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学 位 論 文 題 目　Dual effects of microglia on blood brain barrier permeability induced by systemic inflammation

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博士論文の要旨

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論文題目　　Dual effects of microglia on blood brain barrier permeability induced by systemic inflammation

Microglia are the sole immune cells that reside in the central nervous system in embryonic stage. Microglia are activated in neurological diseases such as multiple sclerosis, Alzheimer's disease, and epilepsy, and are involved in the pathology by releasing neurotrophic factors and cytokines that act on neurons, glia and blood vessels. Production of inflammatory cytokines from activated microglia increases blood-brain barrier (BBB) permeability and may promote immune cell infiltration and brain inflammation. On the other hand, in autoimmune diseases such as systemic lupus erythematosus (SLE) and severe infections are known to increased BBB permeability, activated microglia and associate with psychiatric symptoms such as cognitive impairment, anxiety and depression. However, it is not clear how the activation of the immune system in these diseases acts on microglia and causes abnormalities in the central nervous system. Therefore, the purpose of this study was clarifying how microglia reacts to systemic inflammation separated by BBB and how it affects the change in permeability of BBB.

I observed the response of microglia to systemic inflammation using Murphy Roths Large-lymphoproliferation strain (MRL/lpr) mice, known as autoimmune model mice, and lipopolysaccharide (LPS)-treated mice in which inflammation was induced by intraperitoneal injection of LPS. *In vivo* imaging using a two-photon microscope revealed that microglia migrated and accumulated around the cerebral blood vessels with systemic inflammation. Chemokine signal inhibition by chemokine receptor inhibitors revealed that C-C motif chemokine ligand 5 (CCL5)- C-C motif chemokine receptor 5 (CCR5) signal pathway plays a crucial role in the migration of microglia to

the vessels. Visualization of BBB permeability by intravenous administration of fluorescent dextran tracer revealed that the contact between microglia and vessels plays an important role in BBB permeability changes. It was found that daily injection of LPS promoted the leakage of the 10-kDa fluorescein dextran into the brain parenchyma. It was also found that microglial accumulation around the vessels occurred before permeability changes. In addition, transgenic mice that can induce microglia-specific expression of diphtheria toxin by the withdrawal of doxycycline were used to evaluate BBB permeability during microglia ablation. Microglia ablation increased leakage in the early phases of systemic inflammation, and conversely suppressed leakage during prolonged inflammation. These results suggest that microglia protect leakage at the early phase of inflammation and worsen leakage during chronic inflammation. To clarify the details of microglia functions, microglia were isolated and collected from MRL/lpr mice, and gene expression was comprehensively analyzed. As a result, I found increased expression of phagocytosis-related genes and tight junction genes. The observation of microglia and vessels in MRL/lpr mice with an electron microscope revealed that microglial processes expressing tight junction molecule Claudin-5 (CLDN5) were in direct contact with vascular endothelial cells. Furthermore, immunostaining the formalin-fixed brain slices of mice with daily LPS injection showed that CLDN5 was transiently expressed during early phases of inflammation and that the expression of Cluster of Differentiation 68 (CD68), a phagocytic marker, increased during prolonged inflammation. In addition, it was found that Aquaporin-4 (AQP4), which is a constituent molecule of BBB, was included into the microglia phagocytic vesicles. This result suggests that microglia are involved in the failure of the BBB structure by phagocytosing astrocyte end-feet. Moreover, it was found that leakage during prolonged inflammation was significantly suppressed by administering minocycline that inhibits microglia activation.

In conclusion, I found that microglia have two functions for BBB integrity.

One is the BBB protective effect of early systemic inflammation, and the other is the BBB injury effect during chronic systemic inflammation. During the early phases of systemic inflammation, microglia expressed CLDN5 and accumulated around the vessels to suppress the increased BBB permeability. In contrast, microglia became a phagocytic phenotype in prolonged inflammation, and they disrupt BBB structure by phagocytosing the astrocyte end-feet. In addition, the CCL5-CCR5 pathway was identified as an important signal for migration of microglia to the vessels and BBB protection during early phases of inflammation. From the results of this study, vessel-associated microglia are expected as a therapeutic target for central nervous system symptoms caused by systemic inflammation.

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博士論文審査結果

Name in Full
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Title
論文題目　　Dual effects of microglia on blood brain barrier permeability induced by systemic inflammation

本論文は、脳内の免疫細胞であるミクログリアが血液脳関門の機能を制御すること、およびそのメカニズムを初めて明らかにした研究である。

血液脳関門（BBB）は、脳環境を体循環系の環境と隔離する構造で、脳内外への物質の行き来を制限することで脳内の環境を一定に保つ働きがある。感染症や自己免疫疾患などの全身性炎症によって BBB 機能は低下することが知られている。しかし、全身性の炎症がどのように BBB 機能を破綻させるのか、詳細なメカニズムは不明であった。

出願者は、生きたマウスの脳を観察することができる生体２光子顕微鏡を用いて、全身性の炎症が誘導されたモデル（MRL/1pr）マウスや lipopolysaccharide (LPS)投与マウス大脳の同一部位のミクログリアを経日的に観察し、その動きと血管の透過性を観察することで、ミクログリアの BBB への影響を調べた。その結果、血管にはミクログリアが多く密着しており、炎症によってミクログリアが更に血管に集積すること、それに伴って BBB の透過性が変わることを明らかにした。ミクログリアを除去したマウスでは、炎症早期に BBB 透過性が増加し、後期では BBB 透過性が抑制されていた。遺伝子の網羅的解析を行った結果、早期では細胞接着分子クローディン５（CLDN5）がミクログリアに発現し、後期では貪食関連分子 CD68 がミクログリアに発現することがわかった。電子顕微鏡を用いてミクログリアと血管の微細構造を観察したところ、CLDN5 陽性ミクログリアが血管内皮細胞に密着しており、ミクログリアが血管の漏れをシールする可能性が示された。

ミクログリアは血管内皮細胞から放出されるケモカイン CCL5 に応答して血管に引き寄せられ、CLDN5 を発現することが示された。CCL5 を阻害すると、ミクログリアの血管への集積は起こらず、結果的に血液の漏出が早まる現象も確認された。一方、後期ミクログリアは BBB を構成するアストロサイトの突起を一部貪食していることも明らかにした。アストロサイトの突起は BBB の維持に重要であることが知られており、この構造の破綻を引き金として血液の漏出が起こる可能性が示された。さらに、ミクログリアの活性化を抑制する抗生物質をマウスに投与したところ、ミクログリアの血管集積と初期の BBB 保護に影響を及ぼすことなく、慢性期の BBB 破綻を抑制できることも実証された。

本研究成果は、ミクログリアが BBB 機能の制御に重要な役割を担うことを示した生理的・臨床的にも意義深い研究であり、博士学位にふさわしい成果と評価できる。