Microglia biology - A brief introduction

Selected topics regarding the ontogeny of microglia and its role in neurodevelopment and disease

Daniel Bormann – 18.10.2021

JC - Applied Immunology – WS 21/22

Arge Ankersmit

Topics of todays seminar:

I The birth and youth of microglia – conceptually and ontogenetically

-> Early 20th century: “The big bang” of microglia research
-> The ontogenetical origin of microglia and microglial diversity in the young brain

II Microglia as gardeners of neural forests?

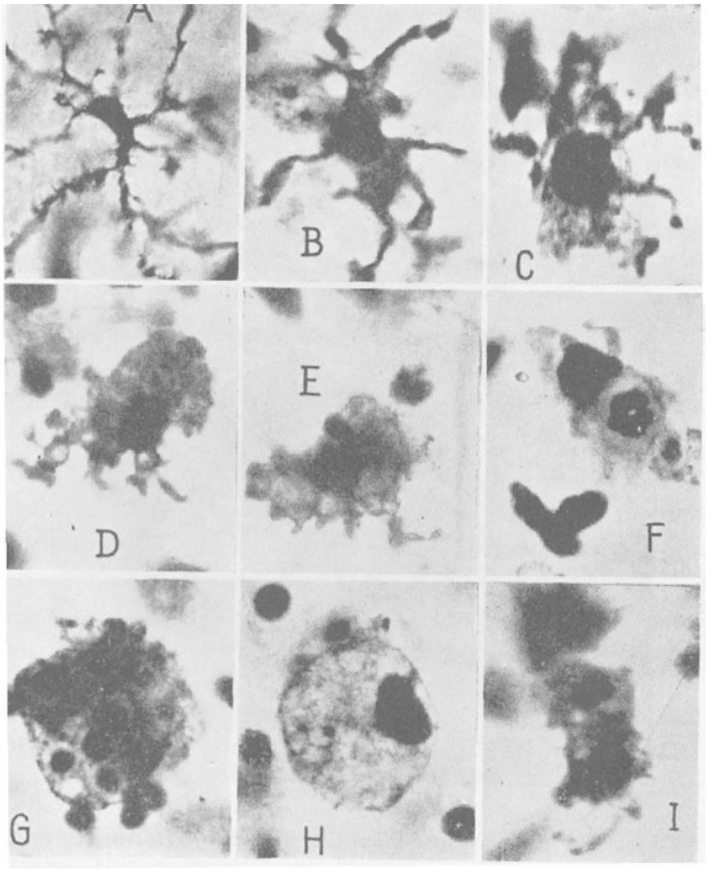
-> “Subtractive” functions of microglia beyond injury response and pathogen clearance
-> Microglia as producers of trophic/antiapoptotic factors

III Microglia as responders to and inducers of CNS injury

-> Selected roles of microglia in chronic and acute responses to disturbances of CNS homeostasis

I The birth and youth of microglia – conceptually and ontogenetically

Brief historical outline



Pio del Rio Hortega (1882 – 1945) *one of the fathers of Glia Biology:*

-> Major contribution to the classification of Neuroglia

-> One of the first notions of ->the **mesodermal origin of microglia**,

->their **relation to leukocytes**,

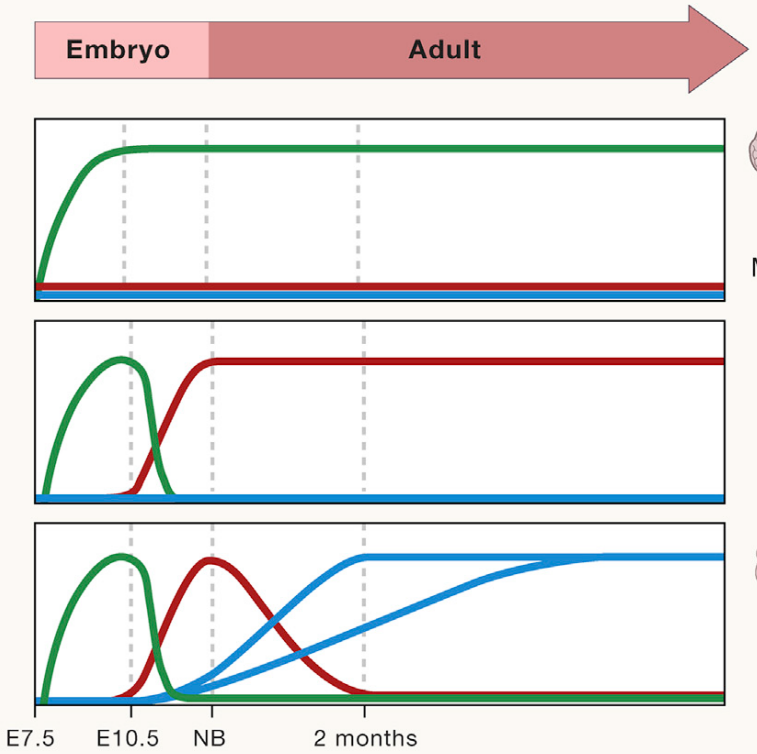
->**phagocytotic** *and* **migratory** *capabilities*

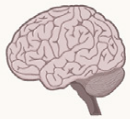
-> role in **neurodevelopment and synaptic pruning**.

-> among other discoveries

Fig. From: Garaschuk O., Verkhratsky A. (2019) Microglia: The Neural Cells of Nonneural Origin. In: Garaschuk O., Verkhratsky A. (eds) Microglia. Methods in Molecular Biology, vol 2034. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9658-2_1

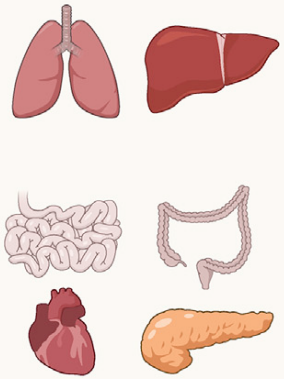
The ontogeny of Microglia





Microglia
Most BAM

Microglia stem from c-Kit-lo, CD41-lo erythroid myeloid progenitors



In contrast to other tissues The CNS is not repopulated by waves of myeloid progenitors and Peripheral monocytes !

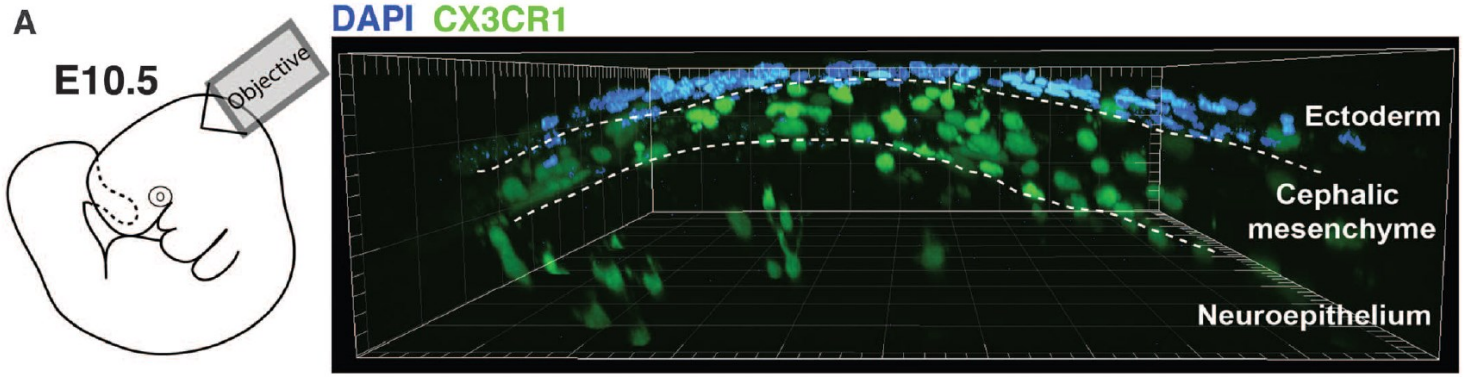
Fast

Slow

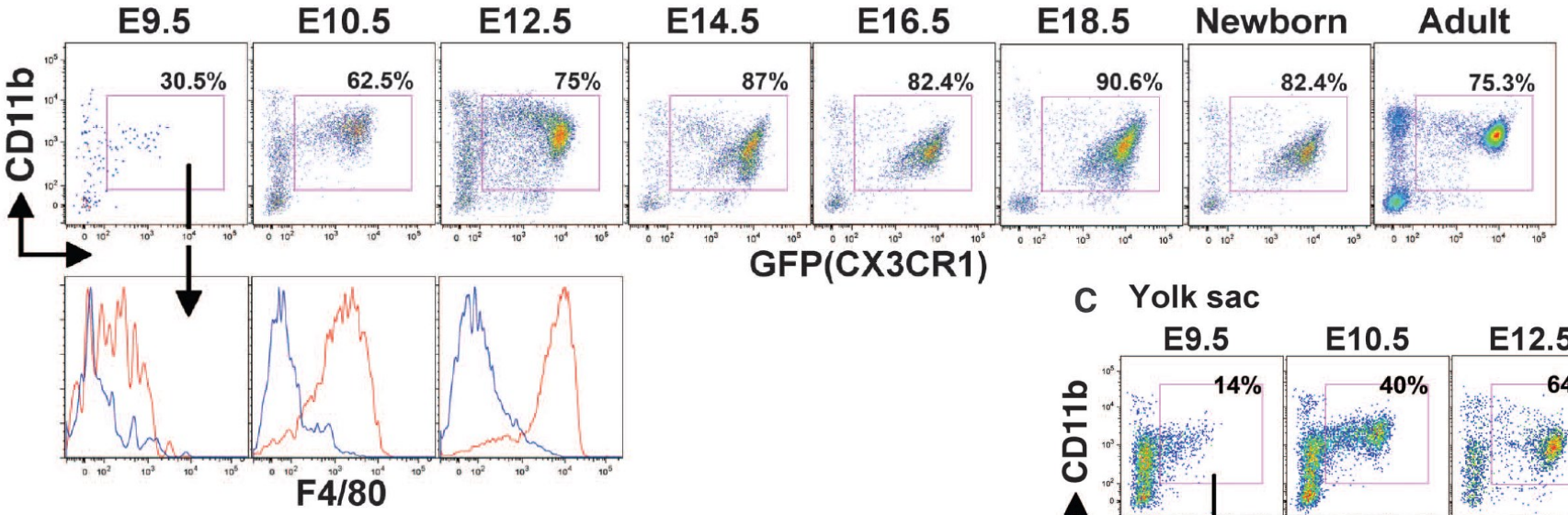
Wave	Progenitor	Site of generation > maintenance	Disseminator	Presence	Macrophage product
Transient primitive	eEMP (cKit^{lo} CD41^{lo})	YS	preMF	Embryo	Microglia, most BAM
Transient definitive	EMP (cKit^{+})	YS > FL	Fetal monocytes	Embryo	Most tissue MF
Definitive	HSC	AGM > FL > BM	Monocytes	Embryo / adult	Barrier tissue MF

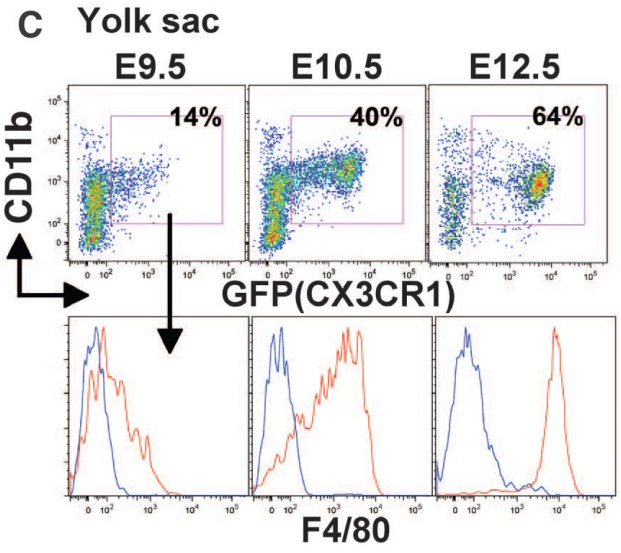
Titel Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell* (2019) 179(2):292-311. Epub 2019/10/05. doi: 10.1016/j.cell.2019.08.053. PubMed PMID: 31585077. *der Präsentation ODER des Vortragenden*

The birth and infancy of a microglia cell



TOP: *Brain rudiment from Cx3cr1-gfp+ mice -> The fractalkine receptor (CX3CR1) - a marker of early myeloid progenitors and microglia fluoresces green.*

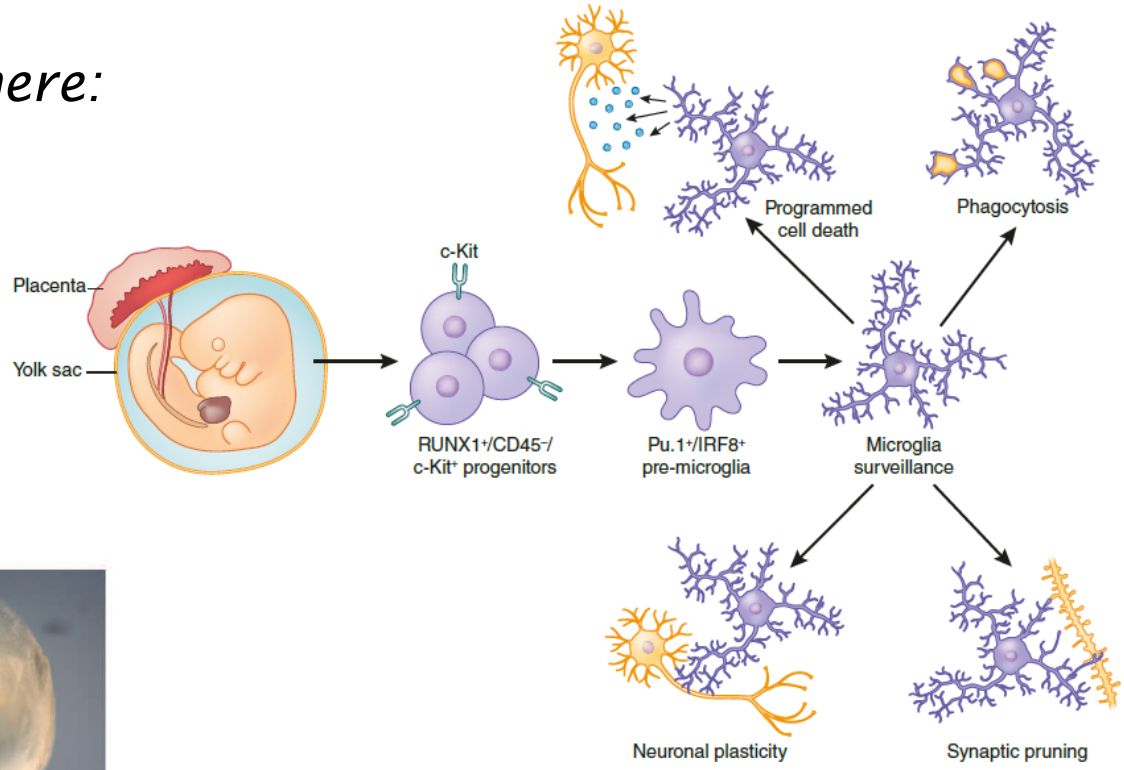


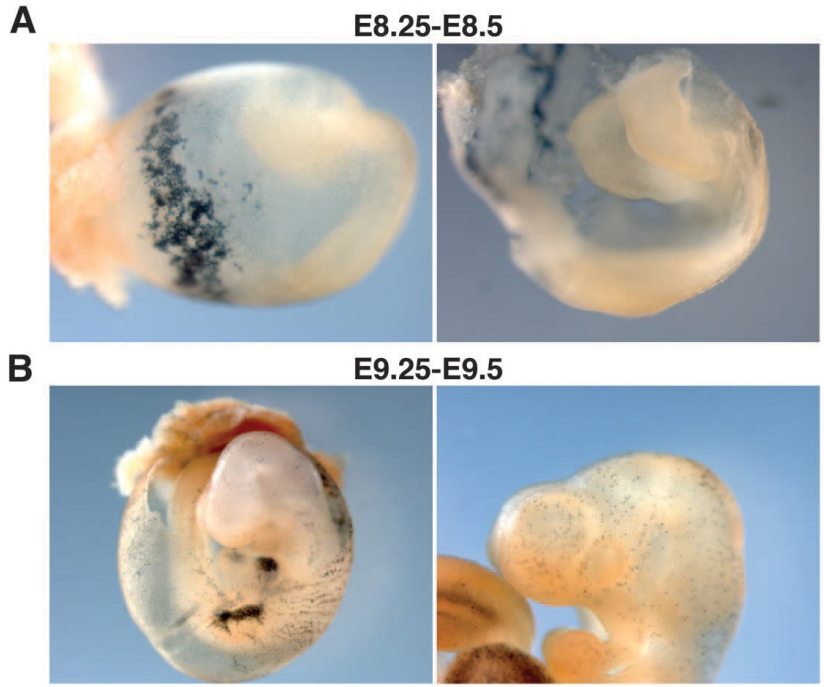


BOTTOM: *Flow cytometry depicting the mature microglia markers CD11b, F4/80 and CXCR1 over different timepoints in the brain and yolk sack*

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. *Science* (2010) 330(6005):841-5. doi: doi:10.1126/science.1194637.

RunX1+ progenitor cells *(here: Lac-Z+ -> dark blue dots) migrate from the YS to the developing CNS via the vasculature*



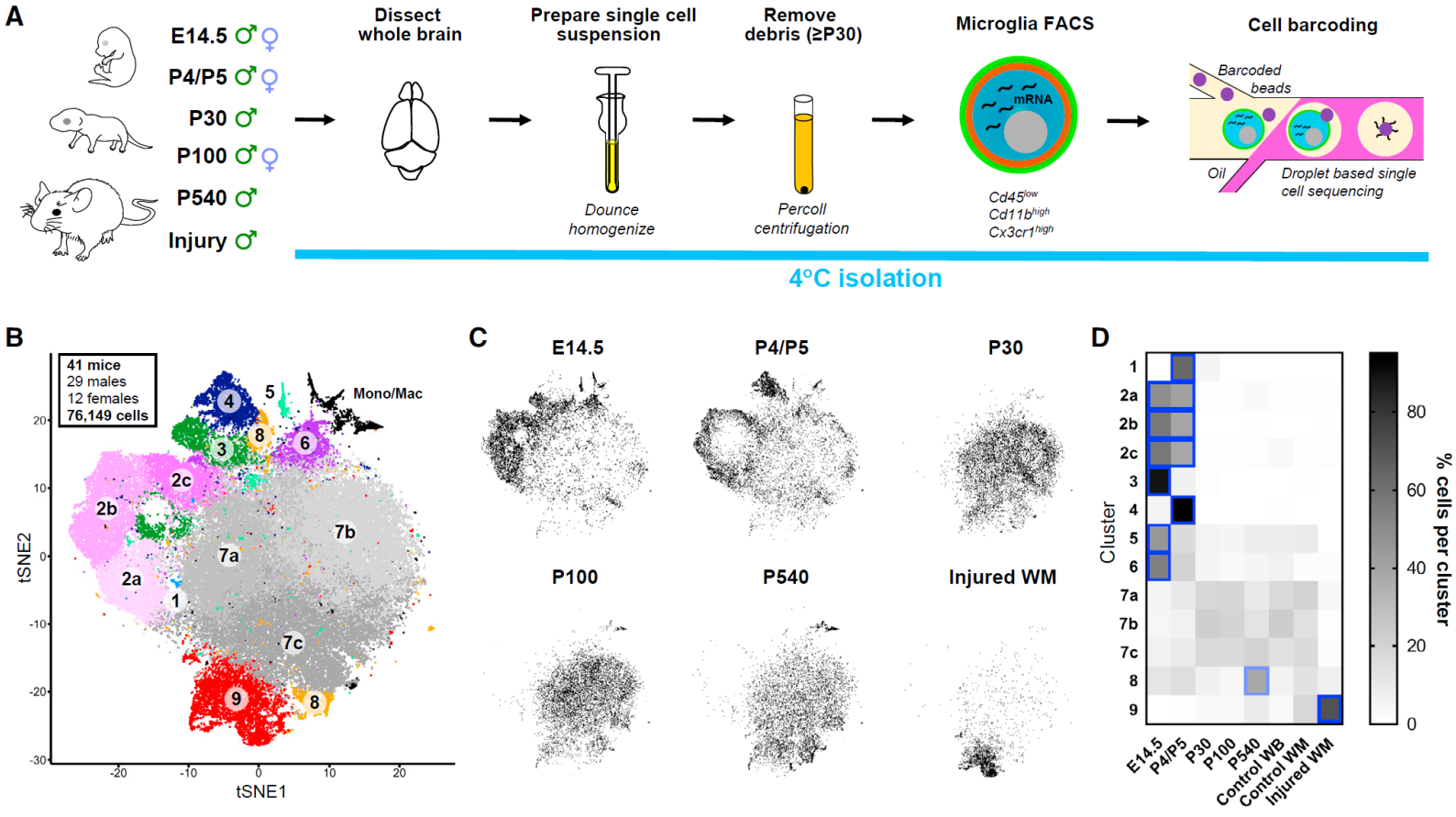


Under normal conditions close to all microglia are derived from yolk-sac precursors, infiltrating the CNS very early (E.8 to 10) without later replenishment from the fetal liver or bone marrow derived progenitors.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. Science (2010) 330(6005):841-5. doi: doi:10.1126/science.1194637.

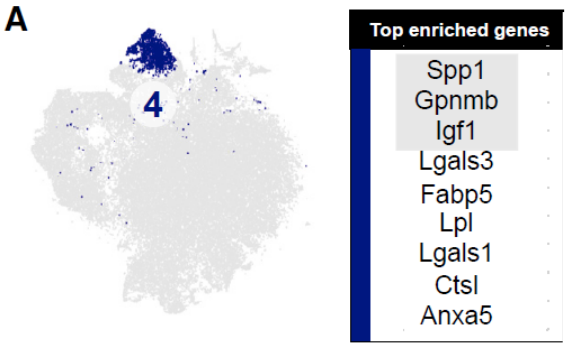
The young microglia in the young brain

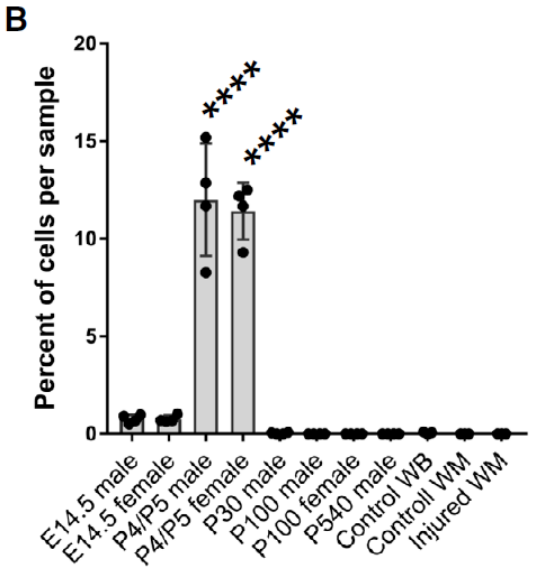
- *Microglia present a unique transcriptional diversity during early postnatal brain development.*

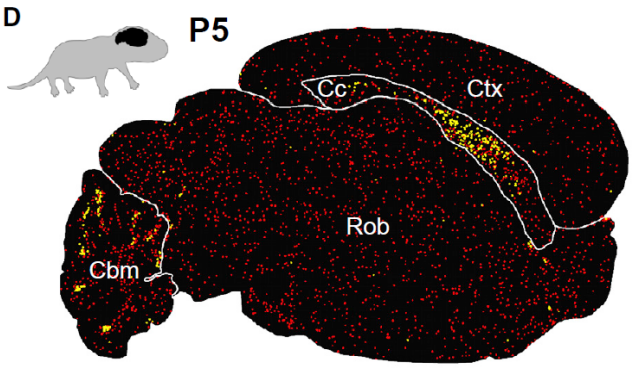


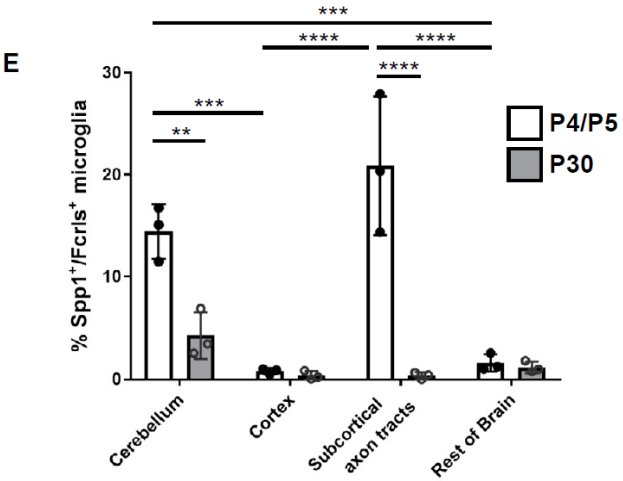
Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. Immunity (2019) 50(1):253-71.e6. Epub 2018/11/26. doi: 10.1016/j.immuni.2018.11.004. PubMed PMID: 30471926; PubMed Central PMCID: PMCPmc6655561.

The young microglia in the young brain









- *At P4/5 a distinct microglia signature emerges associated to:*

Immune recognition, Lysosomal activity, Phagocytosis and Metabolic activity

This microglia subset appears to be associated spatially to axon tracts (AT-Microglia)

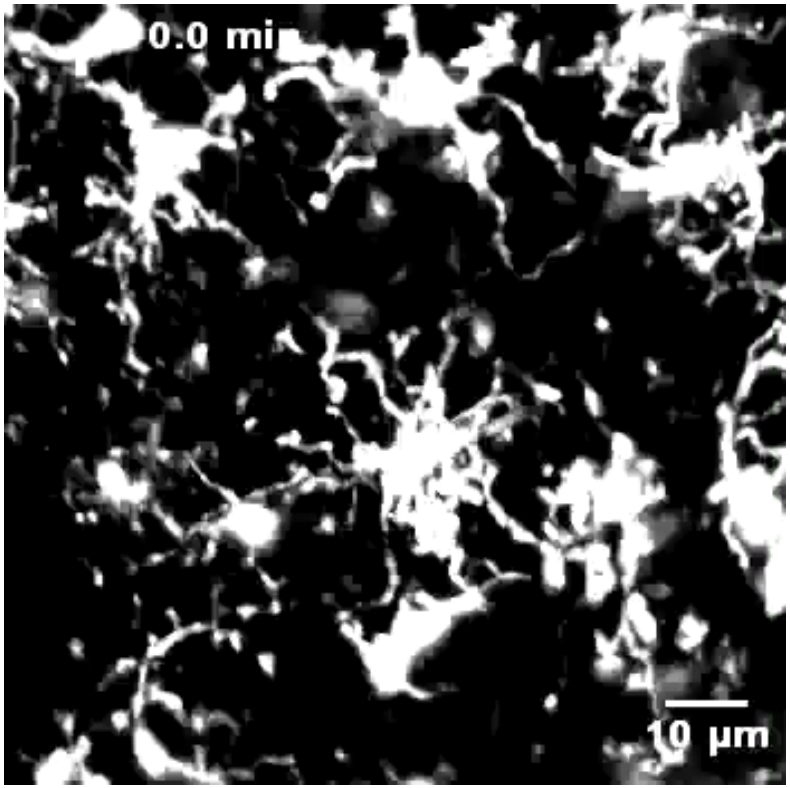
Spp1: secreted phospho protein 1
Igf1: insulin like growth factor 1
Gpnmb: glycoprotein (transmembrane) NMB

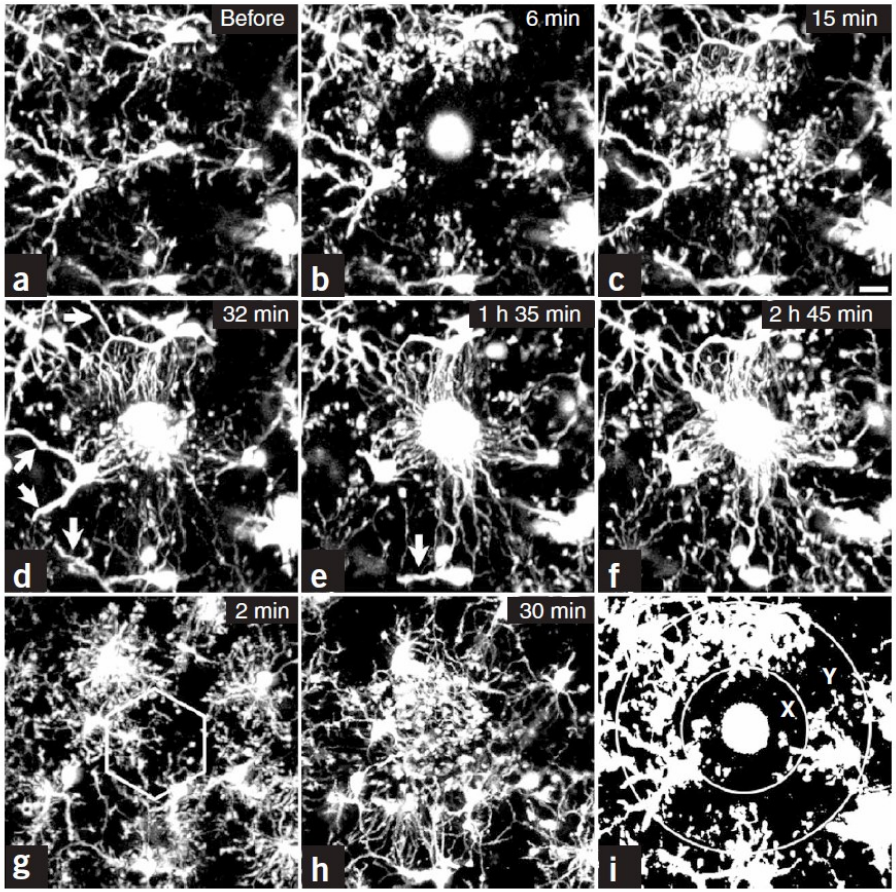
Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. Immunity (2019) 50(1):253-71.e6. Epub 2018/11/26. doi: 10.1016/j.immuni.2018.11.004. PubMed PMID: 30471926; PubMed Central PMCID: PMCPmc6655561.

II Microglia as gardeners of neural forests?

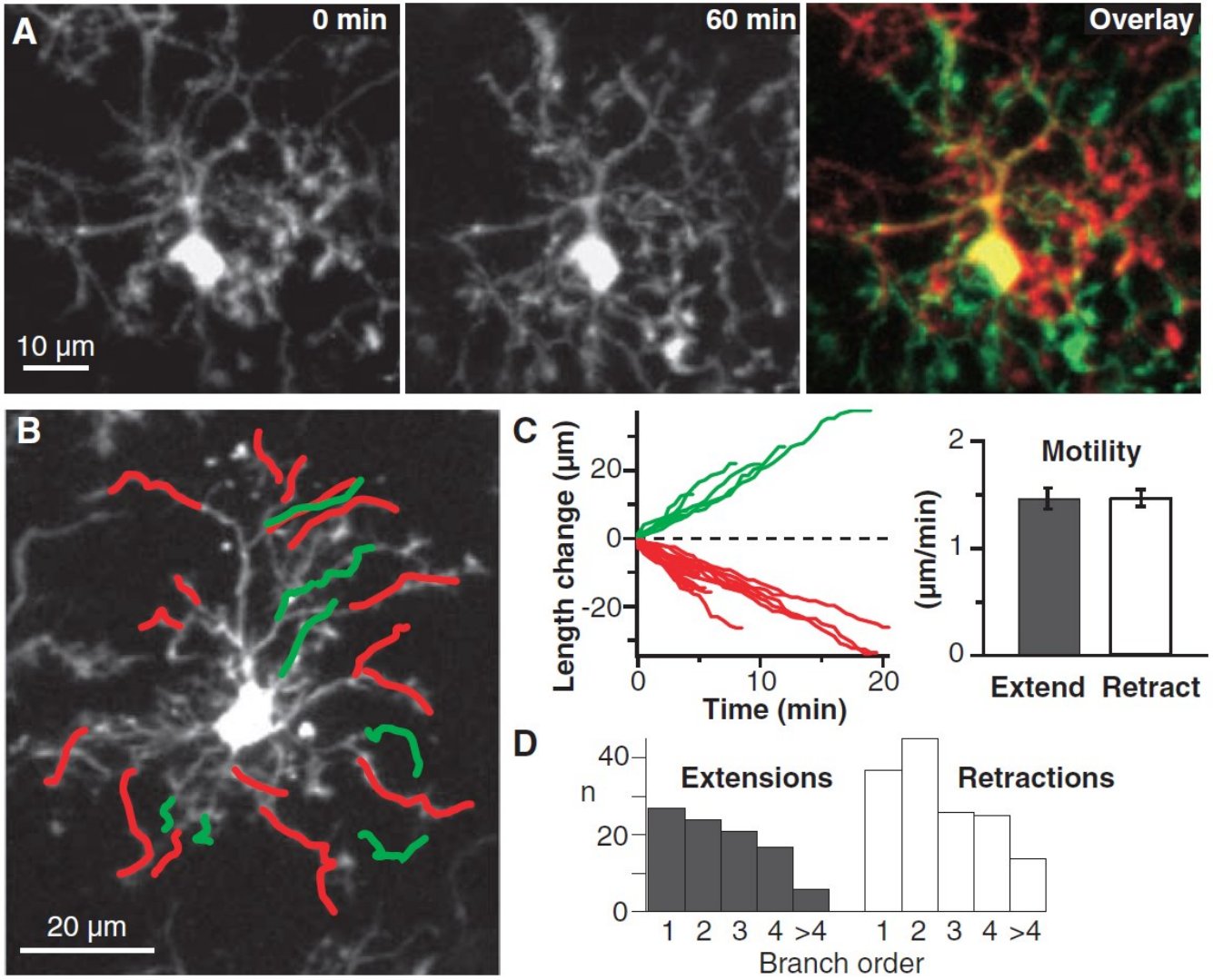
Gardeners of neural forests?

- *Microglia respond rapidly to brain tissue injury, guided by DAMP chemotactic gradients (e.g. ATP).*





Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci (2005) 8(6):752-8. Epub 2005/05/17. doi: 10.1038/nn1472. PubMed PMID: 15895084.tel der Präsentation ODER des Vortragenden

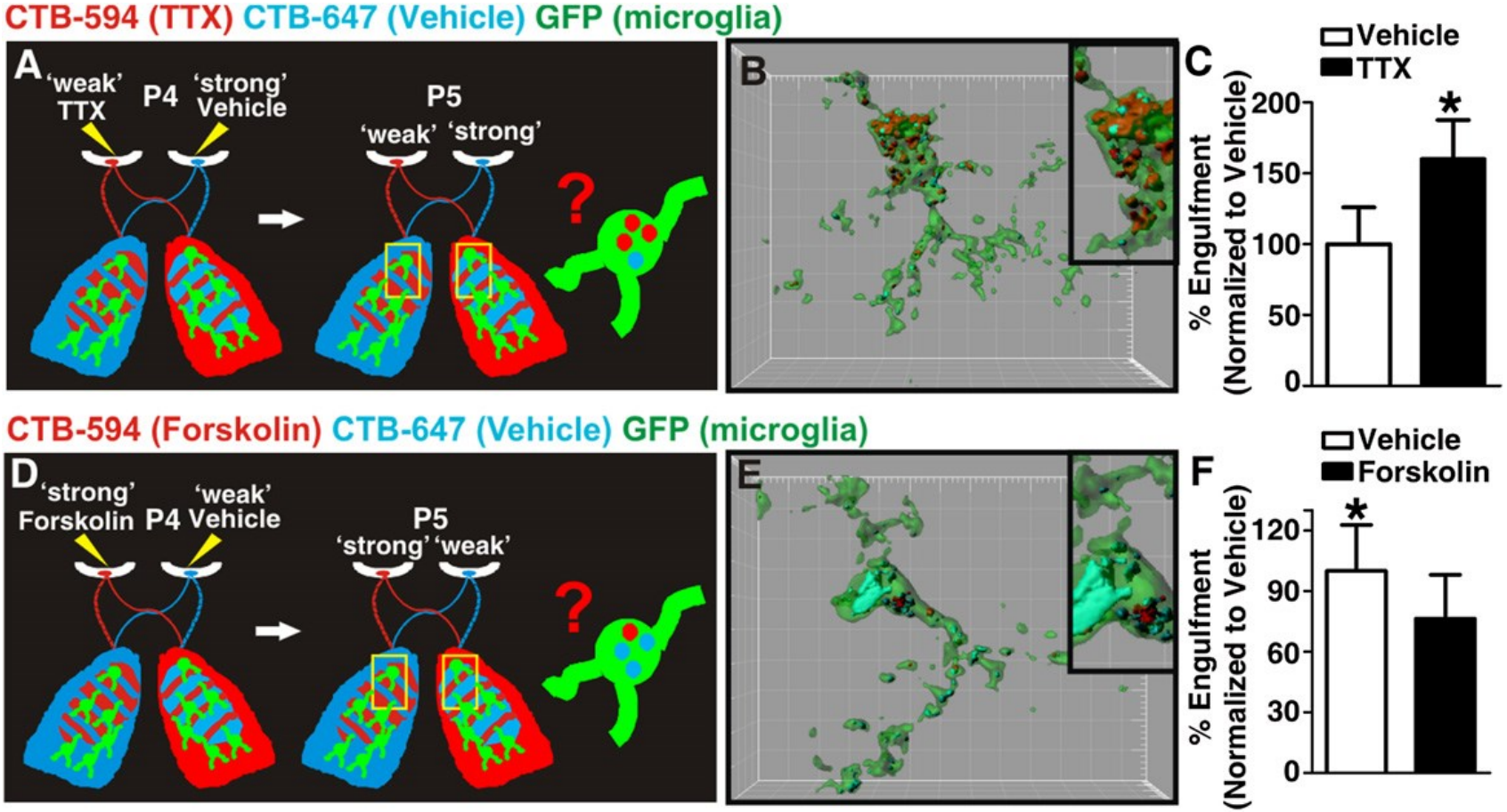


Even in the absence of injury microglia constantly survey their microenvironment

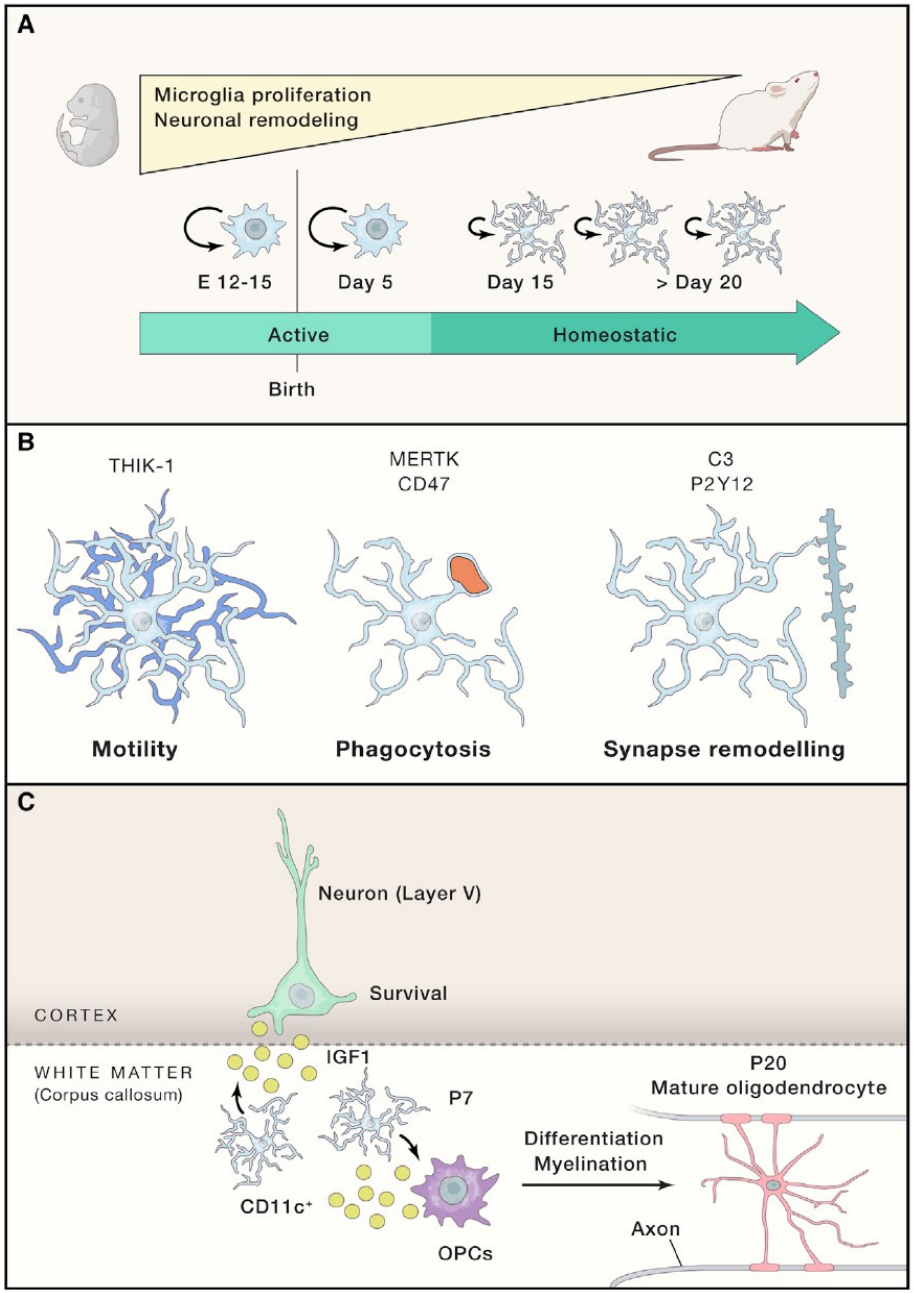
Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science (2005) 308(5726):1314-8. Epub 2005/04/16. doi: 10.1126/science.1110647. PubMed PMID: 15831717.

The young microglia in the young brain – Gardeners of neural forests?

Beyond injury induced phagocytic activity microglia sculpt postnatal neural circuits in an activity and complement dependent manner



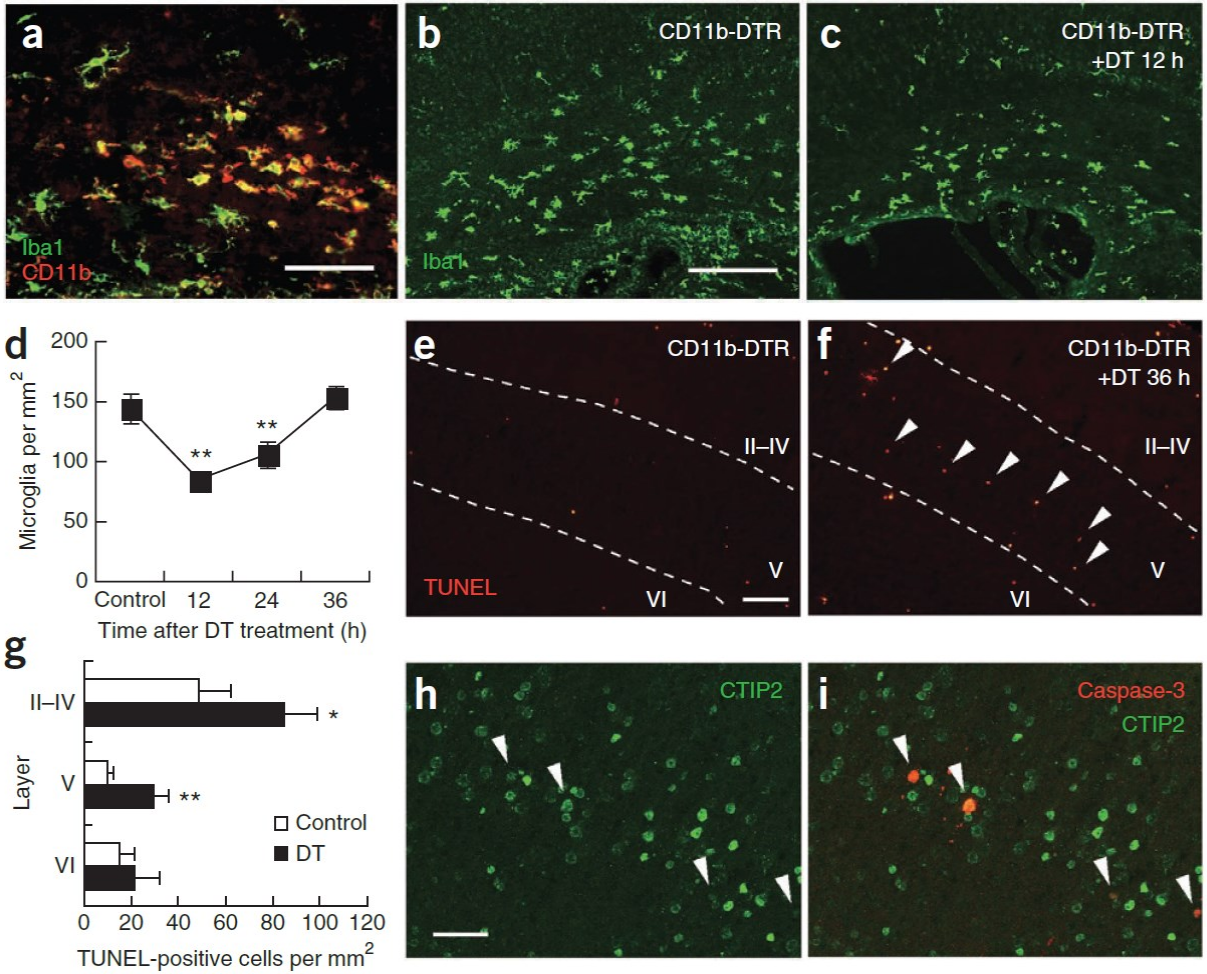
Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron (2012) 74(4):691-705. Epub 2012/05/29. doi: 10.1016/j.neuron.2012.03.026. PubMed PMID: 22632727; PubMed Central PMCID: PMCPmc3528177.

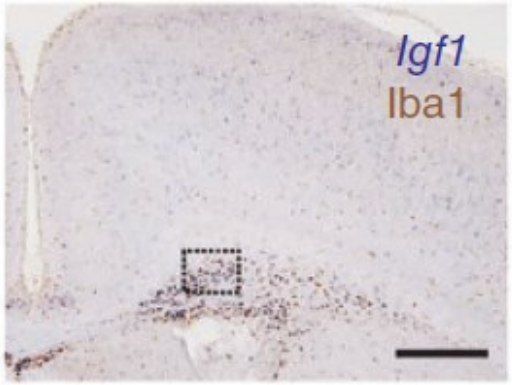


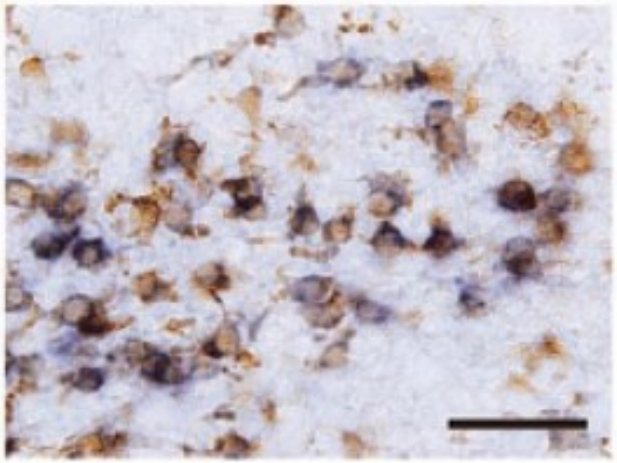
To summarize so far: *Microglia populate the CNS early, expand rapidly (to 10% of all brain cells), shape it activley and remain in a state of dynamic homeostasis.*

Microglia appear to produce various trophic factors, necessary for neuronal survival and OLG differentiation and axon myelination

Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell* (2019) 179(2):292-311. Epub 2019/10/05. doi: 10.1016/j.cell.2019.08.053. PubMed PMID: 31585077.



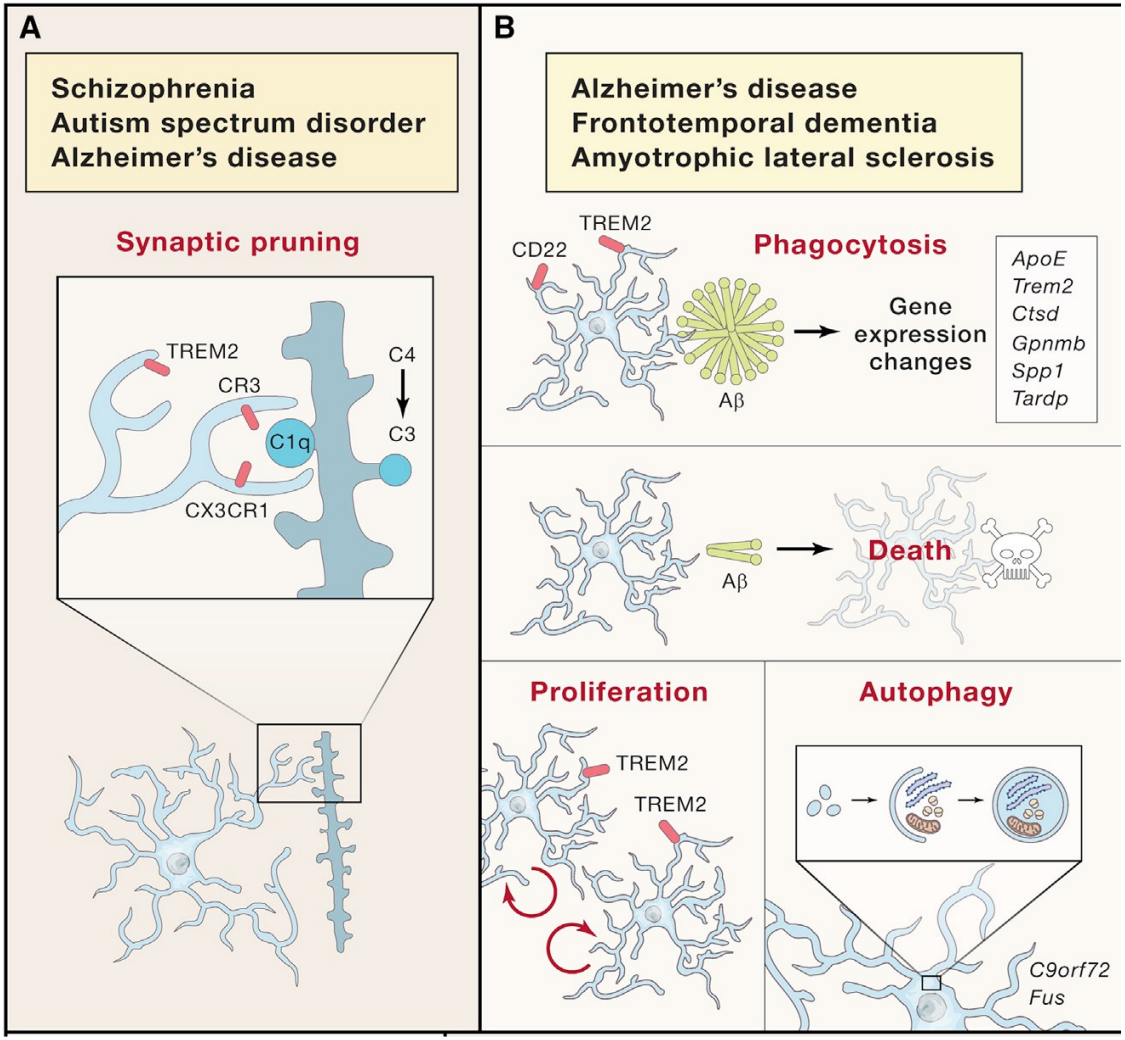




- *Selective, transient depletion of microglial cells induces neuronal apoptosis particularly in layer V interneurons*
- *Microglia adjacent to layer V neurons produce Igf1 which acts as a local trophic factor*

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, et al. Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci (2013) 16(5):543-51. Epub 2013/03/26. doi: 10.1038/nn.3358. PubMed PMID: 23525041.

III Microglia as responders to and inducers of CNS injury



A: Excessive or otherwise dysregulated phagocytosis of synapses (blue) and neurons involving receptors for complement, fractalkine, and purines may contribute to neurological and psychiatric diseases.

B: Microglia dynamically respond to extracellular protein aggregates – hallmarks of neurodegeneration

Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell* (2019) 179(2):292-311. Epub 2019/10/05. doi: 10.1016/j.cell.2019.08.053. PubMed PMID: 31585077.

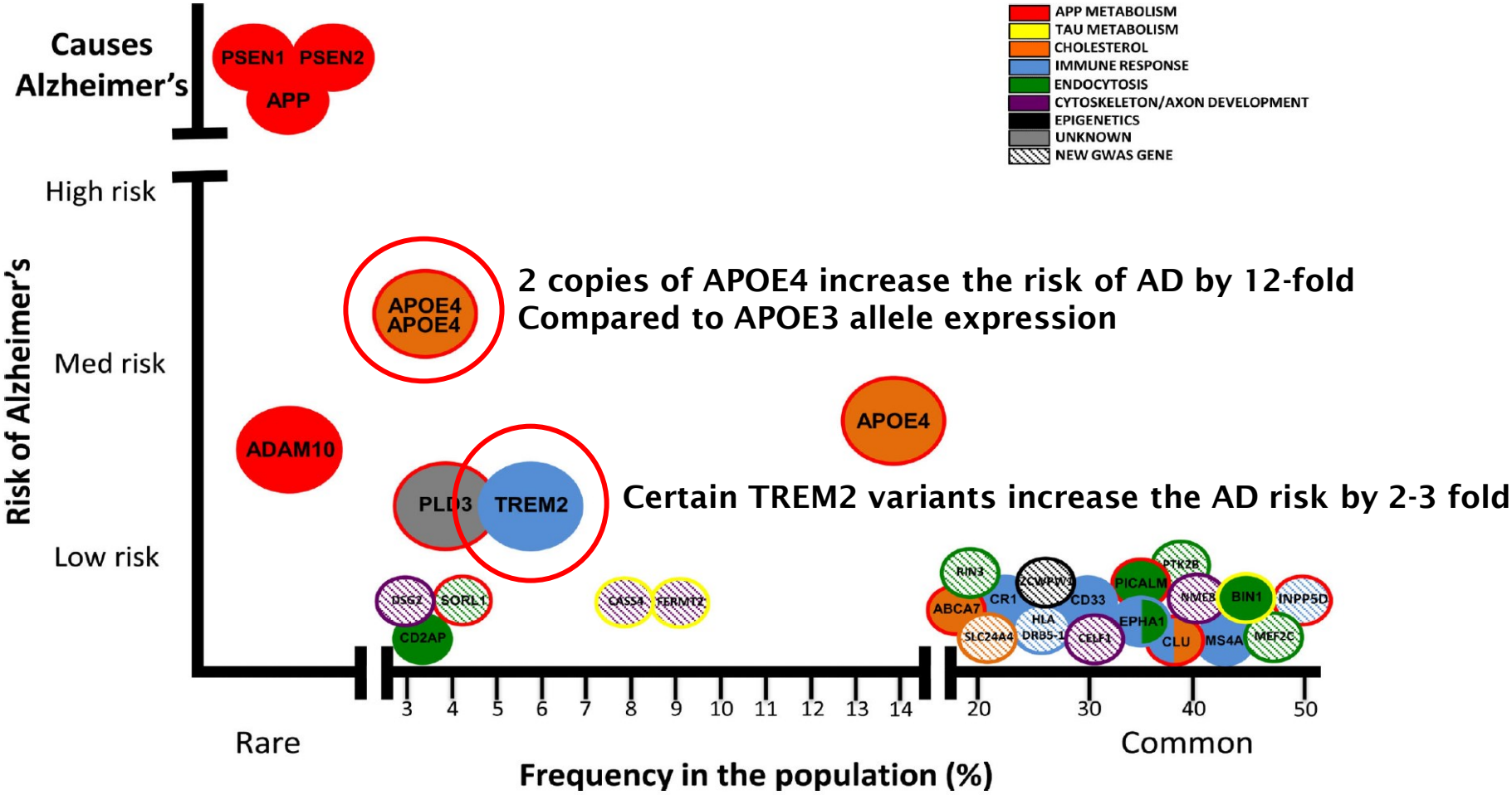
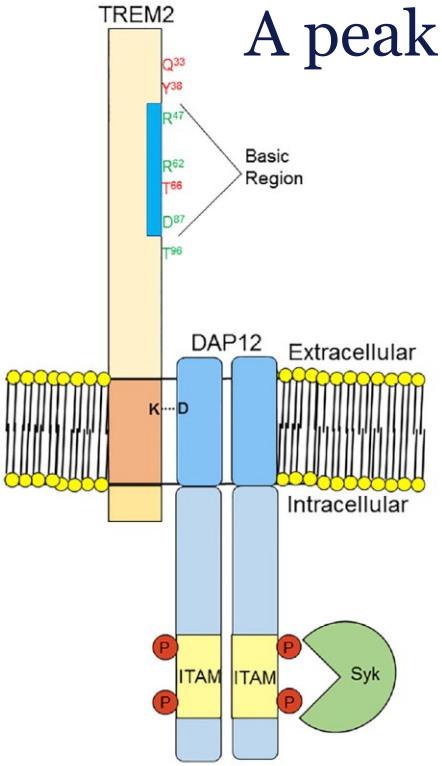


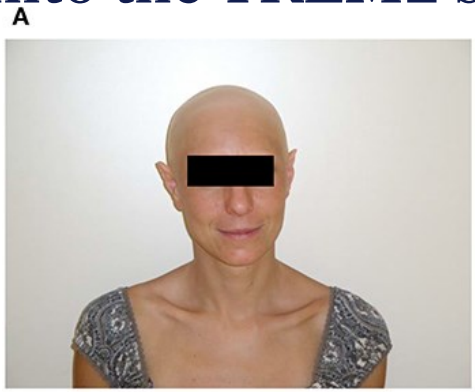
Figure 1. Rare and common variants contribute to Alzheimer's disease risk
Figure updated and modified from (149).

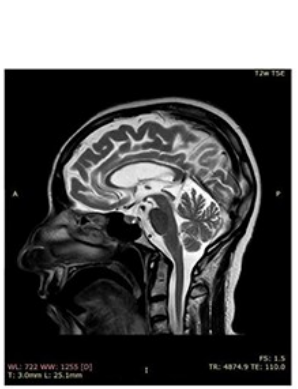
Almost half of the low risk but highly frequent risk alleles associated to late onset AD are enriched in, or related to myeloid cells

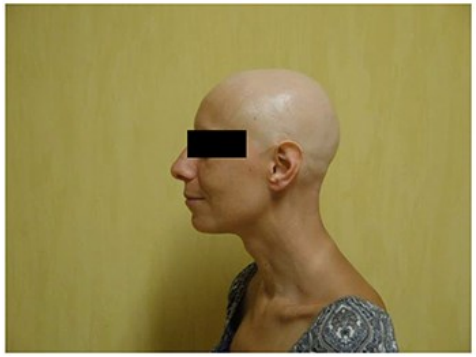
Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry (2015) 77(1):43-51. Epub 2014/06/22. doi: 10.1016/j.biopsych.2014.05.006. PubMed PMID: 24951455; PubMed Central PMCID: PMCPmc4234692

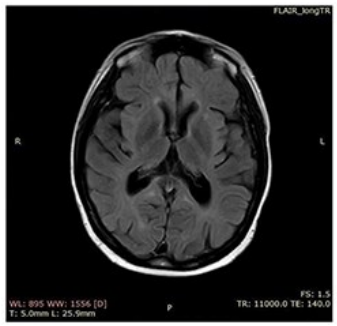
A peak into the TREM2 story

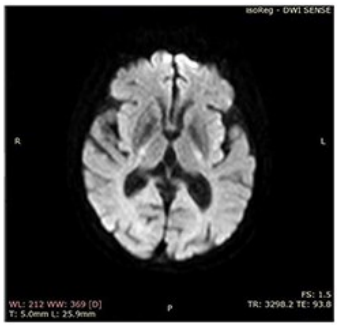












-> V-type immunoglobulin (Ig) domain-containing transmembrane protein - **expressed in many mononuclear phagocytes**, *hitherto unclear ligand receptor interactions.*

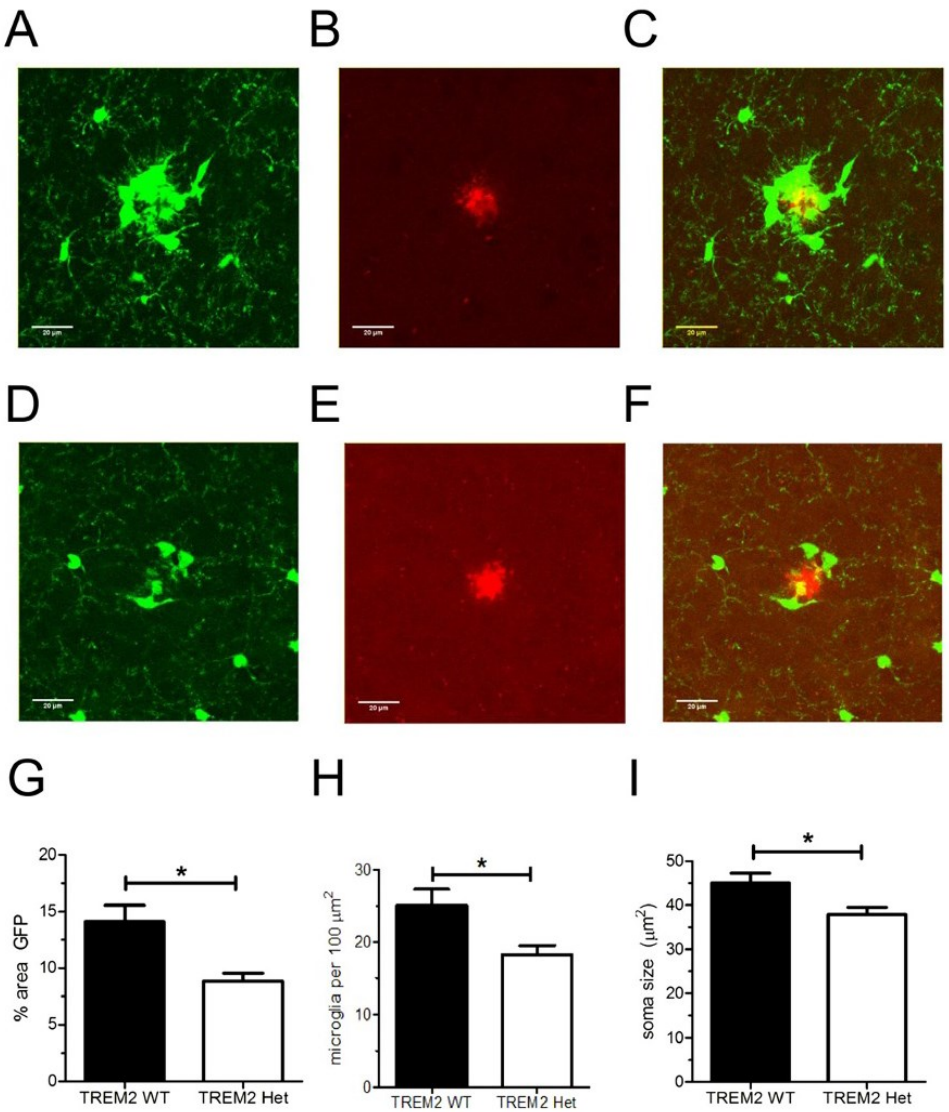
-> The basic **extracellular patch associates with anionic ligands**, *including moieties found on apolipoproteins, such as* **APOE**

-> Signaling through the adaptor protein **DAP12 -> ITAM -> Syk**

-> Loss of function mutations *of the genes encoding* **TREM2 and DAP12 lead to P***olycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy (PLOSL)* **= Nasu-Hakola disease**

Errichiello E, Dardiotis E, Mannino F, Paloneva J, Mattina T, Zuffardi O. Phenotypic Expansion in Nasu-Hakola Disease: Immunological Findings in Three Patients and Proposal of a Unifying Pathogenic Hypothesis. Front Immunol (2019) 10(1685). doi: 10.3389/fimmu.2019.01685.

Many of the TREM2 associated risk allels are associated to a decrease in TREM2 signaling efficiency in microglial cells

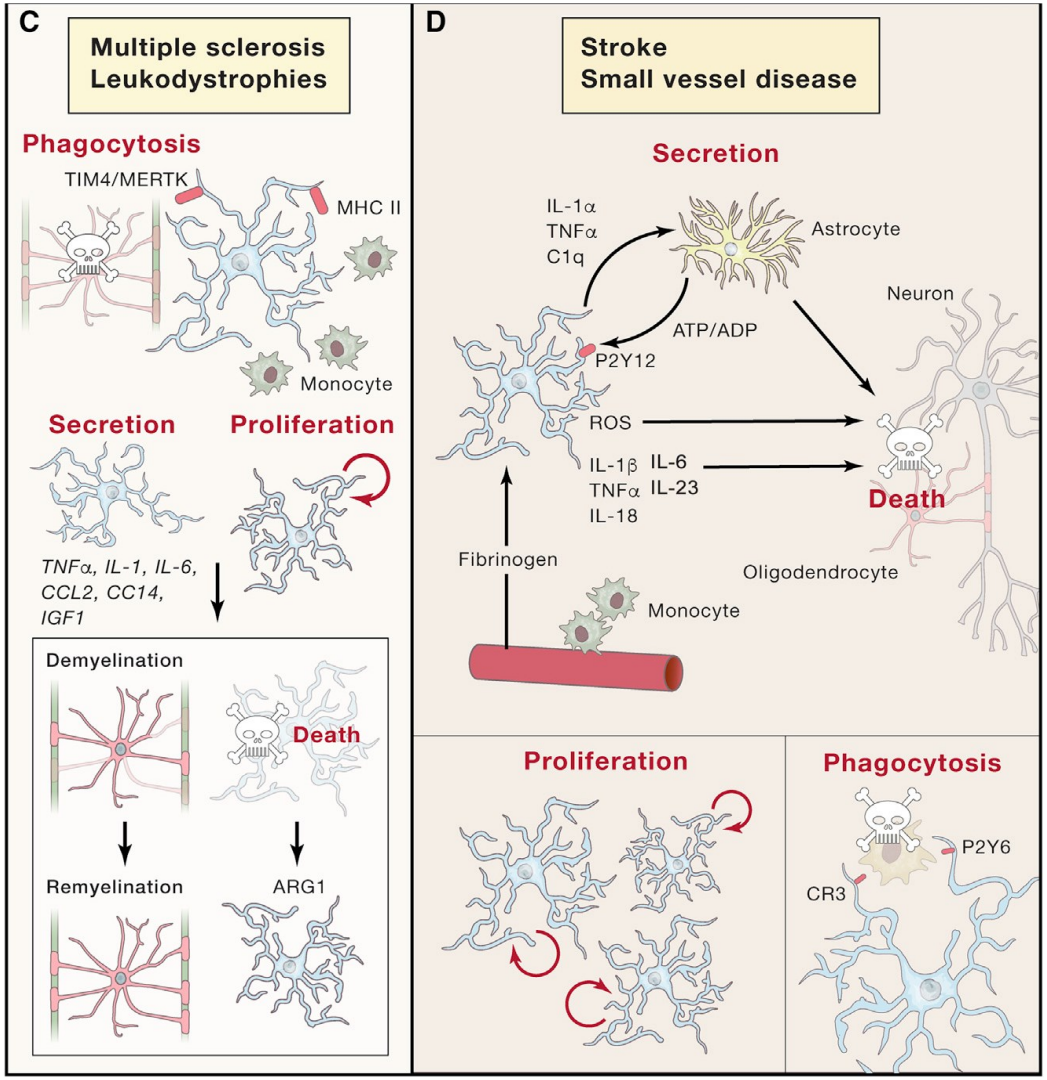


Even a partial knockdown of TREM2 decreased plaque-associated microglia in a APPPS1-21 mouse model of AD

-> These findings where replicated Independently in full TREM2 KOs

-> Moreover the decreased coverage of plaques by microglia was associated to increased neuronal dystrophy and p-tau accumulation.

Ulrich JD, Finn MB, Wang Y, Shen A, Mahan TE, Jiang H, et al. Altered microglial response to Aβ plaques in APPPS1-21 mice heterozygous for TREM2. Mol Neurodegener (2014) 9:20. Epub 2014/06/05. doi: 10.1186/1750-1326-9-20. PubMed PMID: 24893973; PubMed Central PMCID: PMCPmc4049806.



„Classic“ proinflammatory reactive microglia in detrimental neuroinflammation are associated to the release of cytokines like IL-1b, TNF-a, IL-18, IL-6, or IL-23, reactive oxygen species (ROS) and nitrogen species.

Microglia can also acquire a pro-regenerative phenotype and express arginase 1 (ARG1) and IGF1.

Disease-associated microglial phenotypes with specific transcriptomic signatures can be detected in AD and MS brains

Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell* (2019) 179(2):292-311. Epub 2019/10/05. doi: 10.1016/j.cell.2019.08.053. PubMed PMID: 31585077. *tel der Präsentation ODER des Vortragenden*

-> Recognition, phagosome formation and ingestion are a tightly regulated process.

→ Phosphatidylserine (PS) receptors such as Scavenger Receptors (CD36, SRA-1, etc), αvβ3 integrins, MerTK, TIM-1 and 4, BAI1, and Stabilin1 and 2 play vital sometimes non overlapping roles.

→ Loss of function of these receptors critically deregulates phago- and efferocytosis in microglia

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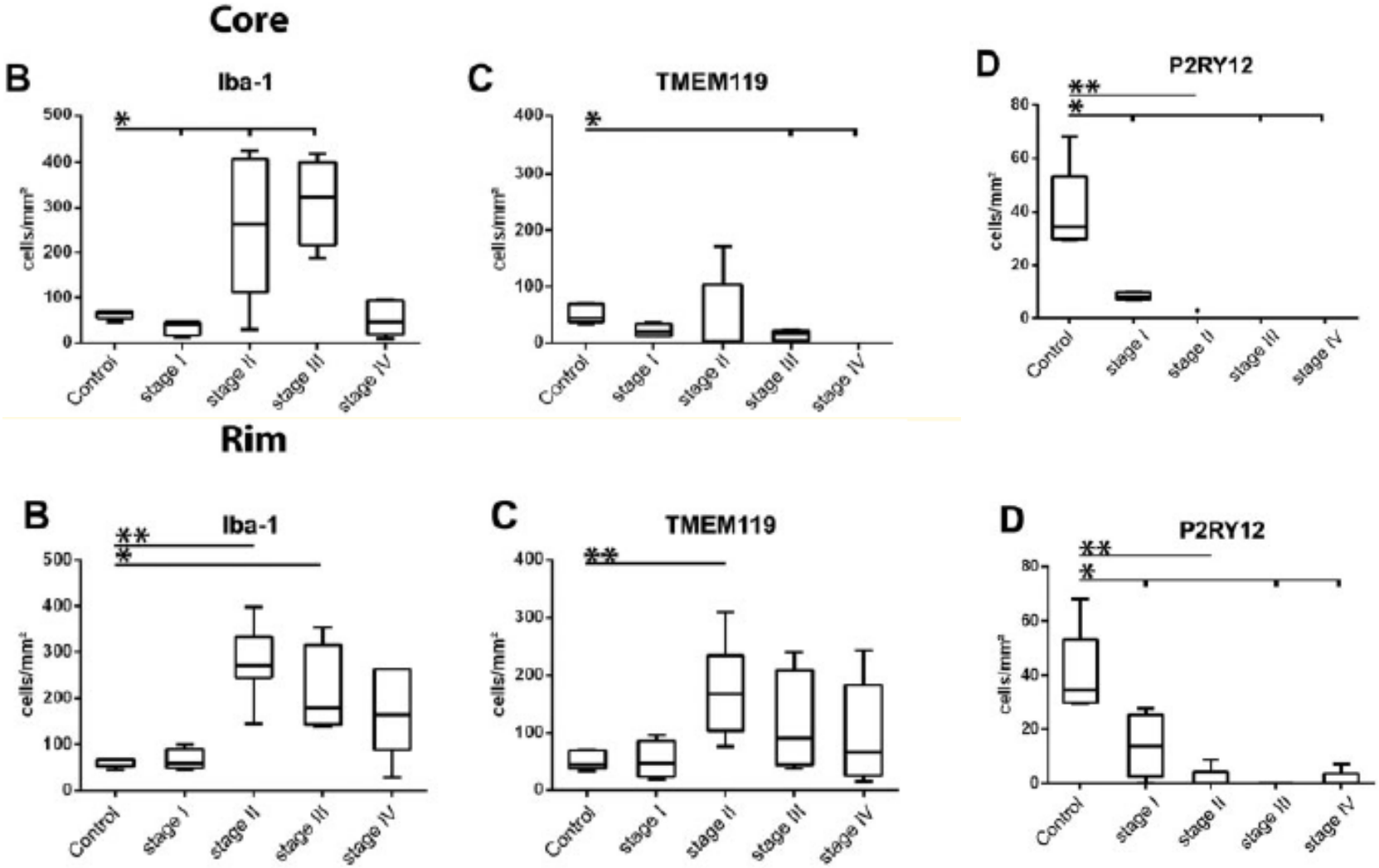
BRAIN
A JOURNAL OF NEUROLOGY

Acute and non-resolving inflammation associate with oxidative injury after human spinal cord injury

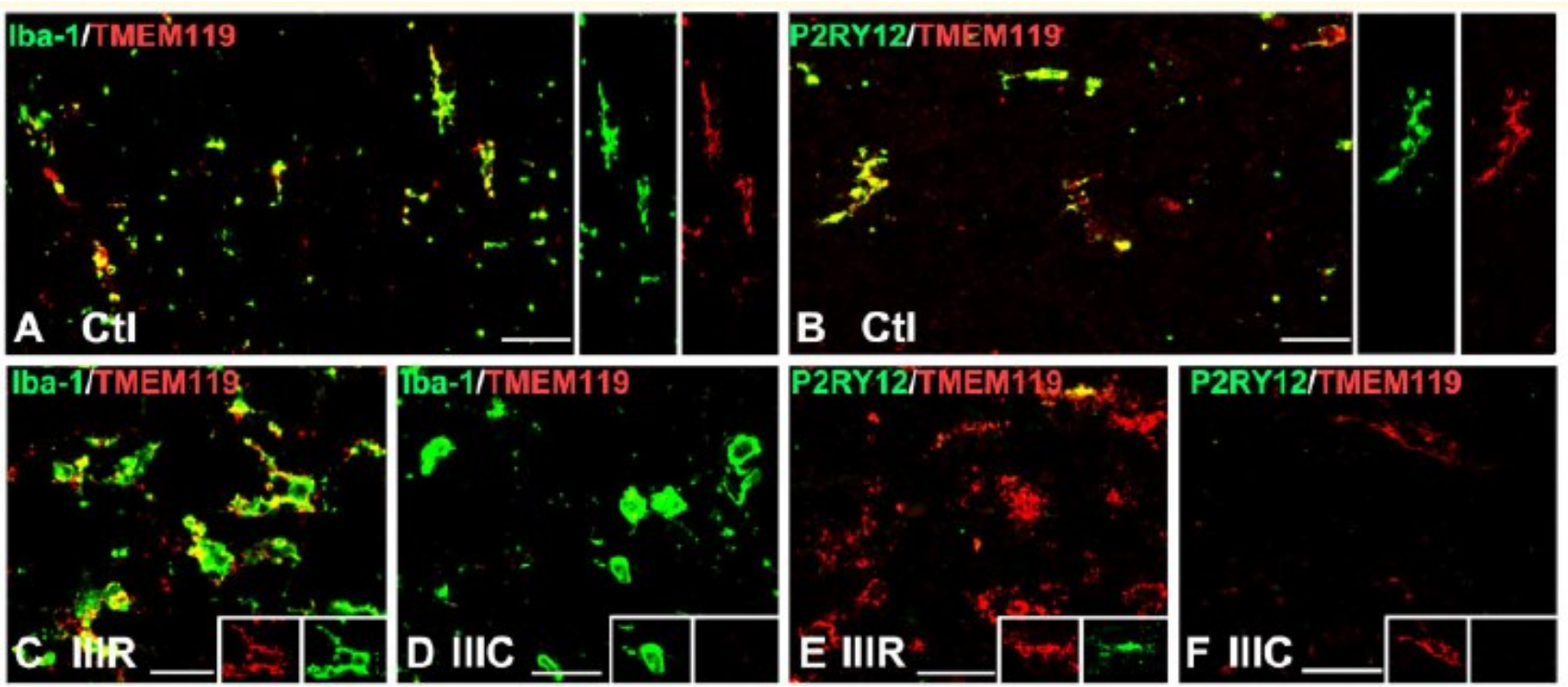
Tobias Zrzavy,[1] Carmen Schwaiger,[2] Isabella Wimmer,[1] Thomas Berger,[1] Jan Bauer,[3] Oleg Butovsky,[4,5] Jan M. Schwab,[6,7,8,9] Hans Lassmann[3] and Romana Höftberger[2]

Traumatic spinal cord injury is a devastating insult followed by progressive cord atrophy and neurodegeneration. Dysregulated or non-resolving inflammatory processes can disturb neuronal homeostasis and drive neurodegeneration. Here, we provide an in-depth characterization of innate and adaptive inflammatory responses as well as oxidative tissue injury in human traumatic spinal cord injury lesions compared to non-traumatic control cords. In the lesion core, microglia were rapidly lost while intermediate (co-expressing pro- as well as anti-inflammatory molecules) blood-borne macrophages dominated. In contrast, in the surrounding rim, TMEM119$^+$ microglia numbers were maintained through local proliferation and demonstrated a predominantly pro-inflammatory phenotype. Lymphocyte numbers were low and mainly consisted of CD8$^+$ T cells. Only in a subpopulation of patients, CD138$^+$/IgG$^+$ plasma cells were detected, which could serve as candidate cellular sources for a developing humoral immunity. Oxidative neuronal cell body and axonal injury was visualized by intracellular accumulation of amyloid precursor protein (APP) and oxidized phospholipids (e06) and occurred early within the lesion core and declined over time. In contrast, within the surrounding rim, pronounced APP$^+$/e06$^+$ axon-dendritic injury of neurons was detected, which remained significantly elevated up to months/years, thus providing mechanistic evidence for ongoing neuronal damage long after initial trauma. Dynamic and sustained neurotoxicity after human spinal cord injury might be a substantial contributor to (i) an impaired response to rehabilitation; (ii) overall failure of recovery; or (iii) late loss of recovered function (neuro-worsening/degeneration).



Traumatic SCI induces a prompt and lasting downregulation of the homeostatic microglial marker P2RY12. Microglia (TMEM119 +) are maintained for a prolonged period in the lesion rim

All following figures from:Zrzavy T, Schwaiger C, Wimmer I, Berger T, Bauer J, Butovsky O, et al. Acute and non-resolving inflammation associate with oxidative injury after human spinal cord injury. Brain (2021) 144(1):144-61. Epub 2021/02/13. doi: 10.1093/brain/awaa360. PubMed PMID: 33578421; PubMed Central PMCID: PMCPmc7880675



(A, B)Controls -> Non lesioned SC: Iba-1/TMEM119 +/+ and P2RY12/TMEM119 +/+ homeostatic microglia

(C) The rim of the lesions contains high numbers of Iba-1 (green) TMEM119 (red) double-positive cells, while (D) TMEM119+ (red) cells are massively reduced in the lesion core.

(E,F): Expression of the homeostatic microglia signature marker P2RY12 is fainting in the lesion rim and lost in the lesion core

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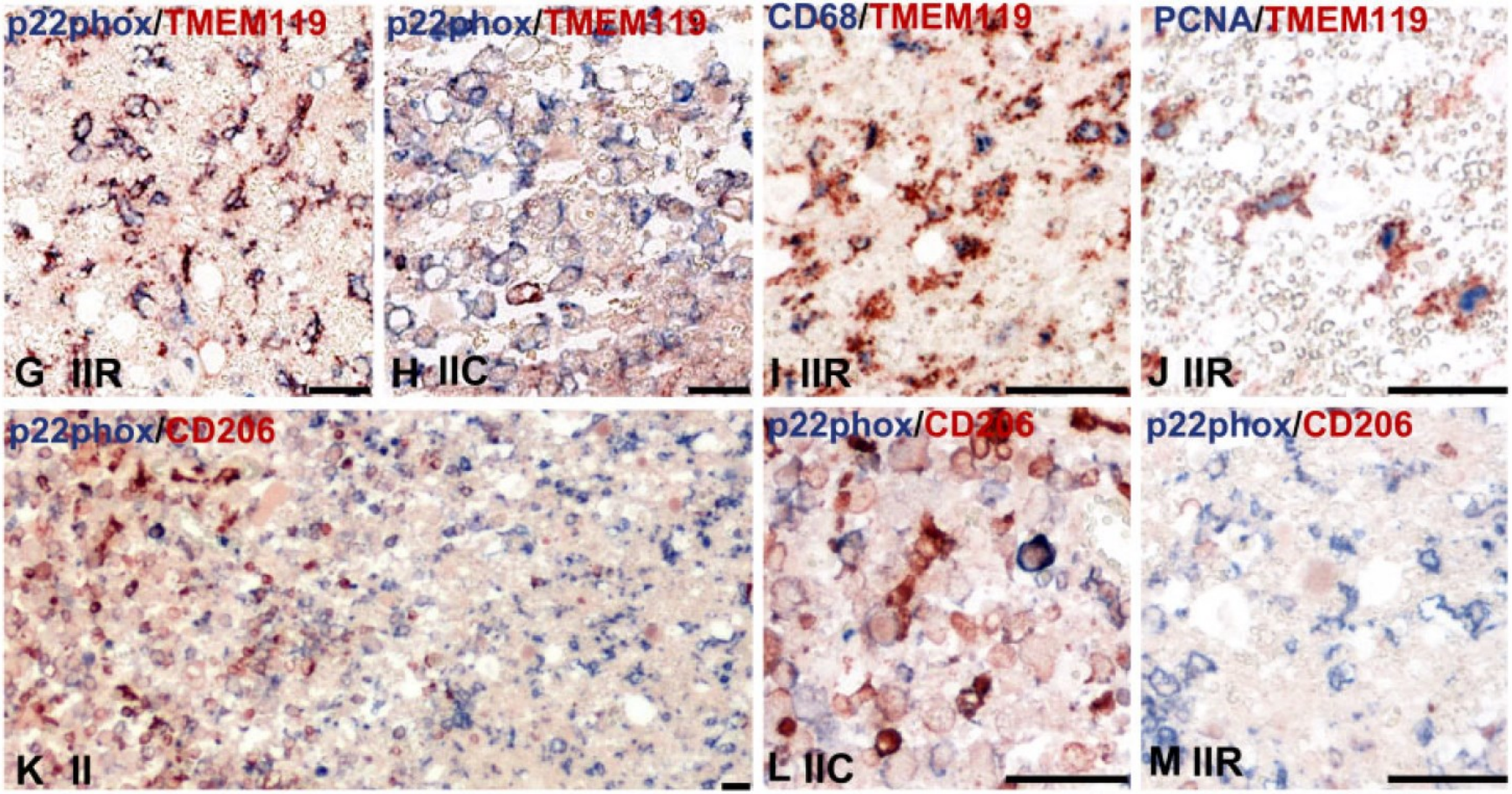
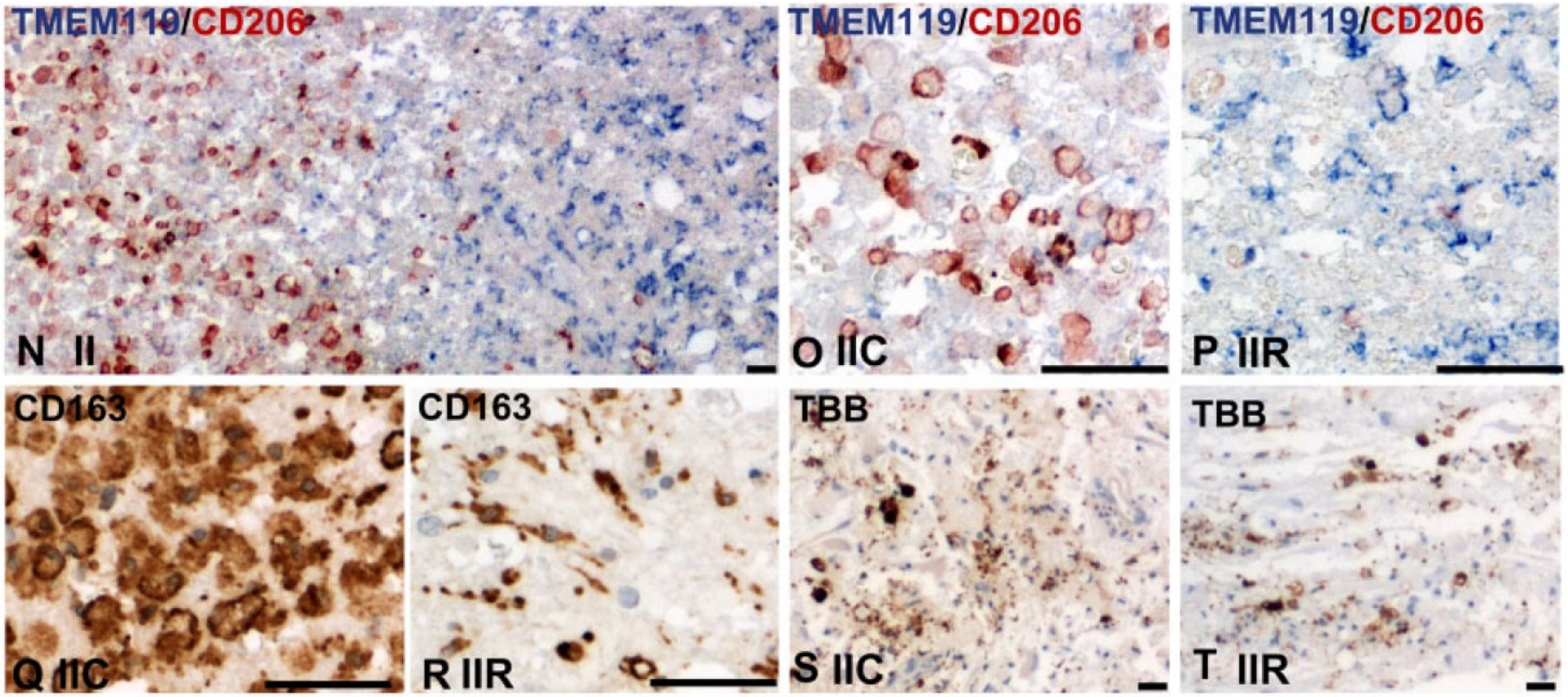


Upper row: In the **lesion rim (R) of SCI:** The majority of microglia (TMEM119 +) coexpresses (G) p22phox (ROS-marker)(blue), (I) the phagocytosis-associated marker CD68 (blue) and (J) proliferating cell nuclear antigen PCNA

Lower row: **(K)** Core (left side): Coexisitance of ROS and anti-inflamm. markers Rim (right side) : Comparatively rim is dominated by ROS markers (L,M: close up)



N: The lesion rim expresses close to no CD206 + (red) Microglia (TMEM119 blue) (O,P: higher magnification)

In the core of stage II lesions (Q), numerous CD163+ macrophages are present; similarly, CD163+ microglia are observed in the rim (R).

Low numbers of TBB+ cells were found in the core (S) as well as in the lesion rim (T)

To summarize:

-> The physiological function of microglia is not restricted to mere phagocytic and cytokine mediated pathogen or damage response

-> Morevocer, microglia appear to fulfill vital functions in maintaining the architecture and intergrity of neural circuits, through involvments in:

-> Synaptic pruning

-> Clearence of apoptotic cells and cell fragments and extracellular aggregates

-> The release of trophic factors

-> ...

-> Microglia are able to entertain long lasting changes of the local microenvironment along various CNS lesions

-> The critical involvment of microglia in evermore neurological and psychiatric diseases emerges, as do potential novel therapeutic targets in microglia biology.

IV Discussion and References

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