Inflammation and Immunity in Stroke

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“In the last two years, I have not had a financial interest/arrangement or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this presentation.”
STROKE

Dirnagl et al. 1999; *TINS* 22:391-7
The inflammatory cascade after brain ischemia

1) Reaction of resident cells: glial reaction
2) Adhesion and infiltration of leukocytes
3) Molecular players: cytokines, chemokines, adhesion molecules, TLR, complement...

Dirnagl et al. 1999; *TINS* 22:391-7

Rojas et al, 07 *JCBF* 27:1975
Inflammatory markers as clinical predictors of outcome

Low plasma levels of IL-10 predict bad outcome and neurological impairment
Vila et al. (2003) Stroke

High plasma levels of IL-6 and TNF-α predict bad outcome and neurological impairment
Castellanos et al. (2002) Stroke

Mean ± SD delay to sampling 8.2 ± 5.7 hrs
- Lacunar stroke (n=113)
- Controls (n=43)
Ischemia induces danger signals activating the innate immune system

Innate Immunity
Cell Associated Receptors

Matzinger P, 2007 Nat Immunol

Kariko et al 2004 JCBF 24:1288
TLR expression increases after ischemia (Perez-de Puig et al., unpublished)

TLR-4 contributes to the brain lesion after ischemia in mice
Caso et al., Circulation. 2007

TLRs in monocytes of stroke patients
Urra et al., Stroke 2009

TLR2

TLR4

TLR expression increases after ischemia (Perez-de Puig et al., unpublished)

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Caso et al., Circulation. 2007

TLRs in monocytes of stroke patients
Urra et al., Stroke 2009
How does innate immune receptor activation contribute to inflammation?

Gorina et al., Glia 2011

VCAM-1

Justicia et al., 2006

J Cereb Blood Flow Metab. 26:421-32
The Complement System responds to stroke

The complement system is activated in rodents after brain ischemia

The complement system is activated in patients with acute stroke
The Lectin Pathway
Experimental ischemia/reperfusion in Manose-Binding Lectin (MBL) null and wt mice

Cervera et al., PlosOne 2010
The Lectin Pathway in stroke patients

Manose-Binding Lectin (MBL) :
MBL-sufficient and MBL-low genotypes

Baseline characteristics in the study population (n=135) according to MBL genotype

<table>
<thead>
<tr>
<th></th>
<th>MBL-low</th>
<th>MBL-sufficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=24 (18%)</td>
<td>N=111 (82%)</td>
<td></td>
</tr>
<tr>
<td>Age (mean, SD), yrs</td>
<td>73.9 (12.8)</td>
<td>72.9 (11.5)</td>
<td>0.67</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>9 (37.5)</td>
<td>59 (53.2)</td>
<td>0.16</td>
</tr>
<tr>
<td>Active smoking, no. (%)</td>
<td>3 (12.5)</td>
<td>20 (18.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>12 (50.0)</td>
<td>73 (65.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>Diabetes, no. (%)</td>
<td>6 (25.0)</td>
<td>24 (21.6)</td>
<td>0.72</td>
</tr>
<tr>
<td>Coronary heart disease, no. (%)</td>
<td>2 (8.3)</td>
<td>15 (13.5)</td>
<td>0.49</td>
</tr>
<tr>
<td>Previous stroke, no. (%)</td>
<td>4 (16.7)</td>
<td>19 (17.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Peripheral artery disease, no. (%)</td>
<td>2 (8.3)</td>
<td>9 (8.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Admission NIHSS score, no. (%)</td>
<td></td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>0 to 6</td>
<td>5 (21)</td>
<td>14 (13)</td>
<td></td>
</tr>
<tr>
<td>7 to 17</td>
<td>13 (54)</td>
<td>56 (51)</td>
<td></td>
</tr>
<tr>
<td>&gt;17</td>
<td>6 (25)</td>
<td>41 (37)</td>
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</tbody>
</table>

Cervera et al., PlosOne 2010
**Stroke patients**

60% MBL-low and 30% MBL-sufficient patients reached functional independence (mRS score 0 to 2) ($\chi^2$, $p=0.008$)

% GOOD OUTCOME AT 3 MONTHS

Cervera et al., PlosOne 2010
The complement can activate the coagulation cascade favoring secondary vessel occlusion

**Coagulation activates complement:**
- Platelets phosphorylate C3b and cleave C3
- Trombin and factor XIIa: cleave C3, C5

**Complement has Procoagulant activity:**
- Induces platelet activation
- ↑ Cellular TF expression (C5)
- Modifies phospholipid membranes and facilitates the extrinsic coagulation pathway (TF)

In infections, complement induces local thrombus formation as a barrier to prevent bacterial dissemination in the circulation.

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**Takahashi et al., 2011, Immunobiology 216:96–102**

**Endo et al., 2009 J Innate Immun 2:33-42**

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**Markiewski et al., TRENDS Immunol 2007**

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**Complement and coagulation: strangers or partners in crime?**
The cross-talk between the CNS and the immune system: *stroke-induced immunodepression facilitates infection*


Lymphocytopenia
Apopotosis of thymocytes
Change in cytokine production


Th1

Th2

Martin et al., *Mol Imaging*, 2008


Lymphocytes

Apoptosis

Infection
- Lymphocytes
- T cells

Prognosis
- Activated B cells
- B cell count

Monocytes

Phenotype

- HLA-DR
- TLR4

Function

- TNF-α
- IL-10

Infection

Prognosis

- HLA-DR
- CD49d
- CD86
- TNF-α
- TLR4

Monocyte subtypes

**CD16:** “bad guys” inflammation cancer

**CD14:** main subtype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>(CD_{14}^{\text{high}}CD_{16}^{-})</th>
<th>(CD_{14}^{\text{dim}}CD_{16}^{+})</th>
<th>(CD_{14}^{\text{high}}CD_{16}^{+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>85%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Function</td>
<td>Inflammation</td>
<td>TNF-(\alpha) Non-inflamed tissues</td>
<td>IL-10</td>
</tr>
</tbody>
</table>

Urra et al, JCBFM, 2009
Monocyte subtypes after stroke

Phenotype

Platelet-monocyte complexes

Infection

CD14\textsuperscript{high}CD16-\textsuperscript{}, OR
1,29
1,38
1,40

The organism becomes aware of brain damage and reacts to it through the immune system. The innate immune system is quickly activated and mediates secondary reactions.

But how and why???

Initiation of damage resolution and repair???
Certain immune responses can alter mechanisms of protection and repair

**Inflammation and innate immunity in neurogenesis**

Deficiency in TLR4 in the early postnatal retina results in increased neuronal differentiation

*Shechter et al 08 J Cell Biol 183:393*

**Regulatory T cells protect the brain after stroke**

*Rutkowski et al., Immunol Cell Biol. 2010*

**Complement affects neurogenesis and regeneration**

*CD14++CD16+Tie2+ monocytes*

Phagocytosis / Angiogenesis

*Protective effects in myocardial ischemia*

*Liesz et al, Nat Med, 2009; Planas & Chamorro, Nat Med, 2009*

Therapeutic strategies

Modulate post-stroke immune responses
Downregulate deleterious responses and enhance protective mechanisms

- Attenuate lymphocyte activity
- Promote the action of regulatory lymphocytes
- Prevent complement activation
- Limit proinflammatory cytokines
- Favour antiinflammatory cytokines
- Promote recovery
- Antibiotics
- Combine immunomodulatory and thrombolytic therapies

Will effective treatments in experimental ischemia translate into the clinic?

Urra et al., *Stroke* 2010
Conclusions

• Innate immunity plays an active role in inflammation and brain damage after stroke

• Genetics may affect the features and magnitude of inflammatory and/or immune responses: towards a more personalised treatment?

• Stroke induces a transient immunodepression that favours systemic infections

• The study of blood cells can improve our understanding of the interactions between brain and immune system in stroke

• The modulation of specific immunological targets offers new therapeutic avenues that are being explored

• Inflammation and innate immunity affect neurogenesis and repair. Understanding the intensity of the signals and their time course is essential for the design of novel therapeutic strategies.
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