Stroke Impairs Epithelial Microfold Cells in Intestinal Peyer’s Patches

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• No conflicts to disclose.
Post-stroke infection (PSI)

- Infections produce major complications in stroke patients.
- Overall rate of post-stroke infections (PSI) can be as high as 24-36% in the first week after stroke.
- Elderly stroke patients often succumb to systemic complications, rather than the brain injury itself.
- However, our understanding of the relationship between infection and stroke is still in the earliest stages.

PNA: pneumonia
UTI: urinary tract infection
We correlated the stroke severity (NIHSS) to systemic bacterial burden in the patients with acute ischemic stroke (AIS).

LBP (LPS-binding protein): one of biomarkers for sepsis in AIS patients

Patients with more severe strokes had significantly higher LBP in their circulating blood.
The involvement of the gut in stroke and PSI

Ischemic stroke

Microbiota change (e.g., F:B ratio↑, pathogenic1-4)

Luminal side

Epithelium (e.g., TJ↓5)

Peyer’s patch? (largely unknown7, 8)

Immune cell change (e.g., inflammatory4, 6)

1Ann Neurol 84 (2018) 23-36
3Brain Behav Immun 66 (2017) 23-30
4J Neurosci 36 (2016) 7428-40
5Aging 8 (2016) 1049-63
6Nat Med 18 (2016) 858-9
7Neurosci Lett 29 (2017) 165-170
8Neuroimmunomodulation 16 (2009) 213-8
Peyer’s patch: the primary lymphatic tissue in the gut

- PP is a lymphatic tissue that serves as an immune sensor of the small intestine.
- Composition: follicle-associated epithelium (FAE) + aggregated lymphoid follicles (LF)
- The FAE contains enterocytes and specialized cells named microfold (M) cells, with scarce mucus-secreting goblet cells.
- These M cells are responsible for antigen (e.g., bacteria) uptake toward the underlying immune cells that activate or inhibit the immune response leading to either tolerance or systemic immune cell response.
- Despite the important role of PP and M cells in gut immune responses, it is not characterized because of their rarity in the gut.
- PP and M cell change after stroke?

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Mouse small intestine

Peyer’s patch

Villus

Follicle-associated epithelium (FAE): M cells, enterocytes

Lymphoid follicle (LF): aggregation of immune cells
1. **Mouse information**

   - Young male (Charles River): 2-3 months
   - Aged male (NIA, Charles River): 18-20 months

2. **Timeline**

   - Time (d)
     - 0
     - 1
     - 2
     - 3
   - MCAO (60 min)
   - Sacrifice
     - 1. Macroscopic observation
     - 2. M cell localization
     - 3. Gene expression
     - 4. Immune cell analysis
Stroke decreased number of PPs in the small intestine

(A) Sham Stroke

(B) Young Aged

No of PP/mouse

0 5 10 15

Sham Stroke

No of PP/mouse

0 2 4 6 8

Sham Stroke

Intestinal length

Length (cm)

NS

Sham Stroke Sham Stroke

SI LI
Stroke disrupted epithelial cells in the small intestine

Villus

Villus M cell (red)

Enterocyte (green)

Goblet cell (yellow)
Stroke decreased the size of FAE and M cells
Stroke caused impaired gene expression for maturation, differentiation and function of M cells in PP

1. Mature M cells (M cell-specific marker)
2. Bacterial uptake receptor (e.g., FimH bacteria)

M cell differentiation (RANKL pathway)

Immature/differentiating M cells
Stroke did not alter immune cell profiles in PP follicles

**T cells (CD4⁺ or CD8⁺)**

- **Sham**:
  - CD4: 19.1 ± 47.2
  - CD8α: 52.0 ± 47.2

- **Stroke**:
  - CD4: 19.0 ± 47.2
  - CD8α: 47.2 ± 47.2

**B cells (CD19⁺B220⁺)**

- **Sham**:
  - B220: 84.4 ± 85.2

- **Stroke**:
  - B220: 85.2 ± 85.2

**Dendritic cells (B220⁻CD11c⁺)**

- **Sham**:
  - CD11c: 13.6 ± 11.7

- **Stroke**:
  - CD11c: 11.7 ± 11.7

Fold change (to sham)

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<thead>
<tr>
<th>Cells</th>
<th>Sham</th>
<th>Stroke</th>
<th>NS</th>
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<tbody>
<tr>
<td>CD4⁺ T cells</td>
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<tr>
<td>CD8⁺ T cells</td>
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<td>B cells</td>
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<td>Dendritic cells</td>
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</table>

SH: Sham
ST: Stroke
Conclusion

• We show that the number of PP, the area of FAE and the density of M cells are dramatically reduced following stroke.

• The response of PP in stroke is highly epithelial M cell-specific.

• Our data provides us with a new insight into how brain damage can alter antigen sampling and shape adaptive immunity in the host after stroke, and how we consider oral treatment for stroke.
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