

# DARC shuttles inflammatory chemokines across the blood–brain barrier during autoimmune central nervous system inflammation

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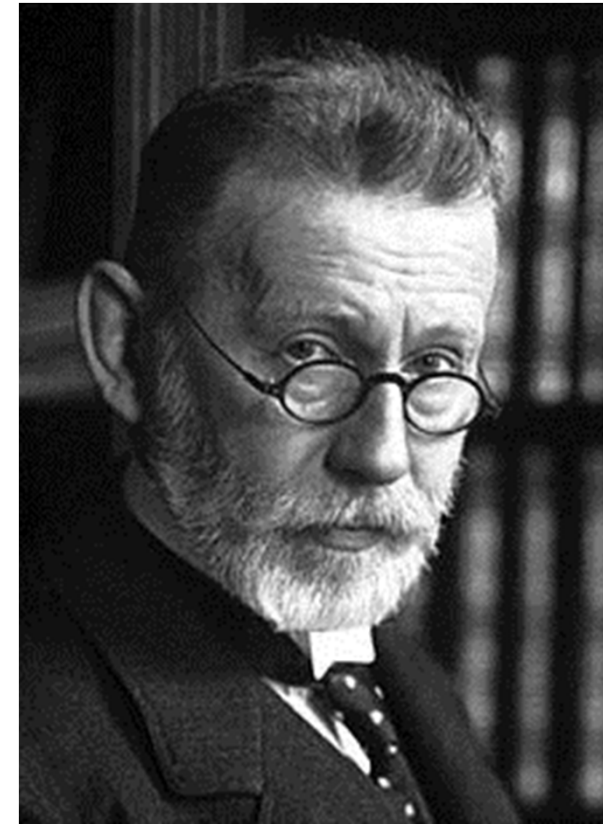
## Immunology of the CNS - History

Ehrlich, 1885 & 1904 dye did not stain brain  
-> BBB

Shirai, Y. (1921) On the transplantation of  
the rat sarcoma in adult heterogenous  
animals. Jap. Med. World 1, 14-15

Murphy and Sturm, 1923 -> sarcoma +  
spleen

Medawar, P.B. (1948) Immunity to  
homologous grafted skin.

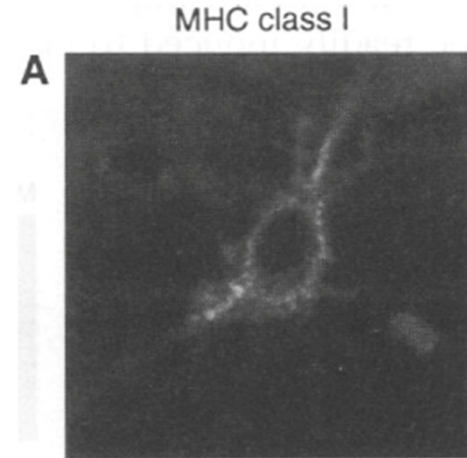


Wikipedia.de

Neumann et al. (1995)  
Galea et al. (2006)

## Immunology of the CNS

- CNS -> "immune privilege"
- Neurons -> physiologically NO MHC I expression
- Loss of electrical activity, virus -> MHC I expression
- T-cell surveillance with prompt clearance (brain-specific mechanisms of apoptosis induction)



Neumann et al. (1995)

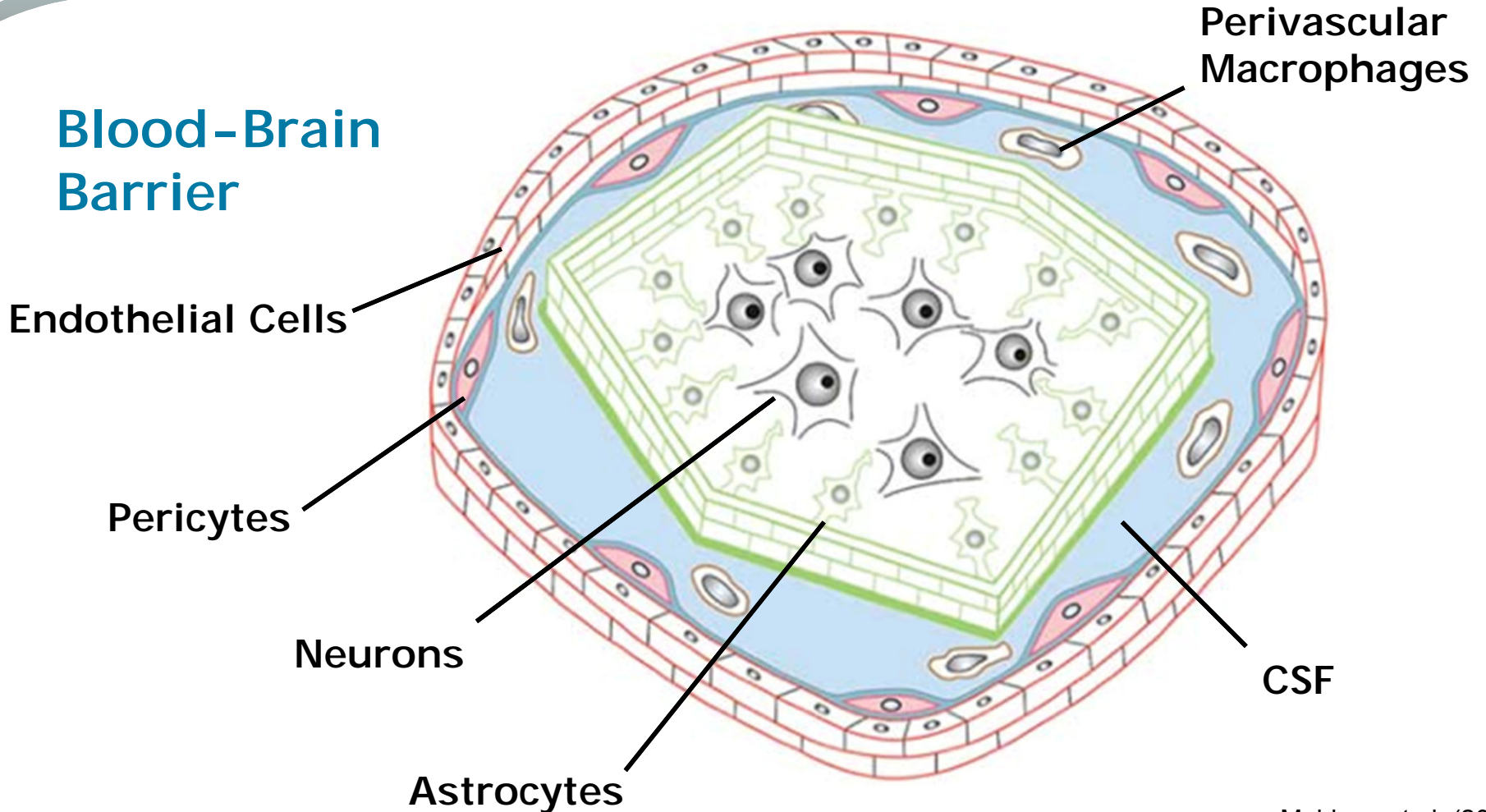
Galea et al. (2006)



# Background



## Blood-Brain Barrier

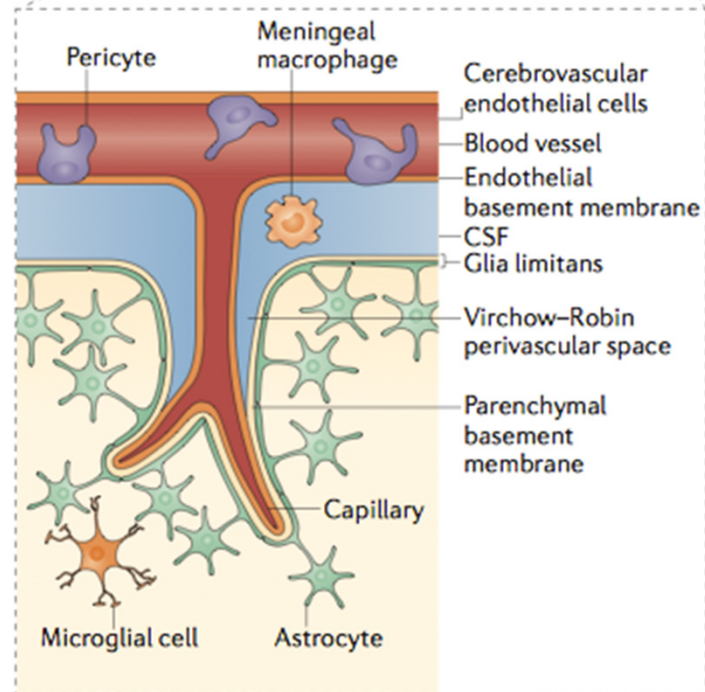
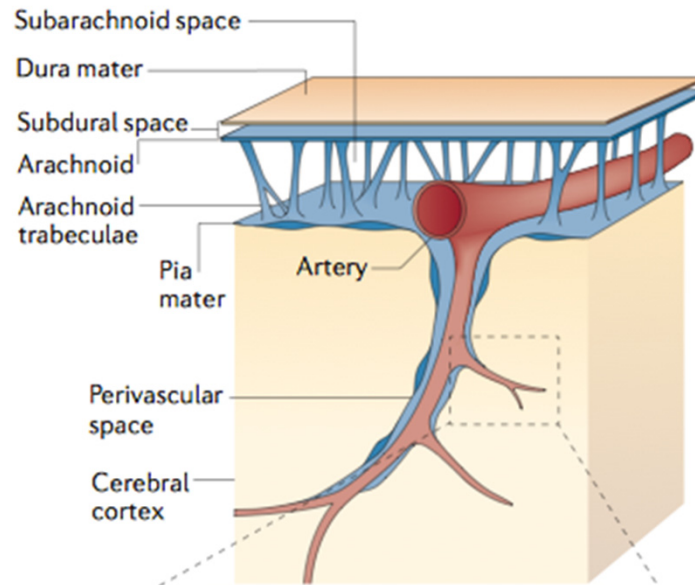


Muldoon et al. (2013)





# Blood-Brain Barrier



## Blood-Brain Barrier

- Highly specialized endothelial cells with low pinocytotic activity, tight junctions and specialized transport mechanisms (GLUT-1 e.g.)
- Heparan sulfates on the luminal side bind chemokines

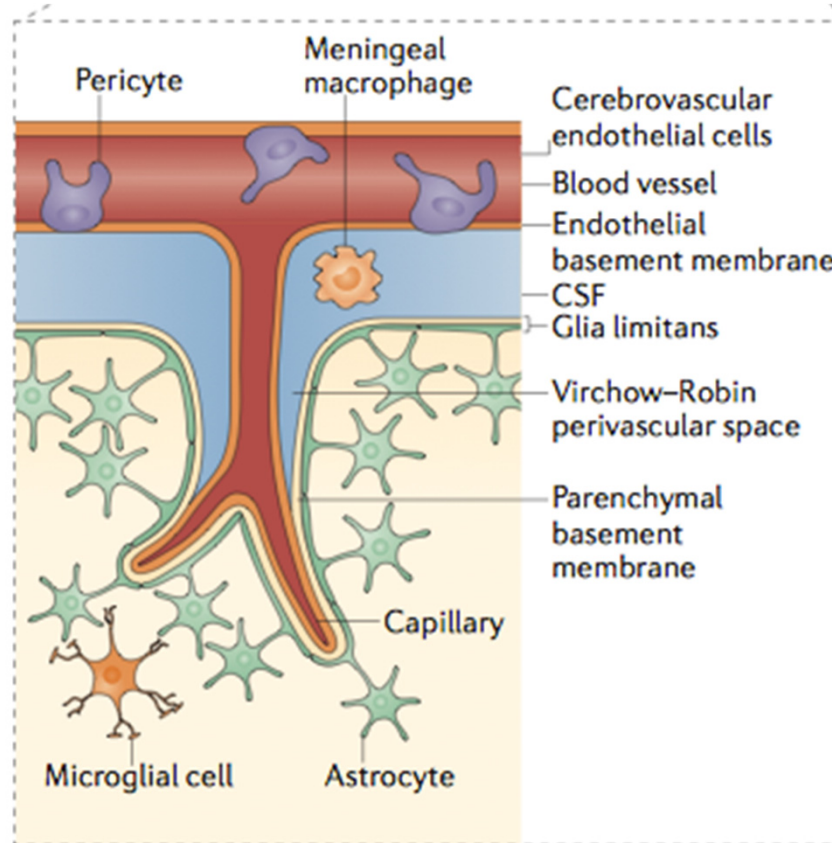
## Multiple Sclerosis

- Autoimmune

- Natalizumab

- Up-regulation of chemokines (

- BUT: CCL2, CCL5, CXCL12 mainly expressed by astrocytes during EAE



disease

is by luminal side

## Blood-Brain Barrier

- Chemokine transport without affecting ECs
- How does that work?



## Duffy antigen receptor for chemokines (DARC)

- =Duffy antigen (serology)
- 4<sup>th</sup> gene to be associated with resistance to Malaria
- Receptor for *Plasmodium vivax/knowlesi*

## Duffy antigen receptor for chemokines (DARC)

- Expression on
  - erythrocytes, cerebellar neurons
  - postcapillary venules and capillary endothelial (lymph nodes, the lung, and the kidney)
  - but not on arterial endothelial cells

## Duffy antigen receptor for chemokines (DARC)

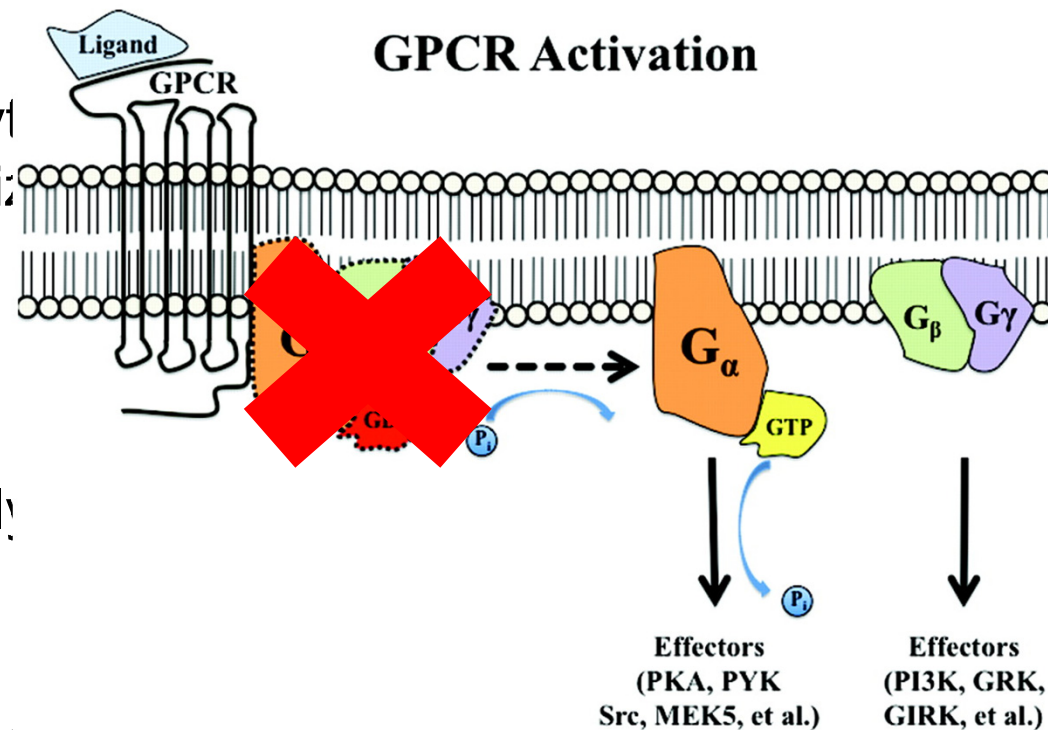
- Lacks intracellular domain to bind G-protein

- Ligand: most inflammatory cytokines  
macropinocytosis-like internalization

- “Sponge theory”

- Knock-out mice phenotypically normal

- Expression on the BBB has not yet been described



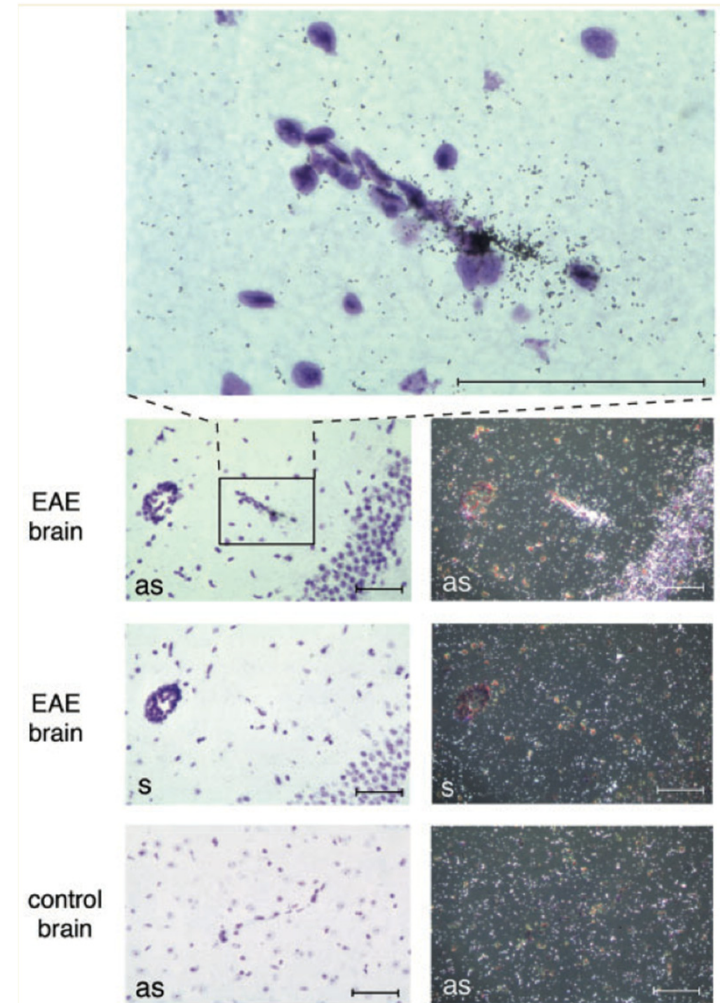
... was to evaluate a possible role of **DARC** in the physiology and pathology of the Blood-Brain Barrier

- Active EAE in wild type DARC<sup>-/-</sup> and chimeric mice
- Human brain tissue from the UK multiple sclerosis tissue bank
- In-vitro BBB studies
- Further in-vitro methods (membrane arrays, ELISA, proliferation assays)



# -Results-

- 2005: Gene-expression analysis of EC during EAE -> up-regulation of DARC
- In-situ hybridization ->>
- On vessels?



Minten et al. (2014)



Christian  
Doppler  
Laboratory

for  
Cardiac and Thoracic  
Diagnosis & Regeneration

# Results



MEDIZINISCHE  
UNIVERSITÄT  
WIEN

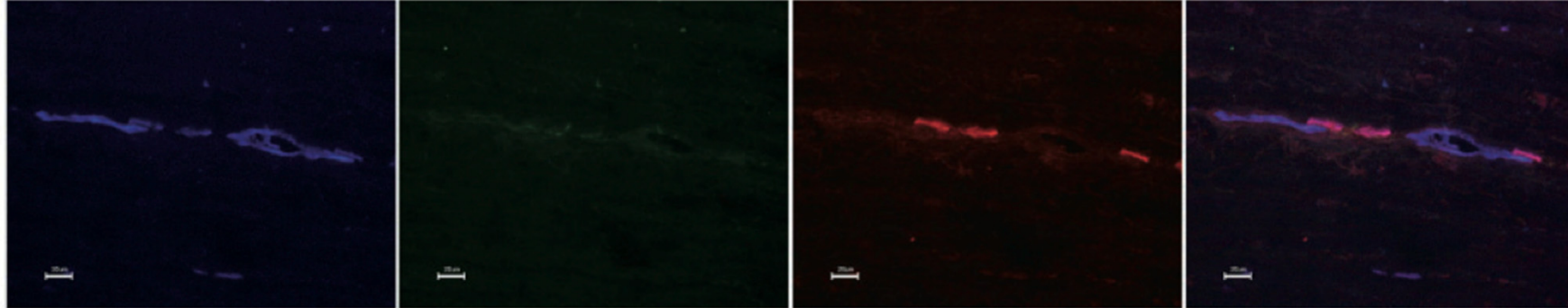
**PECAM 1**

**CD45**

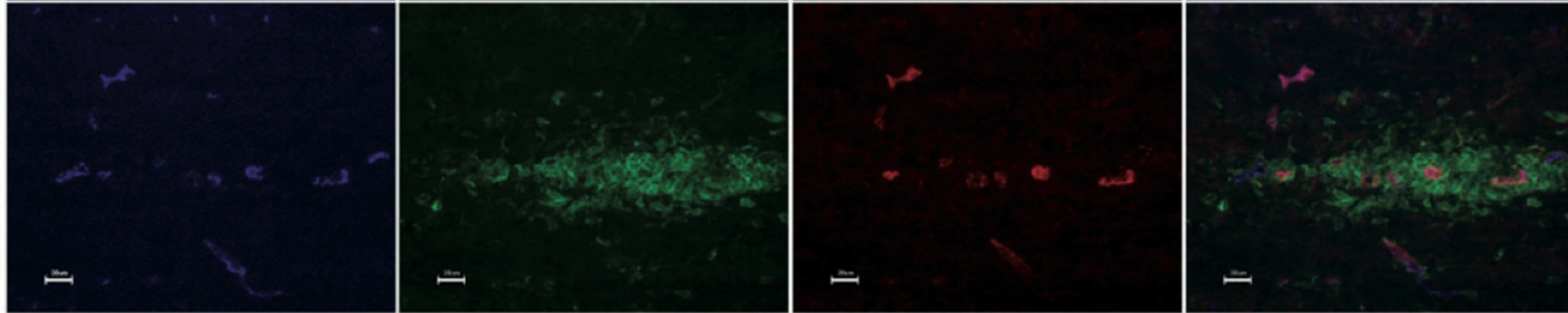
**DARC**

**merge**

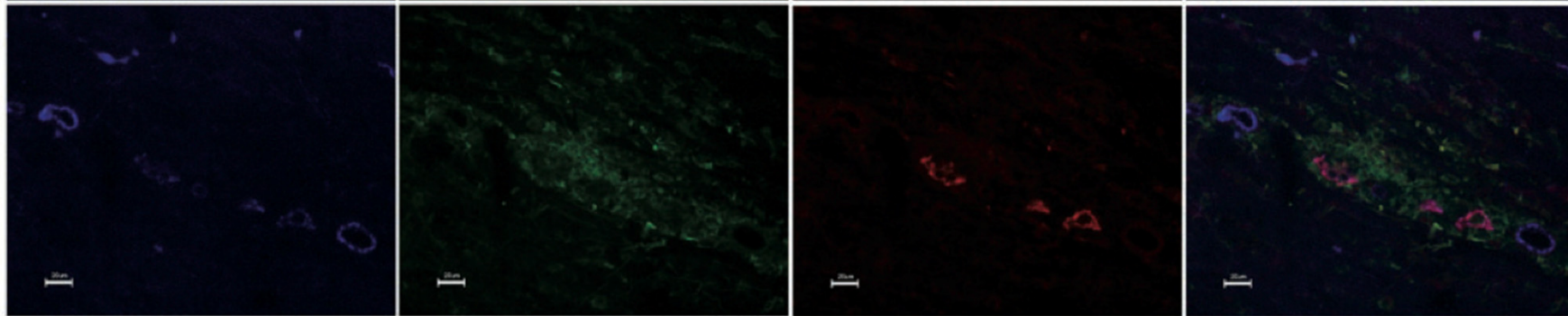
**Day 7**



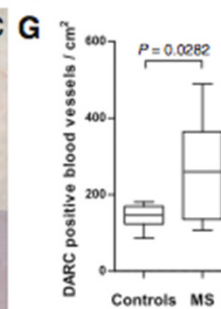
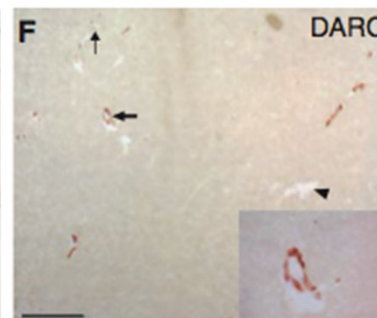
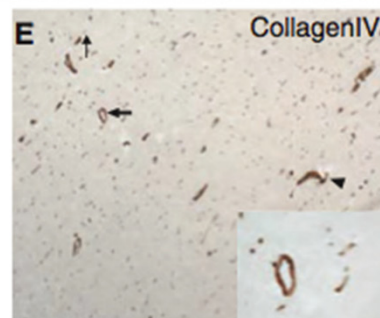
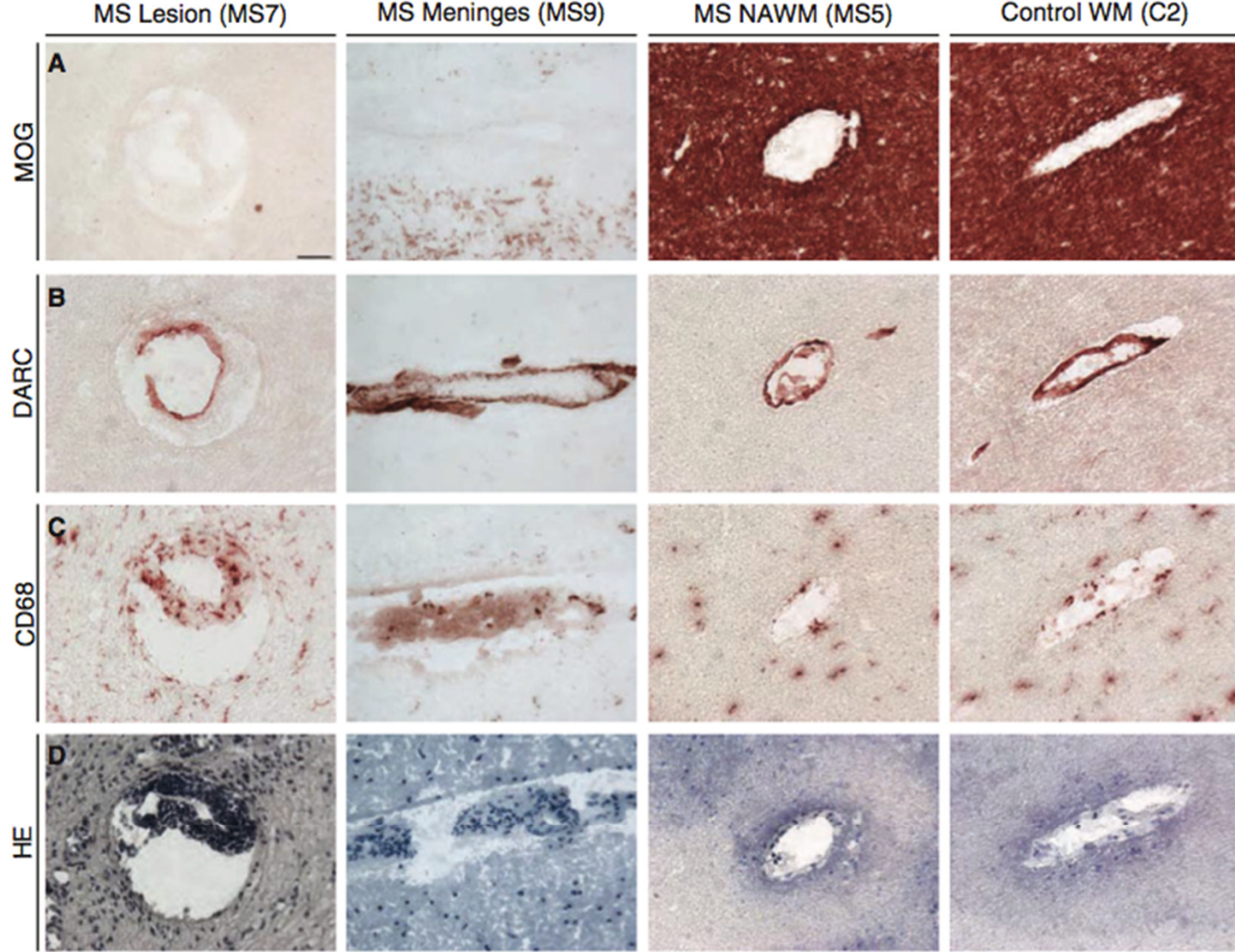
**Day 11**

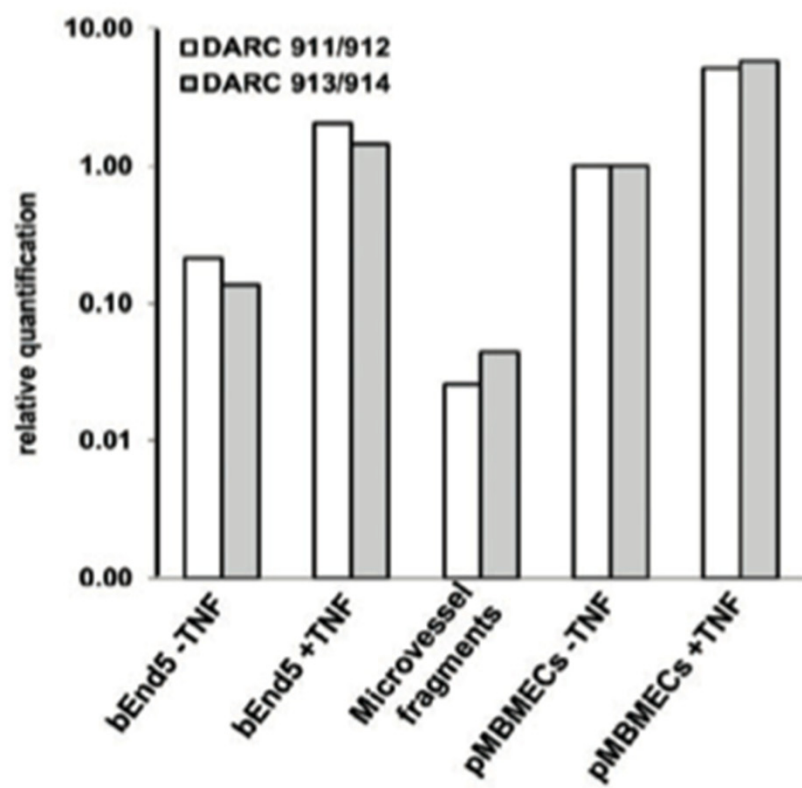


**Day 13**



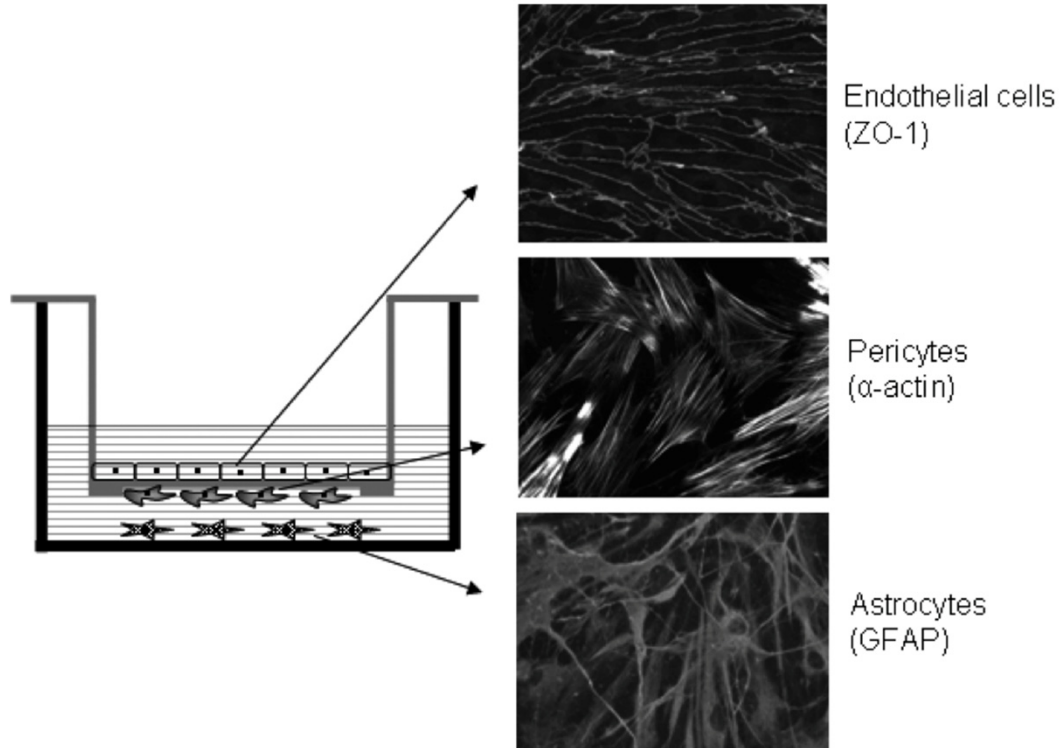




**A**

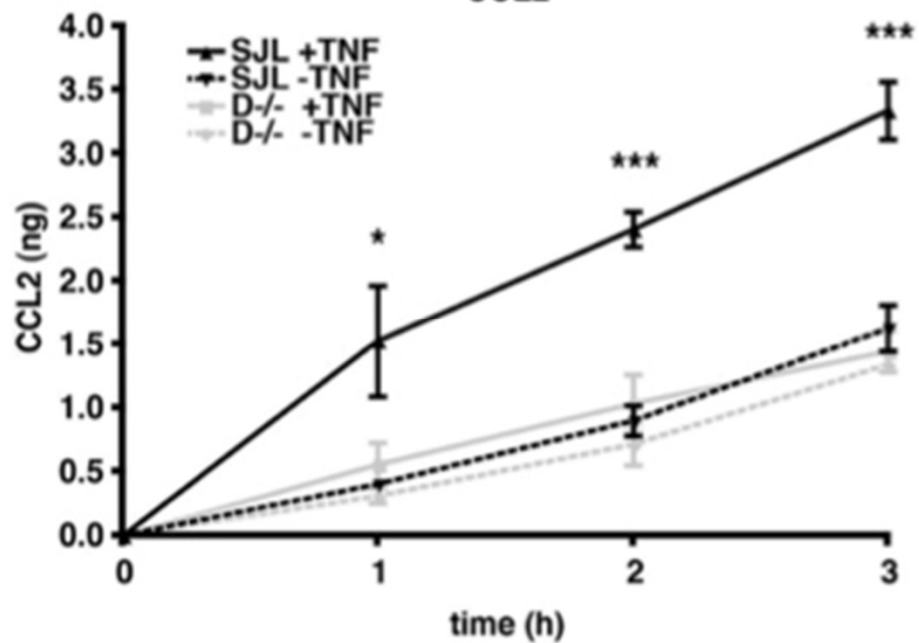


## In-vitro BBB assay

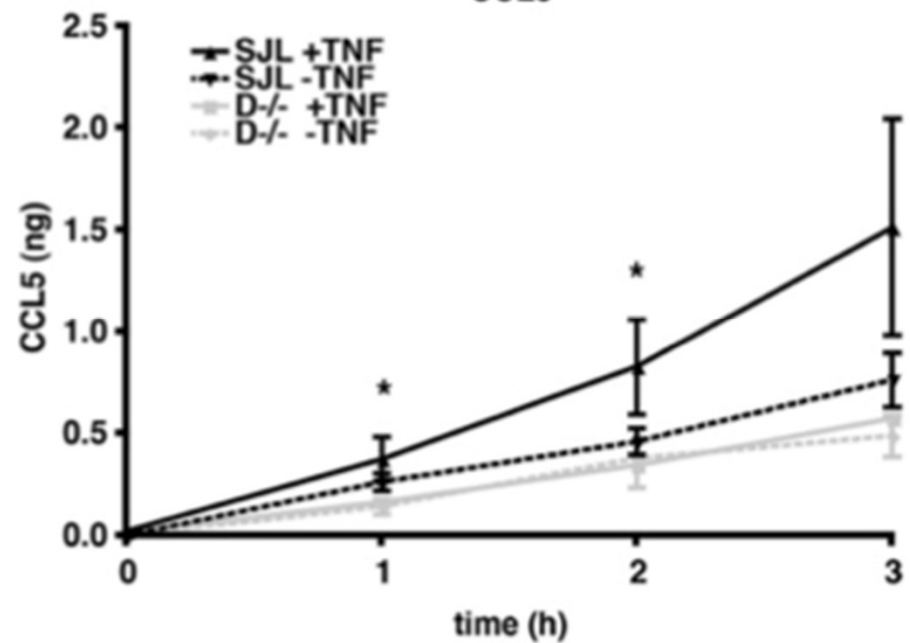


Wilhelm et al. (2011)

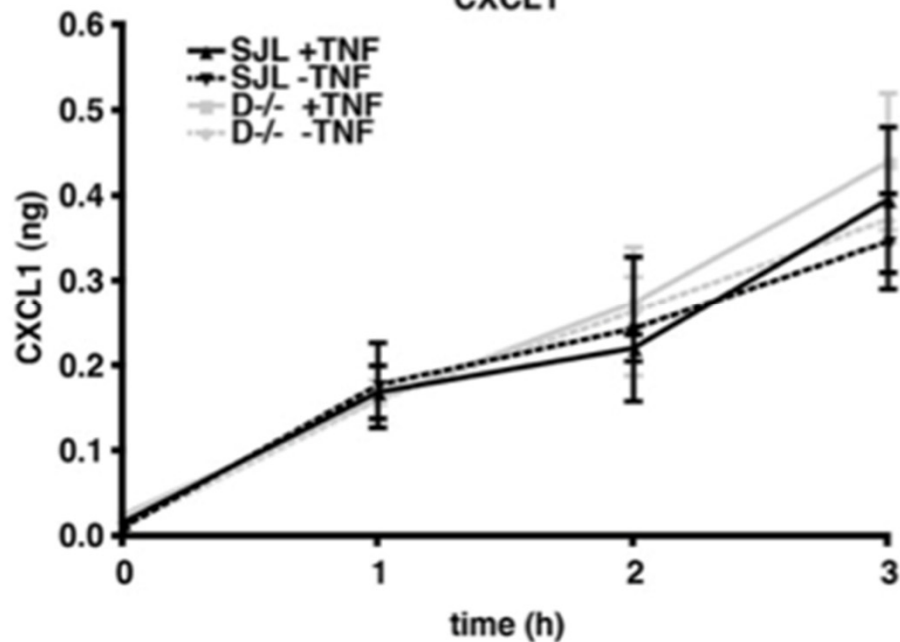
CCL2



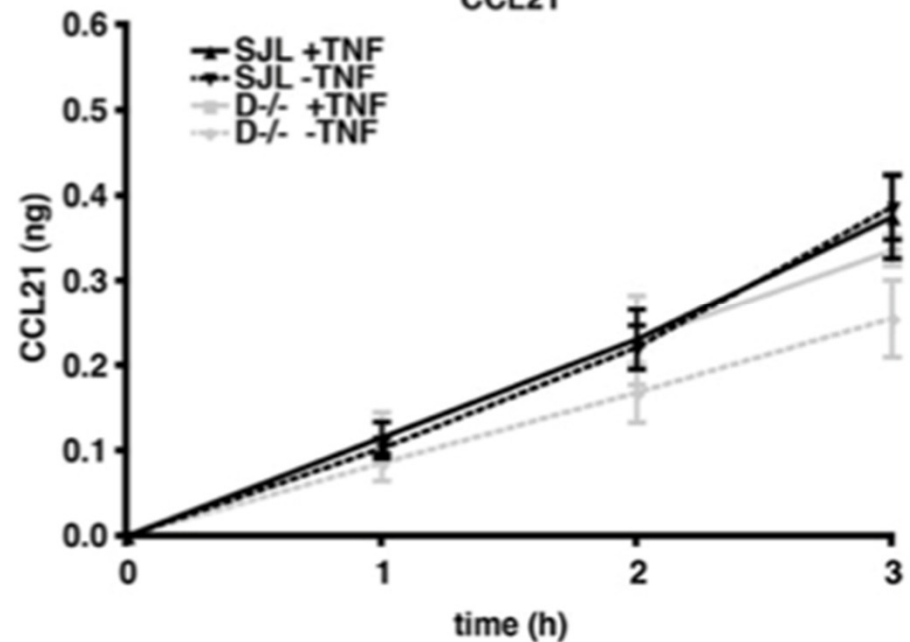
CCL5

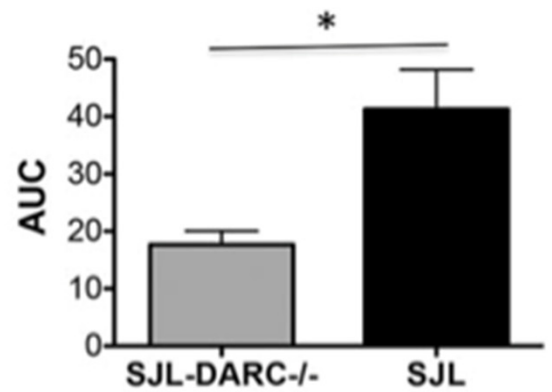
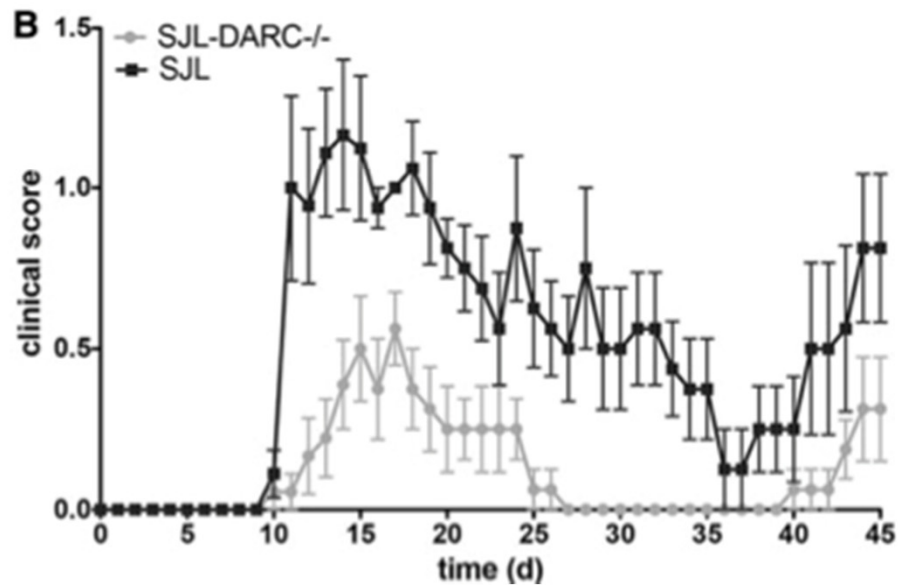
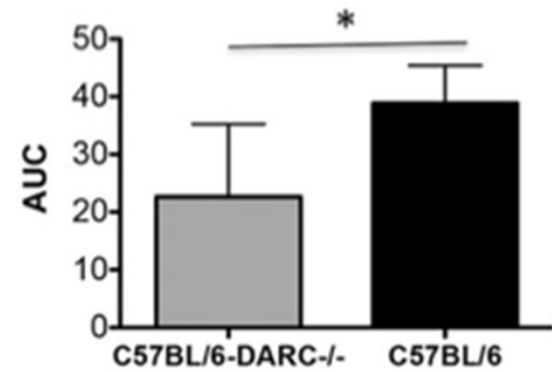
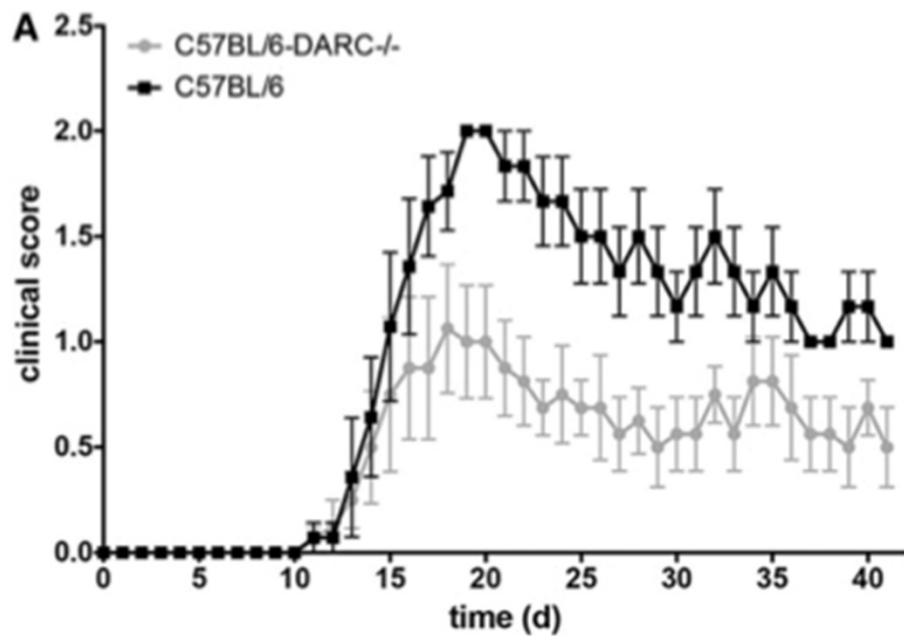


CXCL1



CCL21



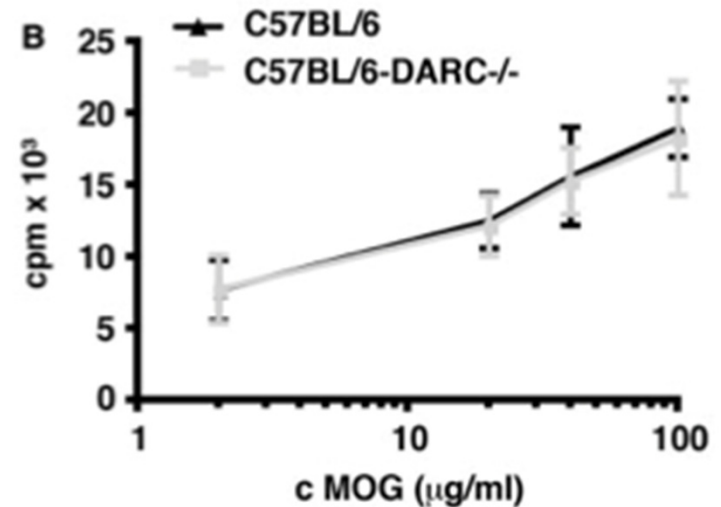
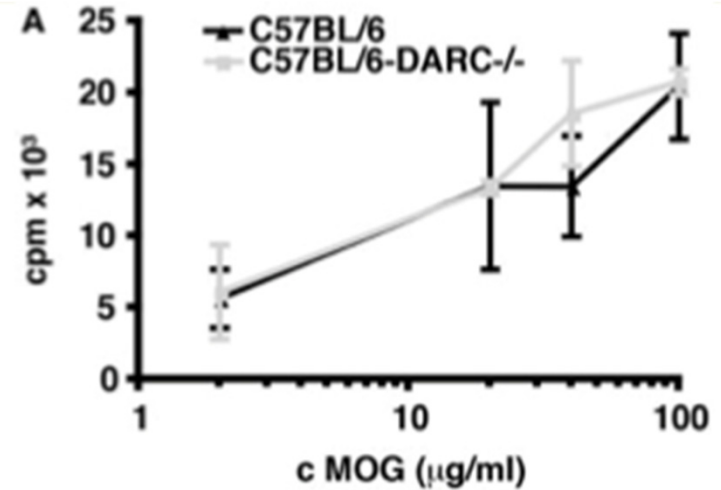


Minten et al. (2014)

Vienna, 2014

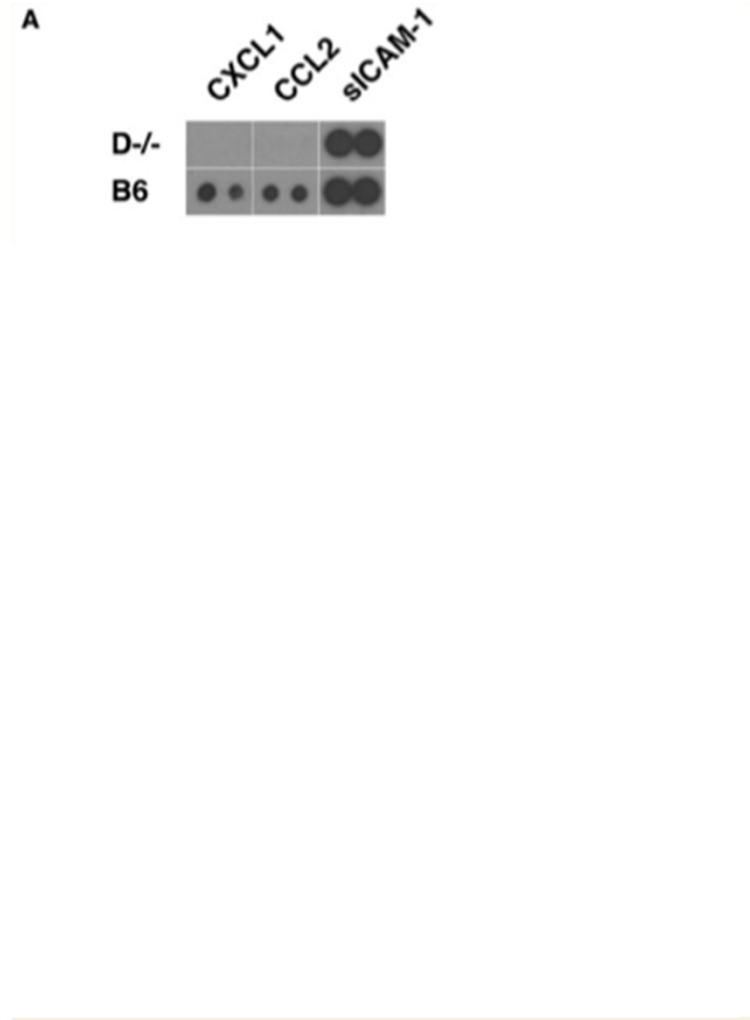
# Results

- DARC -> also on lymph node venules – T-cell priming/proliferation altered?



Minten et al. (2014)

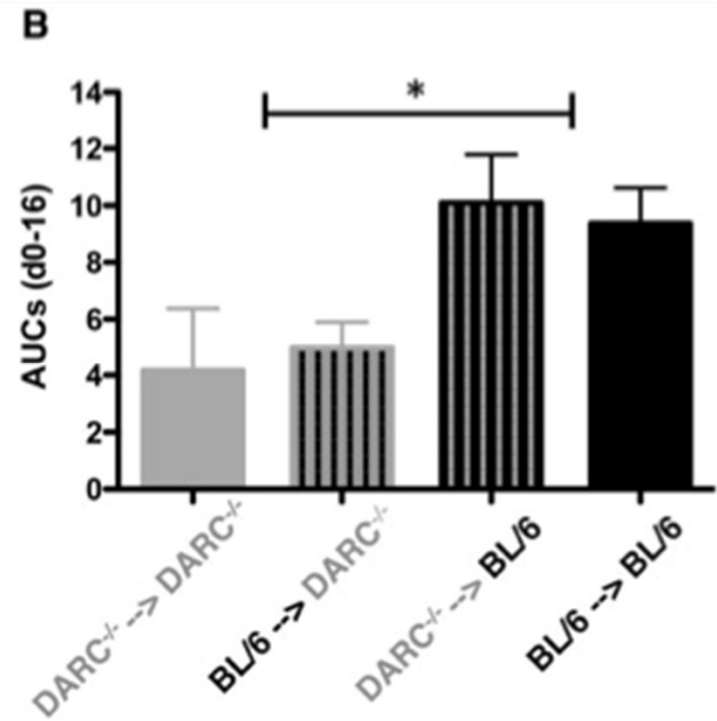
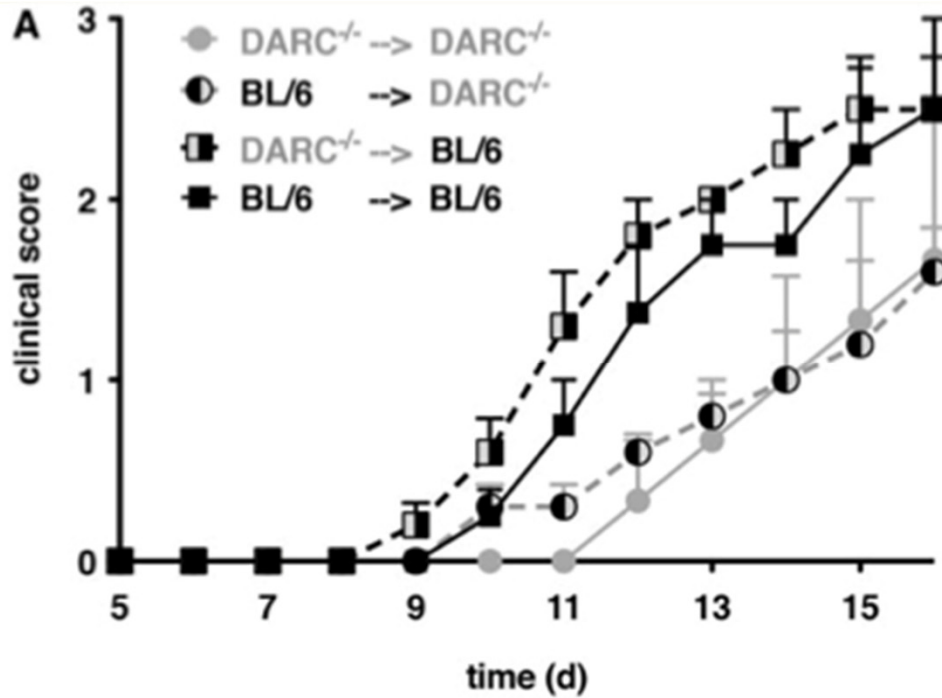
- Plasma cytokine levels
- A: absence of DARC -> lower cytokine levels
- Vascular or erythrocyte DARC responsible?
- B: bone-marrow transplantation experiments



Minten et al. (2014)



# Results



# - Discussion -

- DARC involved in initiation of EAE/MS BUT not the exclusive mechanisms (delayed onset)
- CCL2, CCL5, CXCL-1 affinities: results are NOT in line with data obtained from studies with human DARC – recombinant CXCL-1?
- Difference in clinical course of EAE in BM transplantation experiments
- Erythrocyte DARC -> reservoir rather than sponge

Minten et al. (2014)

**Thank you for your attention!**