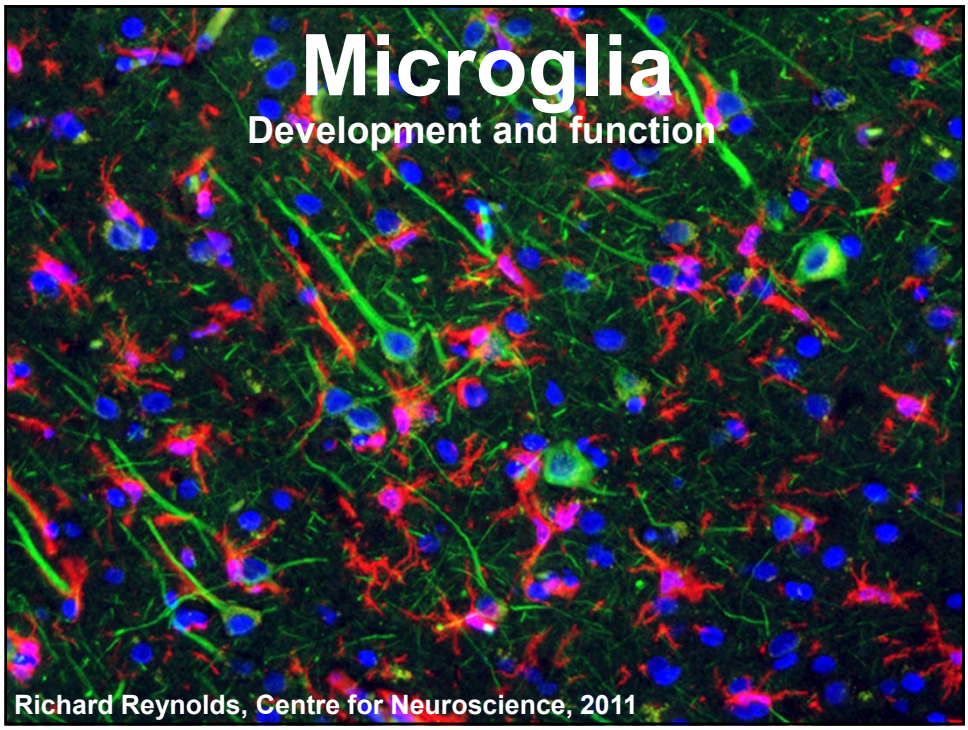
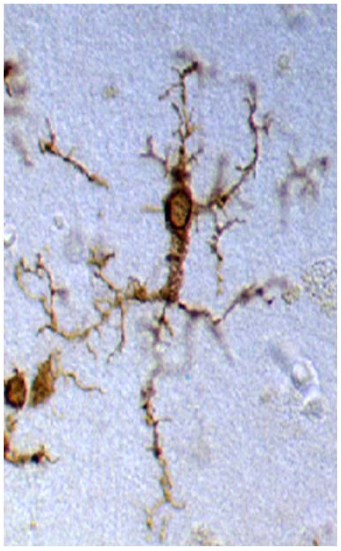


Today's lecture

- Origin of microglia
- Microglial function
- Microglia in neurodegeneration

Microglia

- Resident mononuclear phagocytes (tissue macrophages) of the CNS
- Distributed ubiquitously throughout CNS accounting for 15% of brain cells
- Share many of the properties of macrophages in other tissues
- In the normal adult brain they possess a highly ramified morphology
- General maintenance – remove cellular debris
- Provide trophic support for neurons - neuroprotection
- Primary defense of CNS - first cells to respond to injury or infection



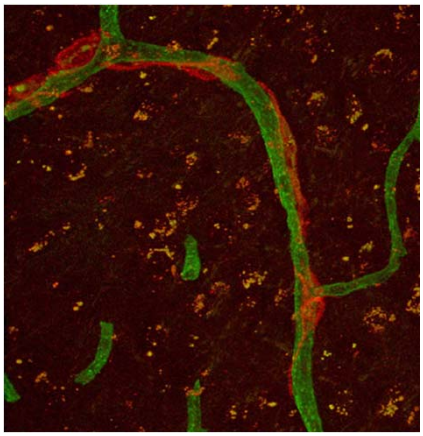
CNS macrophages

- Ramified microglia: Immune surveillance of the brain parenchyma

- Perivascular macrophage: Surveillance of the perivascular space between parenchyma and blood

- Meningeal macrophage: Surveillance of the sub-arachnoid space

- Choroid plexus macrophage: The site of immune cell entry and CSF production in the ventricles

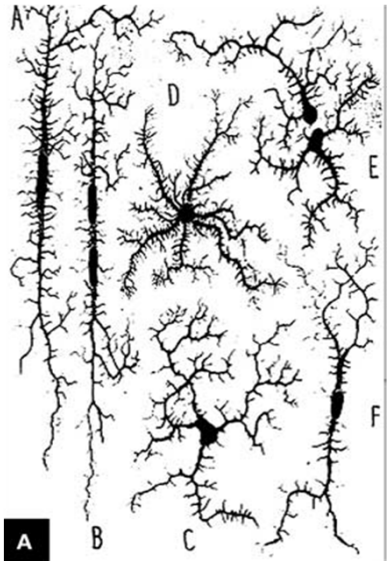


Origins of Microglia

Pio del Rio-Hortega
1882-1945
“The Father of Microglia”

"The microglia or 'mesoglia' is of mesodermal (meningeal) origin, possesses liberal ramified expansions and displays migratory and phagocytic activity. It is more abundant in grey matter than in white, and is found in the general neuroglia-neuronal framework as an annexed element. By reason of its difference in characteristics and origin from nerve cells (first element) and neuroglia (second element), the microglia constitute the true 'third element of the CNS' and it is necessary to separate in all descriptions, microglia from the classical neuroglia, to avoid confusion.”

Normal human cortex

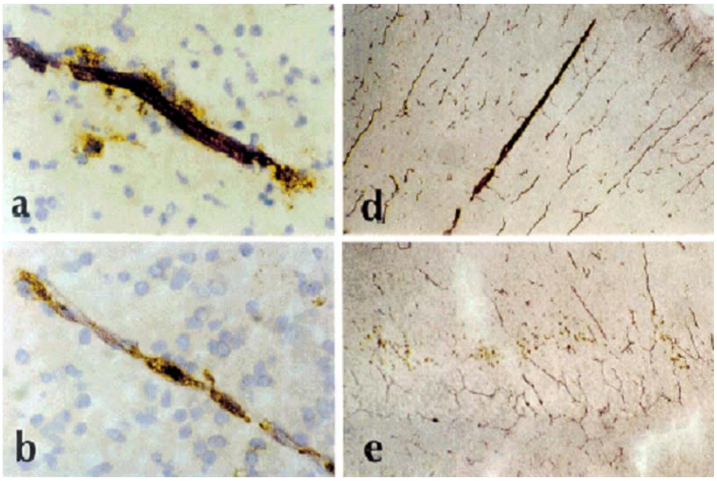


Diseased human cortex

Appearance of microglia coincides with vascularisation in human fetal brain

Cortical vessels

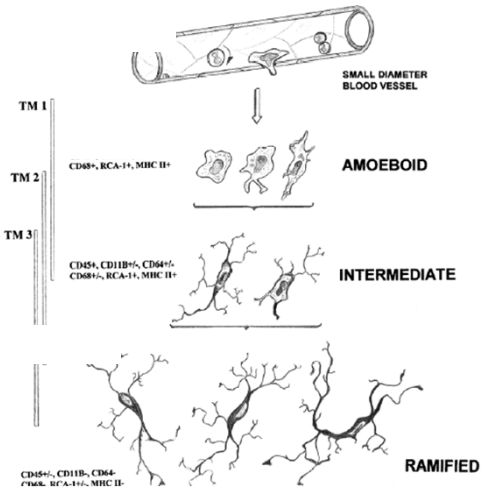
Ventricular zone

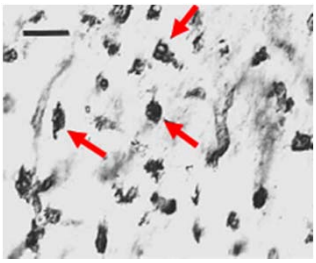


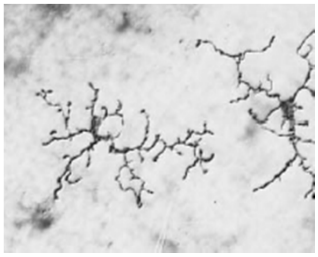
(Rezaie and Male 1999)

Stages of Microglial Development

At least two subpopulations of microglia can be identified during prenatal and early postnatal development. These subpopulations may be discriminated based on heterogeneity in (i) phenotype, (ii) morphological characteristics and (iii) with respect to their distribution within the developing CNS.





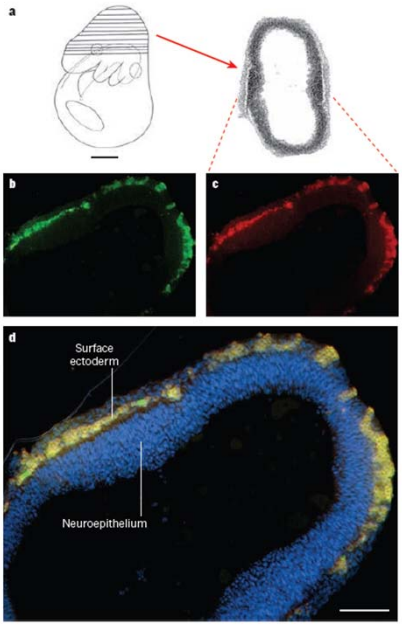


Derived from differing progenitor pools during early and late embryogenesis

Microglia in development

Microglial have a myeloid origin

- differentiation of myeloid lineage cells, including macrophages, fails in mice with a deletion of the transcription factor PU.1
- no microglia are detected in the CNS of PU.1 null mice
- myeloid progenitors of the yolk sac are the source of the embryonic wave of myeloid cells that colonize the CNS
- expansion of numbers occurs through in situ proliferation



Ransohoff & Cardona 2010

Microglial function during development

- crucial scavenger function
- phagocytose debris from naturally occurring cell death
- remove cells in remodelling of fetal brain
- eliminate axonal projections
- appearance correlates with programmed cell death during development
- immune surveillance

Microglia in the adult brain

More than 10% of all cells in the adult CNS are microglia

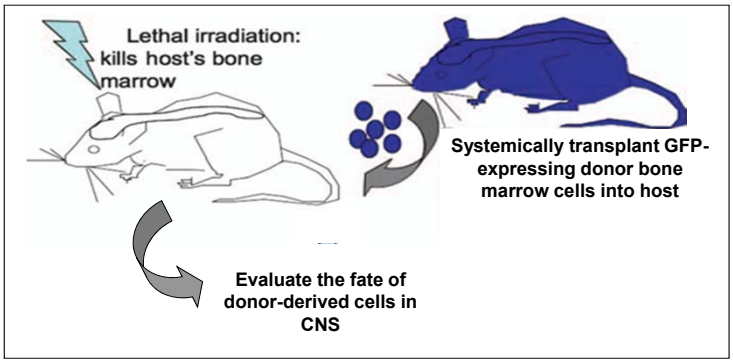
Morphological and phenotypically plastic cells of the CNS parenchyma:

- highly ramified resting morphology
- activated: shorter processes and enlarged soma
- amoeboid: rounded, 'classic phagocytic macrophage'

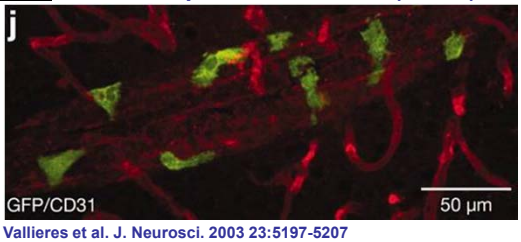
Source of microglia in adult healthy brain?

- proliferation of resident parenchymal microglia
- migration of bone marrow-derived progenitor cells into parenchyma via vasculature and meninges…only under certain conditions (?)

Chimeric studies

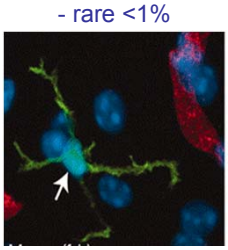


RESULTS: **Amoeboid perivascular cells (99.7%)**



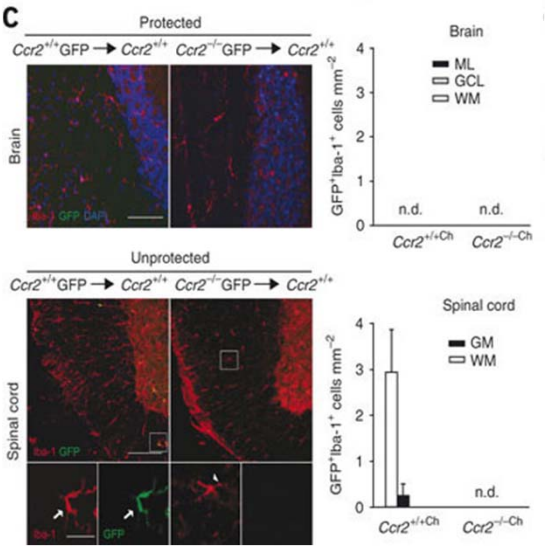
Vallieres et al. J. Neurosci. 2003 23:5197-5207

Ramified microglia
- rare <1%

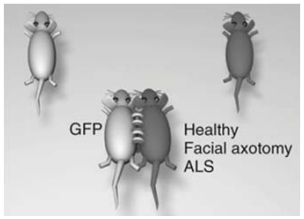


Other irradiation and transplantation studies failed to detect donor-derived microglia

Loss of fur colour due to prolonged irradiation. Shielded mice did not present with GFP-microglia in the brain but only in the spinal cord (unshielded)
Mildner et al, Nature Neuroscience **10**, 1544 - 1553 (2007)

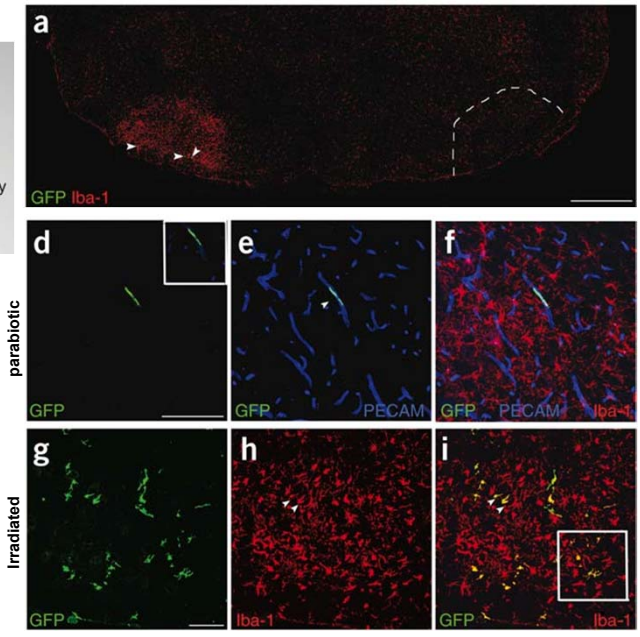


Parabiosis: The conjoining of two animals



Donor mouse with GFP-labelled bone marrow progenitors conjoined with GFP- mouse. Assess whether circulating GFP progenitors generate new microglia in GFP- mouse following nerve damage (facial nerve axotomy) or with disease (model of MND)

Ajami et al, *NatureNeuroscience* **10**, 1538 - 1543 (2007)



Source of microglia in adult healthy brain (no BBB disruption)

Bone marrow progenitors infiltrate the CNS and give rise to new microglia

Nearly all of the donor-derived microglia are located in or around blood vessels

Perivascular macrophages

Derived from bone marrow cells and do not result from resident microglial division

A few microglial-like cells observed in the brain parenchyma

Parenchymal microglia

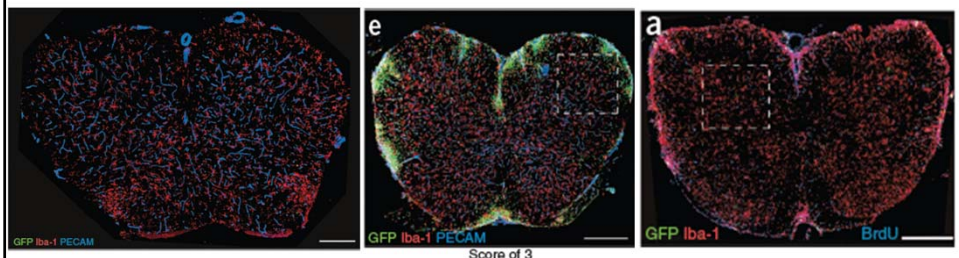
Not derived from bone marrow cells but from proliferation of endogenous microglial cells

Source of microglia in pathological brain (± BBB disruption)

New microglia derive predominantly from endogenous proliferation

Mildner et al (Nat Neurosci 10, 2007)
Circulating monocytes are preferentially recruited to the lesioned brain but only differentiate into microglia if the brain is first irradiated. Cuprizone induced demyelination – intact BBB.

Ajami et al (Nat Neurosci 14, 2011)
Blood born monocytes are recruited to the CNS during inflammation (EAE) but do not contribute to the pool of microglia. Disrupted BBB



Normal spinal cord | EAE disease | EAE recovery

Microglia – a distinct unique population?

Morphological observations:

- Ramified morphology – resting state

Bone marrow chimera studies:

- Parenchymal microglia - stable self-renewing population distinct from perivascular macrophages
- Rarely (if ever) replenished by bone marrow stem cells in contrast to perivascular macrophages

Molecular observations:

- Like other hematopoietic lineage cells microglia express CD45 (leukocyte common antigen)
- Differentiated hematopoietic lineage cells are CD11b+/CD45 high
  Parenchymal microglia are CD11b+/CD45 low
- Suggests a more stable physiology
- CD45 - useful marker to distinguish microglia from macrophages?

Microglial Function

- Surveillance
- Neuroprotection
- Immune response

Microglial Function - Surveillance

Monitor the extracellular environment

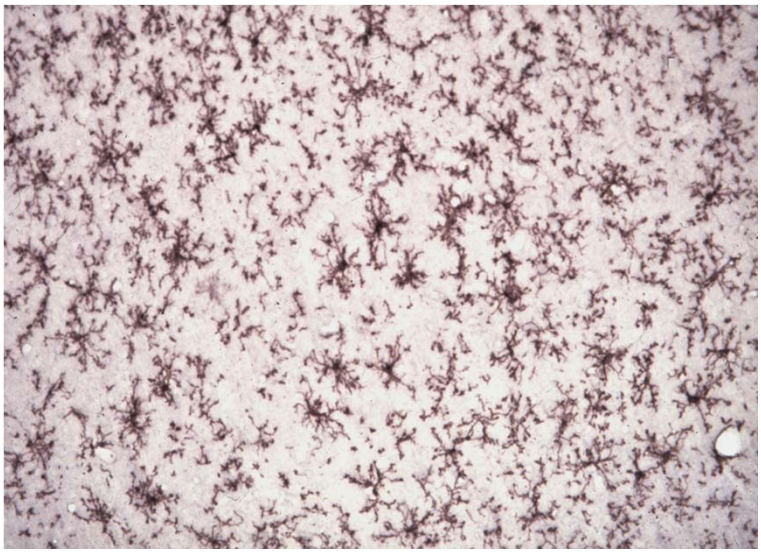
Sense homeostatic disturbances - changes in biochemical composition or structural organisation

Occupy own spatial territory

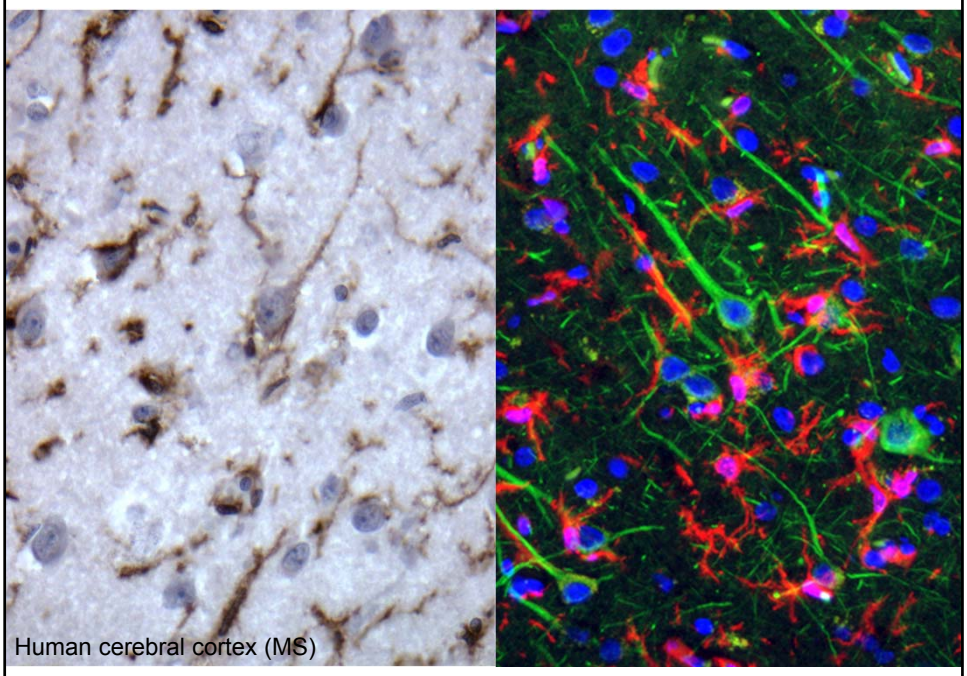
Interact with other cells - their processes directly contact neurons, astrocytes and blood vessels

Any time cells can undergo a transformation into an alerted activated or reactive state

Support endangered neurons or interfere with potential threats to tissue integrity

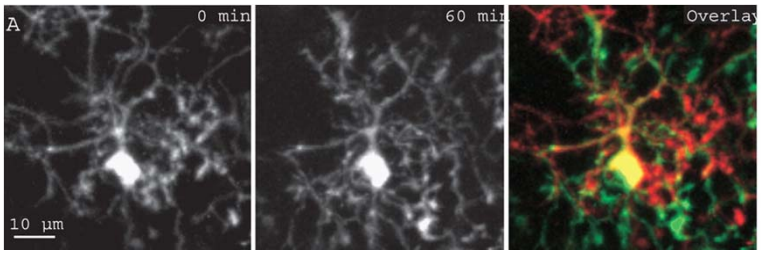


Microglia in the rat cerebral cortex



Resting microglial cells are highly dynamic *in vivo*

Trans-cranial two-photon laser scanning microscopy



formation (green) and deletion (red) of microglial processes

- somata of microglial cells generally remain fixed
- microglial processes are motile, continuously undergoing cycles of de novo formation and withdrawal
- these structural dynamics occur on a time scale of minutes, leading to extensive changes in cell morphology
- microglial processes sample the ECS in a random fashion

All videos available at http://www.sciencemag.org/cgi/content/full/sci;1110647/DC1
Nimmerjahn et al. 2005 Science 308: 1314 -1318

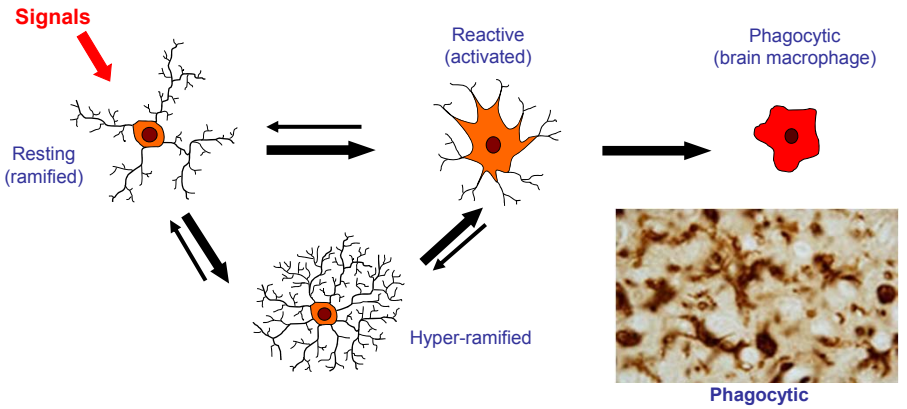
Nimmerjahn et al. 2005 Science 308: 1314 -1318

Microglial Function - Surveillance

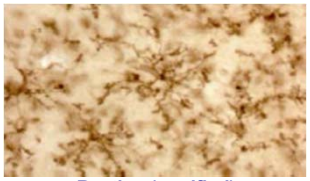
- Any time cells can undergo a transformation into an alerted activated or reactive state
- Support endangered neurons or interfere with potential threats to tissue integrity
- High motility allows microglia to perform a surveillance function by continuously sampling their environment
- They can effectively control the microenvironment and clear the parenchyma of accumulated metabolic products or deteriorated tissue components

Highly branched resting microglia provide the brain with a dynamic and efficient surveillance system

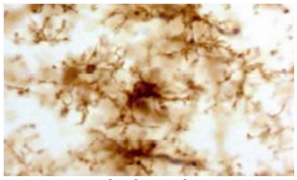
Functional plasticity of microglia



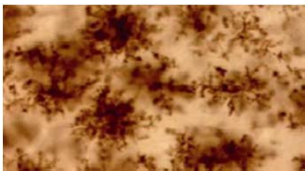
Phagocytic



Resting (ramified)

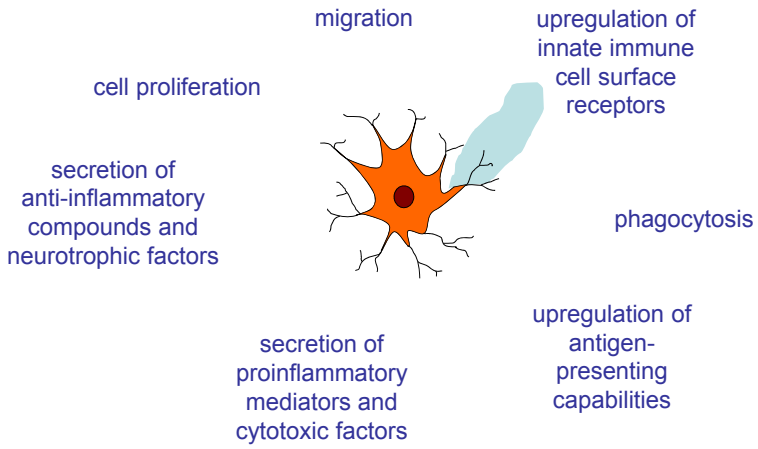


Activated

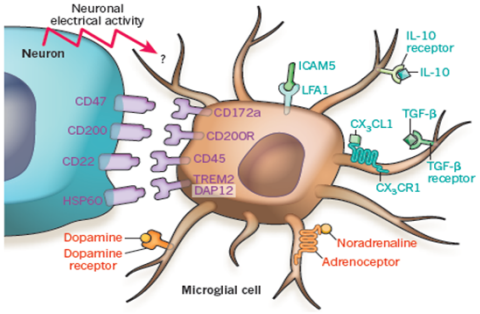


Hyper-ramified "bushy"

Activated microglia upregulate multiple functions and expression of molecular mediators



Neuronal - Microglial Interactions



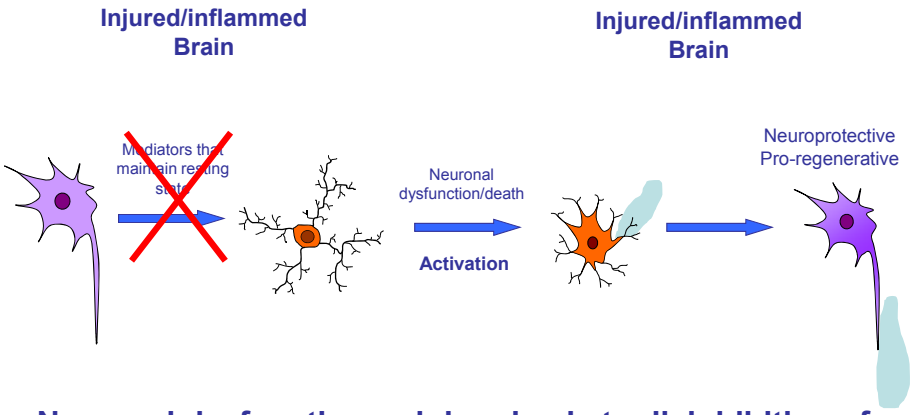
Neurons provide calming inputs to keep microglial cells in a quiescent state:

Interaction of the neuronal membrane protein CD200 with the myeloid cell receptor CD200R on microglia

High levels of fractalkine (CX3C family of chemokines) in normal neurons prevents MG activation - MG express CX3CR1)

Microglial expression of molecules for antigen presentation is inhibited by electrically active neurons

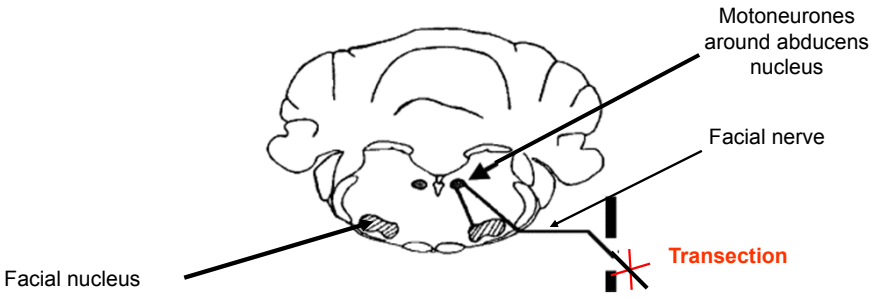
Microglial Function - Neuroprotection



Neuronal dysfunction or injury leads to disinhibition of microglial quiescence

Critical role of MG in neuroprotection – Rat facial axotomy model

Motor neurons innervate muscles that control whisker movement. Axon transection in the periphery results in rapid microgliosis in the facial nerve nucleus in the CNS



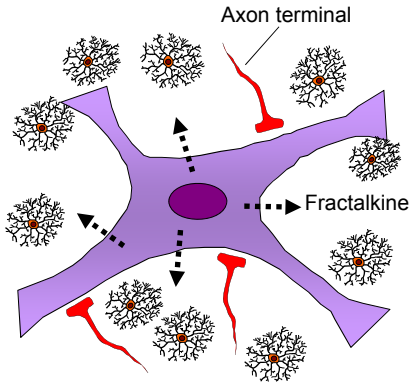
Neurons produce rescue signals that mediate microglial activation

Immediate changes

Axotomy of facial motoneurons in the periphery triggers an immediate microglial response in the facial nucleus

Microglial activation is initiated by fractalkine signalling or massive release of ATP from distressed neurons

Microglia proliferate as more cells required to meet increased trophic demand of injured neurons



Microglial respond to protect and regenerate neurons

Days after axotomy

Axotomised perikarya are surrounded by activated microglia

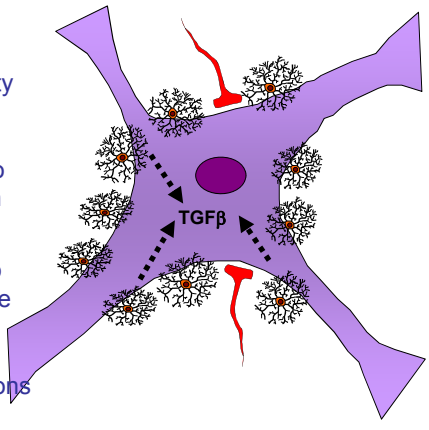
Microglia do not display phagocytotic activity (depends on extent of injury)

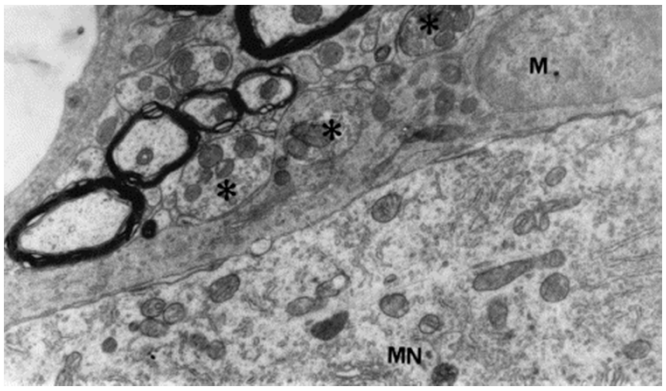
Microglia may release factors (eg TGFβ) to protect neurons and promote regeneration

Microglia displace axosomatic terminals so microglial and neuronal membranes become opposed

- Deafferentation protects axotomised neurons from afferent excitatory impulses

- Facilitates exchange of signalling or trophic molecules between them





Surface of an axotomised adult motoneuron with a microglia cell engaged in “synaptic stripping” ?

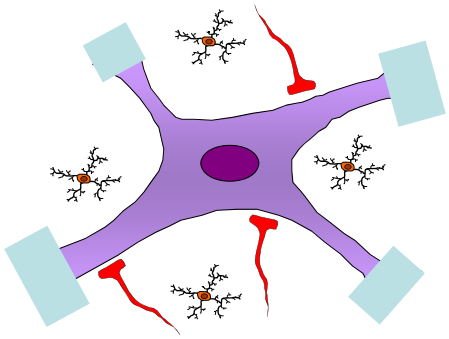
The microglia cell (M) is interposed between the motoneurone (MN) soma separating the detached synaptic boutons (*)

Moran and Graeber (2004) Brain Res. Rev. 44:154-178

Several weeks after axotomy

Microglial activation subsides coinciding with successful regeneration
ie once target reinnervation has been achieved and animals regain whisker movement

Number of microglia decreases – cells die by apoptosis

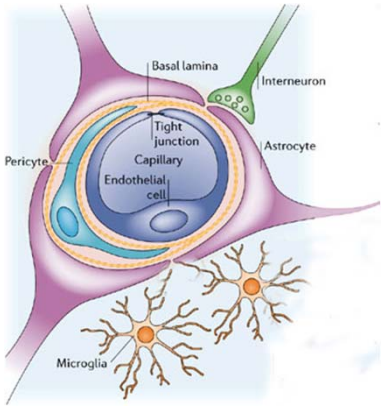


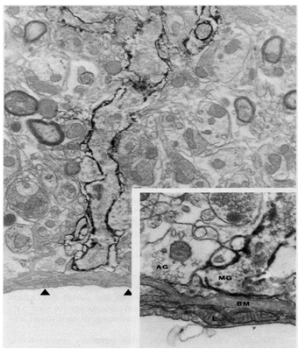
Microglial Function – Immune Response

"Immune-privileged" used to describe reduced exposition of brain to inflammatory events and its inability to mount an immune response

In healthy brain BBB restricts access of immune cells from the blood

Brain exhibits its own innate immune response that resembles many aspects of the classical immune system





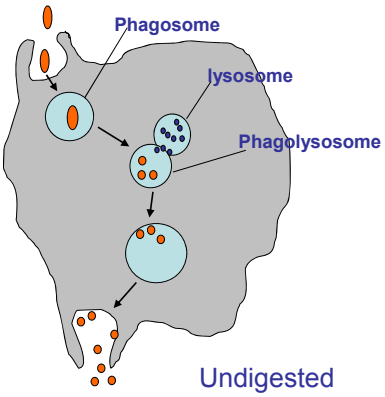
microglial cells have end feet on endothelial cells - placed to communicate with inflammatory cells

Phagocytosis of invading pathogens

Recognise micro-organism through carbohydrate and lipid motifs with PRRs, CR3 or Fc receptors

Extend pseudopodia around microbe and microbe is endocytosed into a phagosome

Cell lysosomes containing microbicidal oxygen metabolites and enzymes fuse with the phagosome to digest the microbe



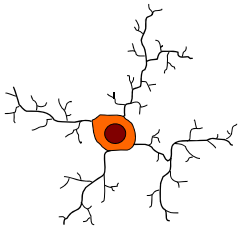
Undigested fragments released to outside

How do MG sense infection or inflammatory stimuli?

Pattern Recognition Receptors
TLR, CR3 (lectin site)

Fc Receptors
Fc γ RI, RII, RIII

Complement Receptors
CR1, CR3, CR4



Cytokine Receptors
IFN-γR, TNFRI/II,
IL12R, IL4R, TGFβRI/II

Chemokine Receptors
CCR2, CCR3,
CXCR4, CX3CR1

Rapid response of microglia to a change in the extracellular environment (ie infectious or inflammatory stimuli) is their constitutive and inducible expression of a large array of surface receptors

Pattern Recognition Receptors (PRRs)

Recognition and elimination of foreign entities - viruses, bacteria, parasites

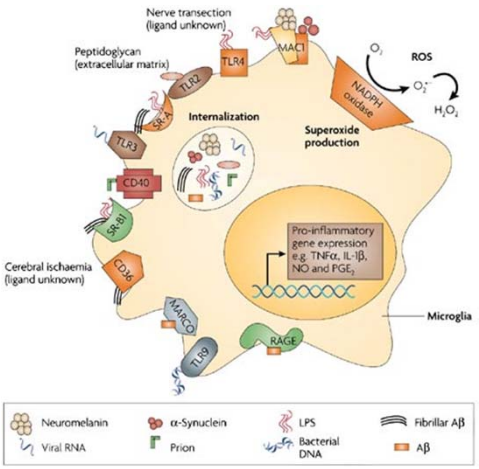
Crucial to the innate immune response

Bind pathogen-associated molecular patterns (PAMPS)

Interact directly with microbial structures:

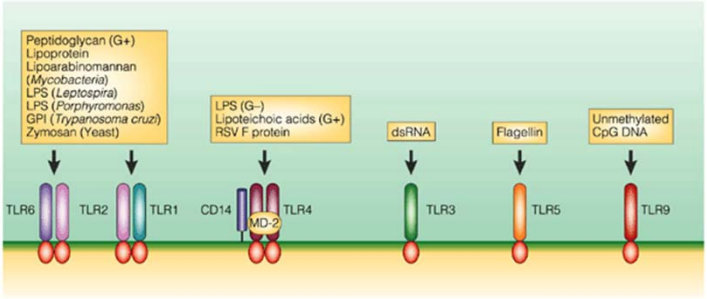
- stimulation of phagocytosis
- induction of cytotoxic mechanisms
- activation of immune response

Chronic inflammation -
eg Crohn's disease, arthrosclerosis,



Block et al 2007 Nat. Rev. Neurosci. 8:57-69

PRRs - Toll-Like Receptors



Microglia express Toll-like receptors TLRs 1-9

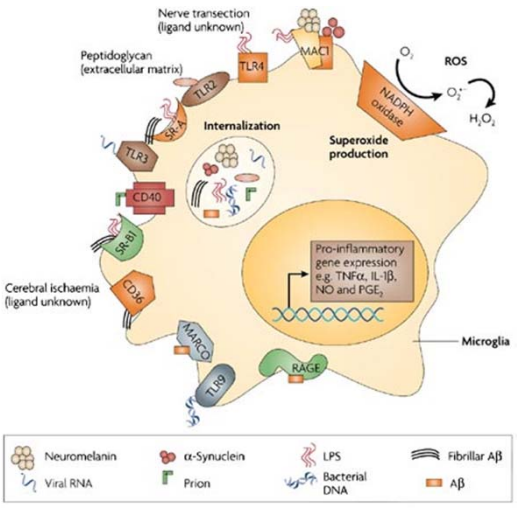
Expression regulated throughout development and in response to pathogens

Linked to microglial activation and neurotoxicity

PRRs - Scavenger Receptors

SR-A1, SR-B1 and CD36 - differentially regulated during development

Recognition and endocytosis of pathological substances leading to ROS release and defence against bacterial pathogens.



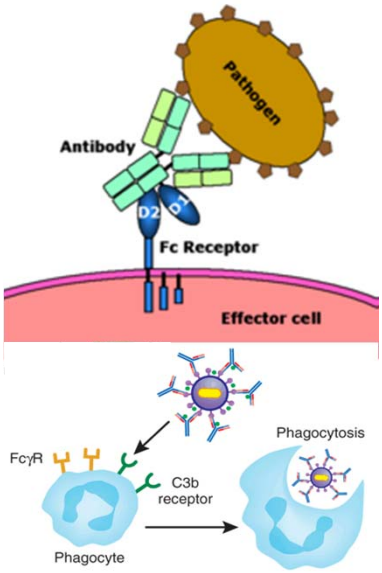
Fc receptors

Expressed by resting microglia but upregulated with activation

Bind the constant fragment (Fc) of immunoglobulins

Mediate or enhance phagocytosis through recognition of serum components on microbes or altered host components

Signalling promotes production of cytokines and ROS – enhancing microglial proinflammatory and cytotoxic functions



Regulation of T-cells responses through presentation of antigen

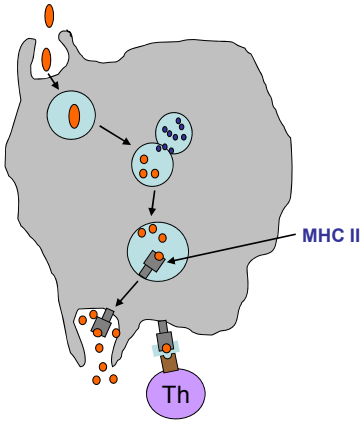
Critical event for generation of protective T-cell responses

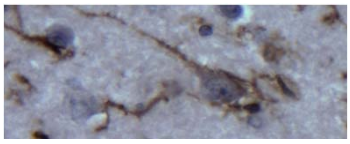
Activated microglia upregulate MHC class II

Antigen expressed on cell surface in association with MHC class II

Interaction between T-cell receptor and MHC class II

Stimulate CD4 T-helper cells to produce cytokines





MHC-II+ microglia in the diseased MS brain

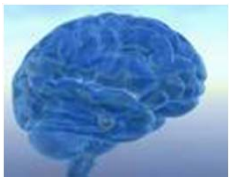
Microglia and Neurodegeneration

All CNS disorders are characterised by microglial activation and the progression and resolution of disease may be contingent on microglial activity

Alzheimer's Disease

Parkinson's Disease

Multiple Sclerosis



HIV-associated dementia

Huntington's disease

Prion disease

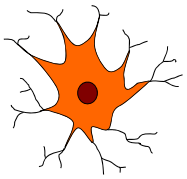
Age

Injury (stroke)

Induction of inflammation and cytotoxicity

Anti-inflammatory cytokines
IL-10, TGF-β, IL-1ra

Chemokines
MIP-1α, MIP1-βmMCP-1, RANTES



Pro-inflammatory cytokines
IL-1, TNF- α, IL-6, IL-12, IL15, IL-18

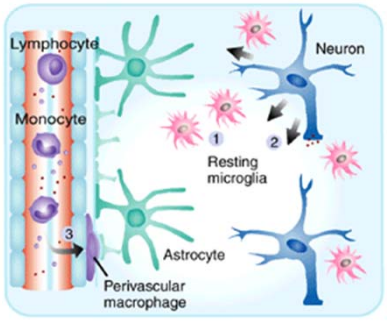
Death receptor ligands
TNF- α, FasL, TRAIL

Cytotoxic Factors
NO, ROS, glutamate,

Prostanoids
PGD2, PGE2, Thromboxane B2

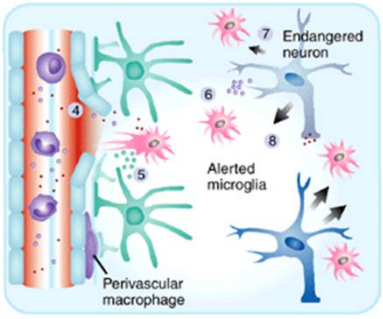
Activated microglia contribute to or reflect neuronal dysfunction in neurodegenerative disease

Healthy tissue



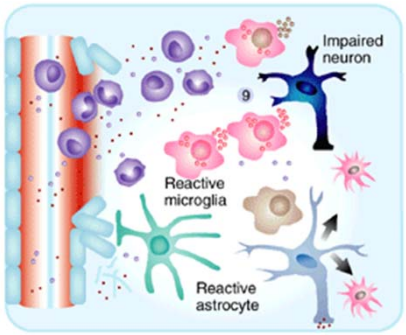
- Surveying environment
- Receive 'calming' signals from neurons

Small local damage



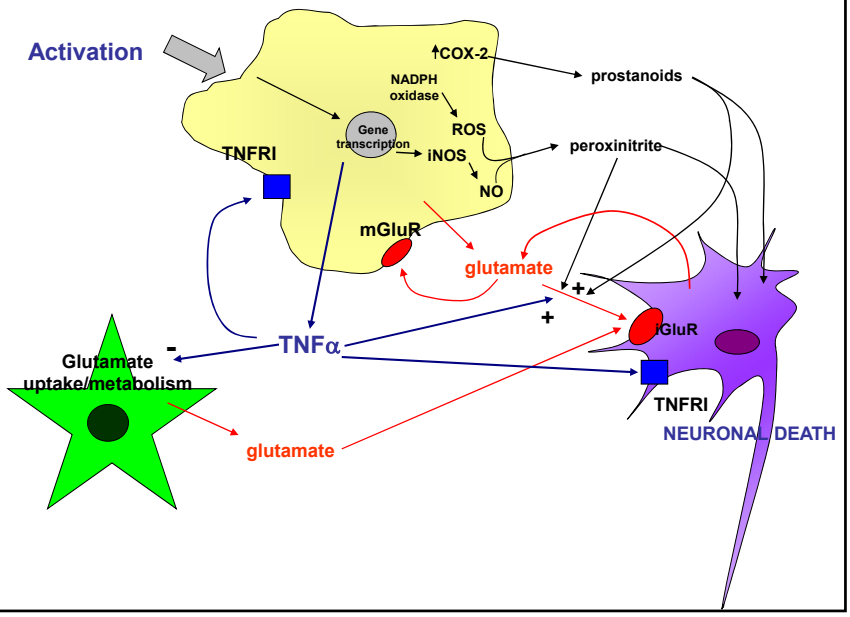
- Respond to vascular or tissue damage by becoming activated
- Produce neurotrophic factors
- Signals from endangered neuron
- May be able to limit damage and restore homeostasis
- Transient or focal activation has no overt symptoms - activation goes unnoticed

Severe insult



- Stronger insults trigger a more drastic changes in functional phenotype
- Surpass level of host tolerance mechanisms become neurotoxic
- Excessive (acute) or sustained (chronic) activation is detrimental to neurons – results in neuronal dysfunction and death
- Fuels microglial activation further

Is sustained or excessive activation of microglia detrimental to neuronal survival?

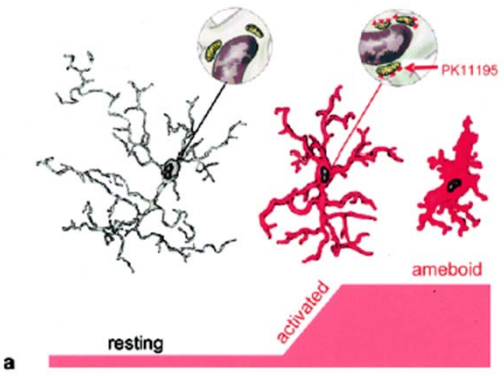


Measuring microglial activation in the brain

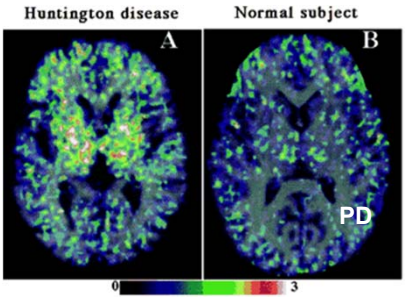
PET imaging of microglial activation as an indicator of inflammation

Expression of peripheral type benzodiazepine binding sites on mitochondrial membranes of activated microglia

Ligand PK11195 - selectively binds to activated but not resting microglia



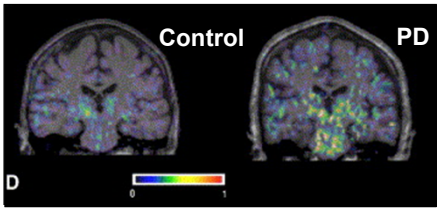
Microglial activation the human brain



Huntingdon Disease

Microglial activation correlates with disease severity

Pavese et al 2006 Neurology 66:1638-1643



Parkinson's Disease

Increased microglial activation in pons, basal ganglion, and cortical regions

Level of microglial activation is:

- Inversely related to density of DA terminals
- Related to motor impairment

Gerhard et al 2006 Neurobiol. Dis. 2:404-412

PET imaging of microglial activation

Early indication of disease onset

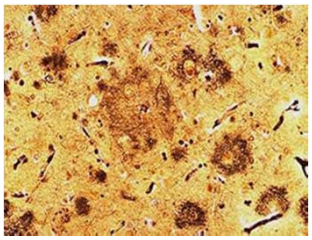
Potential to measure disease progression

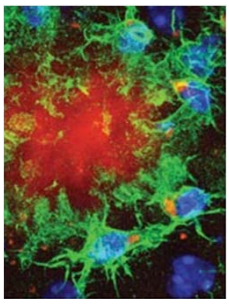
As yet cannot distinguish between beneficial microglial activation and a deleterious phenotype

Microglia in Alzheimer's disease

Neural damage begins in temporal and parietal lobes of cortex, progresses to hippocampus and amygdala

Progressive memory impairment and cognitive decline





Why are microglia implicated?

Accumulate in senile plaques in AD brain and in animal models of AD

Microglial activation increases throughout disease progression

Activated microglia cluster around aggregated Aβ - immune reaction in AD causes neurodegeneration

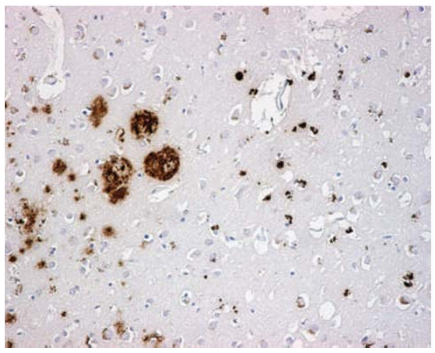
Evidence suggests that microglial activation is a beneficial response in AD

Microglia are recruited to sites of Aβ deposition to clear this neurotoxic peptide

Express receptors that promote the clearance and phagocytosis of Aβ (eg CD36)

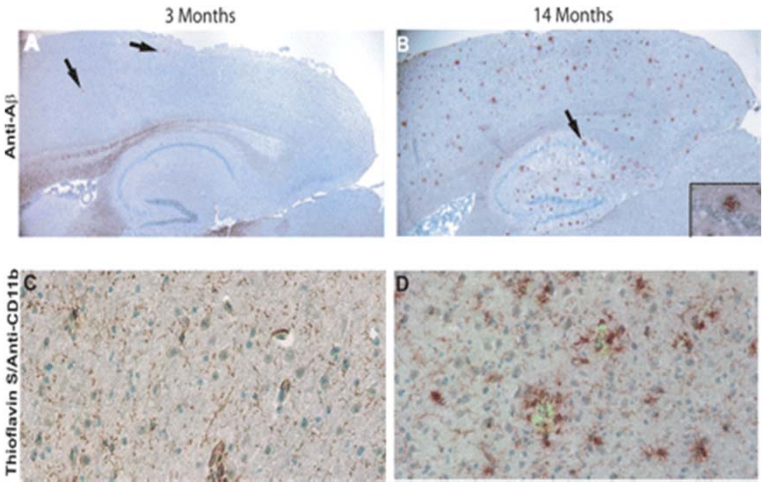
Microglia can restrict senile plaque formation by phagocytosing Aβ (Simard et al., 2006)

Early microglial accumulation in AD may delay disease progression
(El Khoury et al., 2007)



Why does Aβ continue to accumulate, and why does AD pathology progress despite continued microglia recruitment?

Increased number of senile-like plaques and plaque-associated microglia with aging in transgenic PS1-APP mice.



PS1-APP:
APPswe/PSEN1dE9

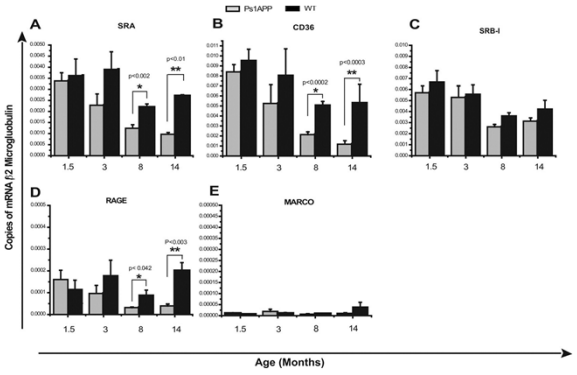
Sparse Aβ deposits
Microglia evenly distributed

Aβ deposits throughout
cortex/hippocampus
Microglia clustered

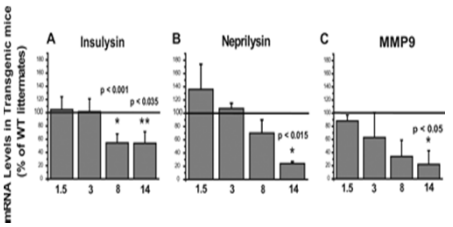
Green – plaques
Brown - microglia

Hickman et al 2008 J Neurosci 28:8354-60

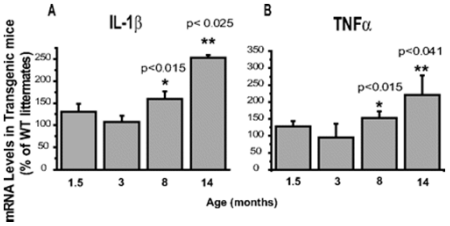
Reduction in expression of Aβ-binding receptors



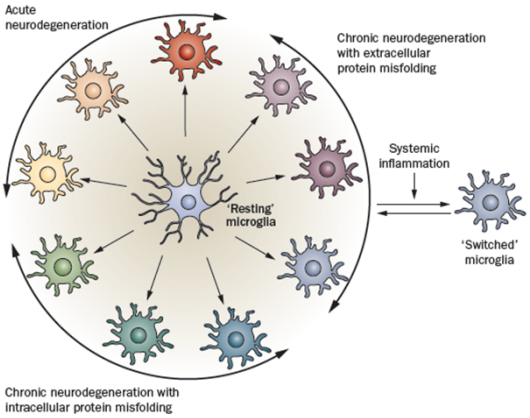
Reduced Aβ-degrading enzymes



maintain ability to produce proinflammatory cytokines

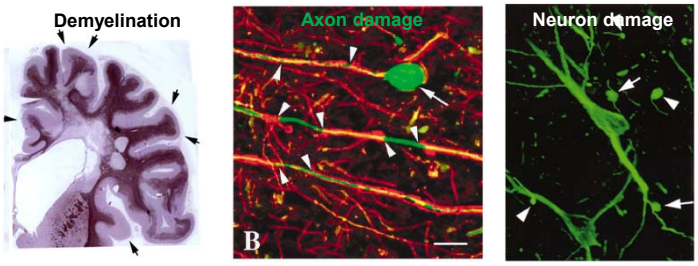


- the slow degeneration of neurons might also activate microglia
- positive feedback loop
- microglia activated by neuronal degeneration secrete neurotoxic molecules
- self-perpetuating degenerative process

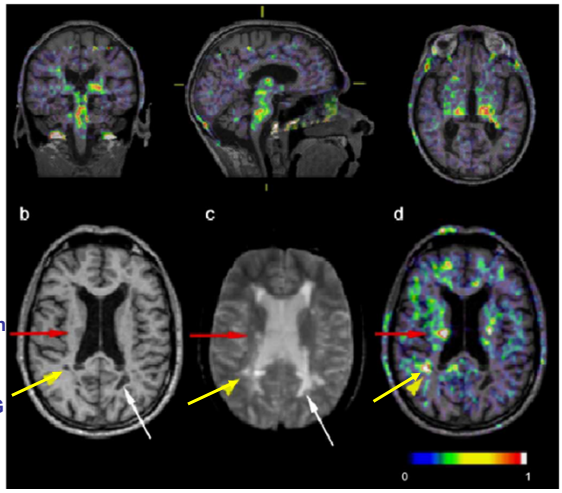


Multiple Sclerosis (MS)

- An immune-mediated disease characterised by demyelination and neuroaxonal loss
- Initial clinical symptoms include fatigue, vertigo, optic neuritis and limb weakness. Disease progresses to ataxia, paralysis and cognitive impairment
- Neurodegeneration and axonal loss due to inflammation and demyelination in white and grey matter
- Demyelination and axonal injury culminating in severe disability



Microglial activation in the human MS brain



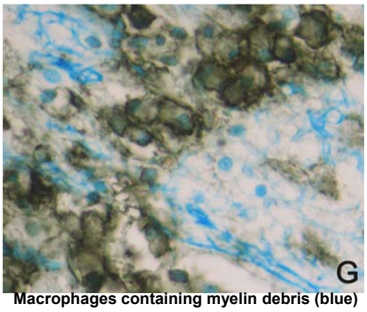
Lesions overlap with areas of MG activation

Black "hole" associated with MG activation

(From Banati et al 2000)

Microglia in MS

- Microglia are central components of MS lesions throughout all phases of the disease course
- Activated microglia evident before damage/lesion

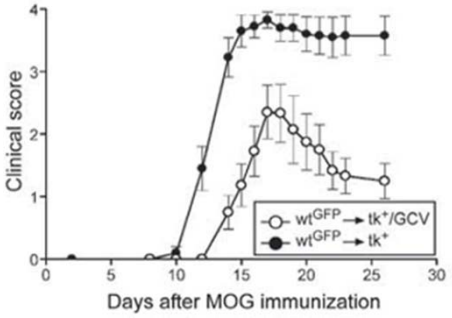


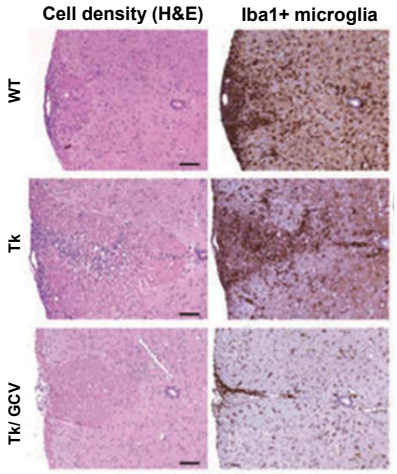
Macrophages containing myelin debris (blue)

- Initial lesions comprise of apoptotic oligodendrocytes and activated microglia before any observable inflammatory response
- Chronic MS lesions characteristic of the progressive phase is dominated by activated microglia and macrophages

EAE is suppressed in the absence of functional microglia

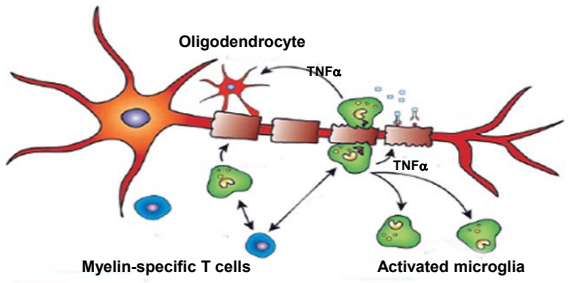
Thymidine kinase enzyme expressed under the influence of CD11b. TK converts gancicolzir into a toxic DNA base-pair analogue resulting in selective cell suicide





Heppner et al, Nature Medicine **11**, 146 - 152 (2005)

The role of microglia in the mechanisms leading to demyelination and axonal injury in MS

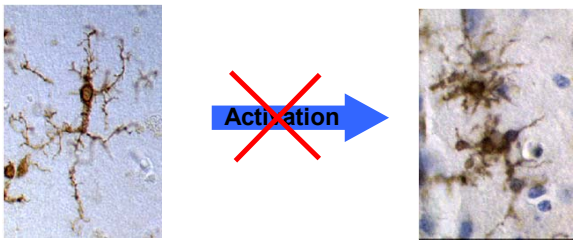


- Activated myelin-reactive T cells migrate into CNS and release inflammatory cytokines which activate microglia
- Activated microglia release cytokines and NO that mediate the initial attack
- Microglia engulf myelin and present to CD4+ T cells causing de novo activation of T cell and microglia
- Myelin activated microglia release of proinflammatory cytokines (IL-1, IL-6 and TNF) which directly damage oligos, myelin sheaths and axons

Agents can reduce microglial activation *in vitro* and *in vivo*

	In vitro effect on microglia	*In vivo* (rodent)
Minocycline	↓ LPS activation	Protective in PD, HD, MS, stroke, brain trauma
NSAIDS	↓ iNOS in LPS activated MG ↓ neurotoxicity	Protective in PD, AD, ALS
Statins	↓ NADPH oxidase activity + iNOS induction in Aβ, LPS activation	↓ Infarct volume in focal ischaemia
Cannabinoids	↓ LPS or Aβ activation	Prevent cognitive dysfunction and neuronal death in Aβ-treated rats
Caffeine	↓ COX-2 expression ↓ ECS glutamate	Neuroprotective in PD, stroke
Vitamin D	↓ iNOS in LPS activated MG	Protective in PD and EAE
mGluR modulators	↓ LPS activation ↓ neurotoxicity	Neuroprotective in PD, ischaemia, traumatic brain injury

Protection against neurodegeneration by reducing MG activation?



Considerations:

Development of optimal therapeutic strategies cannot simply be aimed at suppressing all microglial functions

Therapies for chronic neurodegenerative disease - focussed on mechanisms of microglial activation or amplifying specific reactive phenotypes to promote neuroprotection or neuroregeneration

Learning objectives

- Describe the origin of microglia during development and in adulthood
- Explain the role of microglia in the healthy brain
- Describe how microglia detect changes in the local environment to mount an inflammatory response
- Discuss the role of microglia in neurodegenerative diseases

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