Characterization of microparticles derived from cultured macrophages and cerebrospinal fluid of patients with schizophrenic and affective disorders

E. Marion Schneider
Anesthesiology
University Hospital Ulm, Germany
Inflammation in the brain can be detected in peripheral blood

Leboyer et al. 2012
- "Bipolar disorder can be effectively conceptualized as a multi-systemic inflammatory disease"

Dantzer et al. 2008
- "When activation of the peripheral immune system continues unabated (...) the ensuing immune signalling to the brain can lead to (...) development of symptoms of depression in vulnerable individuals"

- "Immune profile of bipolar disorder and schizophrenia suggests inflammatory disturbances related to neuroplasticity, endothelial function and calcium regulation"

Scientists have known for a while now that inflammation contributes to long-term neurodegenerative conditions such as AD and PD. But lately they have been turning up evidence that inflammation can affect the brain more directly and acutely, and might underlie a wider range of problems, from impaired cognition during infections to depression and even schizophrenia. - See more at: http://www.dana.org/BrainWork/2014/The_Brain_Inflamed/#sthash.QFa2Orpw.dpuf
<table>
<thead>
<tr>
<th>Case</th>
<th>Age[ ], Male/female</th>
<th>Disease</th>
<th>Autoimmune characteristics</th>
<th>Medication A, B, C, D, F</th>
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<tbody>
<tr>
<td>SZ1</td>
<td>[25] male</td>
<td>F20.0</td>
<td>no</td>
<td>B, B, F</td>
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<td>D, F</td>
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<tr>
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<td>F42.2</td>
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<td>[59] female</td>
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</table>
Aims

To characterize inflammatory pathways in enriched antigen presenting cells

To clarify the involvement of damage vs. pathogen related inflammation
Methods

Whole blood → Ficoll separation → culture of plastic adherent cell fraction [<28 days]
↓
Flow cytometry: Cells and microparticles
↓
Enrichment of microparticles
↓
Electron microscopy
↓
miRNA quantification

Reverse phase HPLC
↓
Kynurenine/tryptophan ratio
Transelectron Microscopy Metabolism

Cell enrichment → chemical fixation
↓
microscopy

High Pressure Liquid Chromatography (HPLC)
↓
indoleamine 2,3-dioxygenase (IDO), tryptophan, nitric oxide

Autophagy
Apoptosis
Necrosis
Pyroptosis
Necrosis
Microparticles
Infectious agents

Kynurenine per tryptophan ratio / nitric oxide
↓
Activation of IFN-γ → TH1, NK cell activation

ELISA for biomarkers

D. Fuchs, Clin Chem 2002 vol. 48 (3) p. 3579-581
Cell Culture

plastic adherent cell fraction [<28 days]
ATP stimulation induces microparticle release.

Results

1 mM ATP
Microparticles are derived from the plasma membrane
Mechanism of ATP induced microparticle release

Bianco, F. et al. EMBO J 2009; 28, 1043-1054
Microparticles are derived from the plasma membrane
Western Blot of MPs prepared from cultured antigen-presenting cells (APC) following ATP stimulation
Phenotype analysis

Results
Morphology

M1

M2
M1/M2 phenotype in AD (affective disorder) and SZ (schizophrenia)

Results

**S-100**

**Arginase1**

**TLR3**

**TLR9**
M1/M2 characteristics in AD (affective disorders and SZ (schizophrenia))

Results

![Graphs showing M1/M2 characteristics in AD and SZ](image-url)
Cell types to be detected in CSF

http://www2.hawaii.edu/~johnb/micro/medmicro/medmicro.7.html
Cell derivatives to be detected in CSF


5µm
ATP induced release of microparticles and target cell fusion

P2X7 positive M2 macrophage

Microparticle release

Target cells

MP induce phenotypical and functional changes
Results

Typical microparticle derived from cultured antigen presenting cells
miRNA expression in ATP-induced MP

Four patients’ cultured antigen presenting cells were stimulated with ATP and qPCR was performed for the expression of miRNA species. All patients suffered from AD or SZ.
Summary

An inflammatory phenotype of in vivo activated APC in AD and SZ has been shown.
Prominent release of MPs from cultured APC occurs upon ATP-induced P2X7 ion channel activation

MPs are derived from the plasma membrane.
MPs transport miRNA species related to inflammation and stress or infection induced cognitive impairments.