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


Murphy and Sturm, 1923 -> sarcoma + spleen

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

- Endothelial Cells
- Pericytes
- Neurons
- Astrocytes
- Perivascular Macrophages
- CSF

Muldoon et al. (2013)
Blood-Brain Barrier

Ransohoff and Engelhardt (2012)
Blood-Brain Barrier

- Highly specialized endothelial cells with low pinocytic activity, tight junctions and specialized transport mechanisms (GLUT-1 e.g.)

- Heparan sulfates on the luminal side bind chemokines
Multiple Sclerosis/EAE

- Autoimmune disease
- Natalizumab (anti-\(\alpha_4\)-integrin) ameliorates disease
- Up-regulation of integrins on leukocytes by chemokines (CCL2, CCL5, CXCL1) on the luminal side
- BUT: CCL2, CCL5, CXCL1 mainly expressed by astrocytes during EAE

Minten et al. (2014)
Background

Blood-Brain Barrier

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

Minten et al. (2014)
Background

Duffy antigen receptor for chemokines (DARC)

- =Duffy antigen (serology)

- 4th gene to be associated with resistance to Malaria

- Receptor for *Plasmodium vivax/knowlesi*

Minten et al. (2014)
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; binding results in makropinocytosis-like internalization
- "Sponge theory"
- Knock-out mice phenotypically healthy and normal response to inflammation
- Expression on the BBB has not yet been described

Vienna, 2014
... was to evaluate a possible role of DARC in the physiology and pathology of the Blood-Brain Barrier
Methods

- Active EAE in wild type DARC\(^{-/-}\) 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)

Minten et al. (2014)
- Results -
Results

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

Minten et al. (2014)
Results

Vienna, 2014

Minten et al. (2014)
In-vitro BBB assay

Wilhelm et al. (2011)
Vienna, 2014

Active EAE in wild type and DARC-/- mice

Human brain tissue from the UK multiple sclerosis tissue bank

In-vitro BBB studies

Results

Minten et al. (2014)
Minten et al. (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**

Minten et al. (2014)
- 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!