Systemic immune activation shapes stroke outcome

Adam Denes
Head of Laboratory of Neuroimmunology
Institute of Experimental Medicine
Hungarian Academy of Sciences

Visiting Scientist
University of Manchester
UK

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Chronic diseases increase in developed countries

In 2004, 133 million Americans had at least one chronic condition
"Conventional" risk factors for stroke:

- Hypertension
- Atherosclerosis
- Diabetes
- Obesity
- Smoking

More recently:

- Infection

Inflammation contributes to brain injury

Elevated systemic inflammatory burden promotes brain disease and impacts negatively on outcome

Systemic inflammation is associated with microglia activation and neuroinflammation in humans and rodents

Drake et al., BBI, 2010
Leukocyte accumulation in the choroid plexus of ApoE KO mice fed high fat diet

Focal brain pathology

Interleukin-1 Mediates Neuroinflammatory Changes Associated With Diet-Induced Atherosclerosis
Experimental stroke – induction of focal cerebral ischaemia by MCAo:

- What happens in acute/chronic infection + stroke?
- What are the key effectors/mechanisms?

Does IL-1Ra reduce brain injury in aged animals with chronic systemic inflammation?

- Corpulent rats: obese, insulin resistant and atherosclerotic
- Lean controls
- Animals aged 16 months when submitted to transient, distal MCAo
Risk of stroke is associated with infectious burden (Elkind et al., 2010)

Increased risk of stroke associated with infectious burden among diabetics (Elkind et al., 2010)

Infectious burden associated with stroke risk and atherosclerosis was independently associated with cognitive performance (Katan et al., Neurology, 2013)

Early infections in tPA-treated patients are associated with worse outcome (Salat et al., 2010)

**Streptococcus pneumoniae** infection induces systemic inflammatory responses that drive atherogenesis and augment cerebrovascular pathologies in ischaemia via IL-1- and platelet-mediated mechanisms

- Larger aortic plaques after infection in C57Bl/6 mice fed atherogenic (Paigen) diet and cerebrovascular inflammation
- Larger brain injury after MCAo in infected mice and rats

Denes et al., Ann Neurol 2014
IL-1 is upregulated systemically in response to infection

Infection induces systemic granulocytosis

Blocking IL-1 by IL-1Ra is protective in infected mice

Platelets are activated in infected mice prior to stroke and platelet aggregates are increased in the brain after MCAo

Increased number of granulocytes in the brain in infected mice after stroke

Platelet GPIba blockade is protective in infected mice and downregulates ischaemia-induced microglial IL-1α

Denes et al., Ann Neurol 2014
Preceding chronic infection leads to larger infarct size in aged mice.

Plasma levels of RANTES, a proinflammatory chemokine, correlate with infarct volume in aged mice.

Dhugana et al., Aging Cell 2013

tPA increases bleeding complications and infarct size in mice with preceding influenza infection after MCAo (filament).

Muhammad et al., Stroke 2011

Systemic inflammation leads to increased brain injury.

IL-1
13.6.2016

How early can we detect BBB injury after stroke with SPECT imaging?

Szigeti et al., JCBFM, 2015

How does preceding systemic inflammation change BBB injury and perfusion after stroke?

Szigeti et al., JCBFM, 2015
Preceding systemic inflammation leads to impaired outcome after stroke

Szigeti et al., JCBFM 2015

Denes et al. J neuroinflammation, 2011

Stroke-associated inflammation / infection:

- Lungs
- Urinary tract
- Gut
- Spleen
- Bone marrow
- etc.
How does the gut microbiome change after stroke?

Experimental stroke in C57BL/6 mice

- Naive
- Sham
- Stroke

4h and 72h survival

Peptococcaceae and Prevotellaceae show significant changes 72h after brain injury independently of effects of surgical stress after PERMANOVA and correction for FDR

What are the mechanisms of brain injury-induced microbiota changes?
Noradrenaline levels in the gut tissue increase 72h after brain injury and correlate with neurological deficit.

Neurological deficit 72h after brain injury correlates with changes in Peptococaceae and Prevotellaceae.

Neuronal NE uptake and release in the cecum are increased 72h after brain injury.

Brain injury alters mucoprotein production in the cecum.
Manipulation of the autonomic nervous system changes the gut microbiota

SPECT / CT

Szigeti et al., JCBFM 2016

SPECT imaging shows that experimental stroke induces rapid and transient changes in the gut barrier

SPECT / CT

Szigeti et al., JCBFM 2016
SPECT imaging reveals lung inflammation within 2h after experimental stroke

Do pattern recognition receptors activated by bacterial products or damage molecules contribute to brain injury after stroke?

- TLR4 KO mice have smaller brain injury after experimental stroke (Caso et al., 2007)

- ASC, a common adaptor protein of several inflammasomes is a key regulator of brain injury after stroke

- Peptidoglycan (major cell wall component of Gram-positive bacteria) does not contribute to brain injury via the NOD2 inflammasome
- NLRC4: activated by flagellin
- AIM2: activated by DNA

Both NLRC4 and AIM2 inflammasomes are key regulators of brain injury after stroke

PNAS, 2015

**What is the source of IL-1 that contributes to brain injury in stroke?**

- Brain injury is reduced in mice transplanted with IL-1αβ KO bone marrow
  
  Denes et al., DMM 2013

- Blood cell-derived IL-1 is a key mediator of ischaemic brain injury
- Non-hematopoietic (microglial?) IL-1 is also important
Selective depletion of microglia by a CSFR1 kinase inhibitor (PLX3397)

- 97-99% of microglia are eliminated from the brain within 3 weeks of treatment
- Microglia depletion is not associated with any sign of illness, weight loss, motor or cognitive impairment

Absence of microglia results in markedly increased infarct size

Szalay et al., Nat Comms, 2016
What early and late (inflammatory) changes contribute to brain injury after stroke?

Novel imaging approaches are required to understand the mechanisms involved.

Remote filament model of Middle Cerebral Artery occlusion and two photon imaging with genetically encoded Ca\(^{2+}\) indicators

- Pre occlusion imaging (15-30min)
- 60min MCAo
- 4h reperfusion
- Sampling every 5min (1-2min long video rec.)

Szalay et al., Nat Comms, 2016
Fast in vivo two photon calcium imaging reveals delayed development of excitotoxicity after cerebral ischemia induced by a remote filament

Microglia interact with neurons in an activity-dependent manner and respond to SD after brain injury induced by cerebral ischemia
Absence of microglia dysregulates neuronal activity after cerebral ischemia I.

Szalay et al., Nat Comms, 2016

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