell IL (1998) Transgenic expression of IFN-alpha in the central nervous system of mice protects against lethal neurotropic viral infection but induces inflammation and neurodegeneration. *J Immunol* 161: 5016–5026
- Basser PJ (1995) Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR Biomed* 8:333–344
- Bells S, Lefebvre J, Prescott SA, Dockstader C, Bouffet E, Skocic J, Laughlin S, Mabbott DJ (2017) Changes in white matter microstructure impact cognition by disrupting the ability of neural assemblies to synchronize. *J Neurosci* 37:8227–8238. <https://doi.org/10.1523/JNEUROSCI.0560-17.2017>
- Blank T, Goldmann T, Koch M, Amann L, Schon C, Bonin M, Pang S, Prinz M, Burnet M, Wagner JE et al (2017) Early microglia activation precedes photoreceptor degeneration in a mouse model of CNGB1-linked retinitis pigmentosa. *Front Immunol* 8:1930. <https://doi.org/10.3389/fimmu.2017.01930>
- Cuadrado E, Jansen MH, Anink J, De Filippis L, Vescovi AL, Watts C, Aronica E, Hol EM, Kuijpers TW (2013) Chronic exposure of astrocytes to interferon-alpha reveals molecular changes related to Aicardi-Goutieres syndrome. *Brain* 136:245–258. <https://doi.org/10.1093/brain/aws321>
- Filley CM, Fields RD (2016) White matter and cognition: making the connection. *J Neurophysiol* 116:2093–2104. <https://doi.org/10.1152/jn.00221.2016>
- Goldmann T, Zeller N, Raasch J, Kierdorf K, Frenzel K, Ketscher L, Basters A, Staszewski O, Brendecke SM, Spiess A et al (2015) USP18 lack in microglia causes destructive interferonopathy of the mouse brain. *EMBO J* 34:1612–1629. <https://doi.org/10.15252/embj.201490791>
- Gullmar D, Seeliger T, Gudziol H, Teichgraber UKM, Reichenbach JR, Guntinas-Lichius O, Bitter T (2017) Improvement of olfactory function after sinus surgery correlates with white matter properties measured by diffusion tensor imaging. *Neuroscience* 360:190–196. <https://doi.org/10.1016/j.neuroscience.2017.07.070>
- Honke N, Shaabani N, Zhang DE, Hardt C, Lang KS (2016) Multiple functions of USP18. *Cell Death Dis* 7:e2444. <https://doi.org/10.1038/cddis.2016.326>
- Kavanagh D, McGlasson S, Jury A, Williams J, Scolding N, Bellamy C, Gunther C, Ritchie D, Gale DP, Kanwar YS et al (2016) Type I interferon causes thrombotic microangiopathy by a dose-dependent toxic effect on the microvasculature. *Blood* 128:2824–2833. <https://doi.org/10.1182/blood-2016-05-715987>
- Kuhlmann T, Remington L, Maruschak B, Owens T, Bruck W (2007) Nogo-a is a reliable oligodendroglial marker in adult human and mouse CNS and in demyelinated lesions. *J Neuropathol Exp Neurol* 66:238–246. <https://doi.org/10.1097/01.jnen.0000248559.83573.71>
- Meuwissen ME, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, Li Z, van Unen L, Heijmans D, Goldmann T et al (2016) Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. *J Exp Med* 213:1163–1174. <https://doi.org/10.1084/jem.20151529>
- Nazmi A, Field RH, Griffin EW, Haugh O, Hennessy E, Cox D, Reis R, Tortorelli L, Murray CL, Lopez-Rodriguez AB et al (2019) Chronic neurodegeneration induces type I interferon synthesis via STING, shaping microglial phenotype and accelerating disease progression. *Glia*. <https://doi.org/10.1002/glia.23592>
- Paloneva J, Manninen T, Christman G, Hovanes K, Mandelin J, Adolfsson R, Bianchin M, Bird T, Miranda R, Salmaggi A et al (2002) Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am J Hum Genet* 71:656–662. <https://doi.org/10.1086/342259>
- Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, Lash J, Wider C, Wojtas A, DeJesus-Hernandez M et al (2011) Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat Genet* 44:200–205. <https://doi.org/10.1038/ng.1027>
- Rodero MP, Crow YJ (2016) Type I interferon-mediated monogenic autoinflammation: the type I interferonopathies, a conceptual overview. *J Exp Med* 213:2527–2538. <https://doi.org/10.1084/jem.20161596>
- Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH (2002) Demyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage* 17:1429–1436

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

