Real time dynamics of innate immune cell migration in mouse brain during inflammation using two-photon intravital imaging

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Directed by Professor Young-Min Hyun

The Master's Thesis
submitted to the Department of Medical Science
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Yu Rim Kim

June 2020

This certifies that the Master's Thesis
of Yu Rim Kim is approved.

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June 2020

ACKNOWLEDGEMENTS

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2020년 7월 김유림 올림.

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ABSTRACT

Real time dynamics of innate immune cell migration in mouse brain during inflammation using two-photon intravital imaging

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Neuroinflammation plays an important role in the pathogenesis of various diseases that involve acute and chronic inflammation, including Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis. There exist diverse types of interactions that can happen between the brain tissue-resident cells and the peripheral immune cells following neuroinflammation.

In this study, I discussed how migratory patterns and morphological changes regarding the interaction between neutrophils and microglia occur during acute inflammation in the central nervous system (CNS).

To directly observe cellular responses using two-photon intravital microscopy, $LysM^{GFP/+}$ and $CX_3CR1^{GFP/+}$ mice were used for the observation of neutrophils and microglia, respectively. Especially, $CX_3CR1^{GFP/+}$ mice were intravenously injected with PE-conjugated anti-Ly6G antibody and were used to observe cellular responses between neutrophils and microglia. In order to investigate acute inflammatory response of the CNS in mice, lipopolysaccharide (LPS) 1.0 mg/kg was intraperitoneally injected into mice for two consecutive days. To monitor cellular responses of the brain tissue via time-lapse imaging, a cranial window chamber was set up for two-photon intravital microscopy.

The number of infiltrated neutrophils in the LPS group was significantly increased compared to the control group. Also, infiltrated neutrophils actively migrated within a 20 μm radius during neuroinflammatory response. Observation of neutrophils and microglia was subsequently conducted to identify possible interactions between neutrophils and microglia. Taken together, these results demonstrated that neutrophils directly come into contact with microglial processes in diverse ways. One result showed that microglial processes touched and surrounded that neutrophil and maintained contact for 30 min. The other result showed that microglial processes were elongated toward colocalization sites of microglia and neutrophils, which were comprised of microglia-engulfed neutrophils. Based on these results, it can be suggested

that interactions between neutrophils and microglia takes place via direct touch between these cells.

Although the mechanism of interaction between neutrophil and microglia was not analyzed, this study suggested that the analysis of immune cell migration using two-photon intravital microscopy give a chance to contribute to research in immunology.

Key words: neuroinflammation, neutrophil, microglia, two-photon intravital imaging

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I. INTRODUCTION

Neuroinflammation is the response of brain cells to infections and other sources of cell death and involves infiltration of blood-derived lymphocytes and monocyte-derived macrophages. The infiltration of immune cells occurs by microglia and glial cell activation and blood-brain barrier (BBB) dysfunction during the pathogenesis of Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis[1,2]. Also, fungal infection of the central nervous system (CNS) is reinforced by fungus-specific risk factors concerning HIV infection, neutropenia, and corticosteroid use[3].

Microglia, the resident macrophages of the CNS, are distinguished from other glial cells such as astrocytes and oligodendrocytes by their gene expression, morphology, and function[4-6]. In contrast to other glial cells, microglia function as the primary reacting cells for regulating neuroinflammatory response by phagocytizing and removing myelin inhibitors, debris and dead cells in the CNS[4,7]. Microglia also takes part in innate and adaptive immunity by regulating immune tolerance[8]. Microglia are functionally and morphologically divided into three forms: the ramified, activated and ameboid morphologies. Ramified microglia, with a small cell body and long branches, have no functional capability of phagocytosis and antigen presentation but maintain an immunologically stable environment. When ramified microglia are stimulated by neurodegeneration, endotoxin, interferon, or endothelial activation, activation pathways cause them to transform into activated microglia. Activated microglia exhibit thicker and more retracted branches and possess the ability exhibit antigen presentation and phagocytosis. Additionally, activated microglia, when changed to the ameboid shape, display free movement during phagocytosis but do not engage in antigen presentation and inflammation[9]. In addition, excessive or long-term activation of microglia induces neuronal death and an increase in pro-inflammatory cytokines[10].

Neutrophils, the first responders against acute inflammation, help organs begin and retain immune reactions[11]. Neutrophils participate in the general immune response by signaling to diverse cell types including endothelial cells, mesenchymal stem cells, lymphocyte and microglia during

neuroinflammation[12]. Additionally, neutrophils are remarkably versatile cells, which mediate inflammation by infectious and sterile injuries, capture microbes with granular protein, and repair sterile wounds[13]. Recent reports have emphasized that neutrophils play a variety of roles in neuroinflammation, where brain resident cells participate in a coordinated fashion[14]. Yet despite intensive investigation about neutrophils and microglia, the interaction of microglia and neutrophils regarding neuroinflammation is debated.

In this study, I mainly conducted intravital imaging using two-photon microscopy. In contrast of confocal microscopy, which uses a single-photon system, two-photon microscopy simultaneously excites two photons in a single event. Therefore, two-photon microscopy has photons with longer wavelengths (700-1,000 nm) and less energy than confocal microscopy. Due to these mechanisms, two-photon microscopy has been known as a powerful tool to closely image live animal tissue for lengthy time periods in three dimensions and great depths. Particularly, two-photon microscopy is able to represent cellular and molecular phenomena including anatomic structure, cytokine gradients, and cell to cell interaction[15,16]. Therefore, two-photon intravital imaging can be a useful tool to investigate immune cell migratory patterns in the brain in a time-lapse manner, which could demonstrate unknown phenomena in immune response concerning neuropathological studies.

In the present study, I aimed to investigate migratory patterns and morphological changes about the relationship between neutrophils and microglia during neuroinflammation *in vivo* using two-photon microscopy.

II. MATERIALS AND METHODS

1. Mice

$LysM^{GFP/+}$ and $CX_3CR1^{GFP/+}$ transgenic mice, in which the lysozyme and the CX_3CR1 gene are replaced by green fluorescent protein (GFP), respectively, were obtained and used for visualizing neutrophil and microglia[17,18]. All mice were kept in a room with a specific pathogen-free (SPF) condition, a light cycle from 7:00 AM to 7:00 PM at 23±2℃, and 55±10% humidity. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC, 2019-0097) of Yonsei University College of Medicine, Korea.

2. Identification of mouse genotype

Genotyping for each strain ($LysM^{GFP/+}$ and $CX_3CR1^{GFP/+}$ transgenic mice) was performed. Mouse genotyping was conducted using a Genomic DNA Prep Kit (BioFACT, Daejeon, Korea). A toe of 7-10 day-old mice was cut, and then DNA extraction from toes was conducted using Genomic DNA Prep Kit. Template DNA (50 ng/μl), primers and 2xTaq PCR master mix2 10 μl (BioFACT, Daejeon, Korea) were mixed to PCR tube. Distilled water up to 20 μl reaction volume was added to PCR tube. Samples were performed in the PCR machine as cycling condition.

3. Cranial window surgery

The cranial window was implanted on the calvaria for intravital brain imaging as described previously[19,20]. Mice were deeply anesthetized using intraperitoneal injection of zoletil (Virbac, Carros, France) at a dose of 30 mg/kg and rompun (Bayer, Germany) at a dose of 10 mg/kg. Body temperature in each mouse was maintained at 37±0.5℃ using a stereotactic instrument with heating system (Live Cell Instrument, Seoul, Korea) during cranial window surgery (Fig. 1). Mice were fixed in a stereotactic instrument (Live Cell Instrument, Seoul, Korea) during all procedures (Fig. 2). A cranial window of 5 mm in diameter was made in the right hemisphere. The head skin and the periosteum on the calvaria were removed from between the eyes to the caudal region of the ears (Fig. 2A, 2B). Between the lambda and bregma regions on the right hemisphere, the circular opening was carved with a micro drill, frequently washed with cool phosphate-buffered saline (PBS), and sealed with a round coverslip (diameter = 5 mm) using tissue glue on the skull using the optical microscope (Fig. 2C, 2D). The metal frame was glued and fixed using dental cement (B.J.M laboratory, Or-Yehuda, Israel) on the borders of the cranial window and skull area for filling imaging area with distilled water (Fig. 2E). The metal frame was assembled with a head fixation device (Live Cell Instrument, Seoul, Korea) attached to the heating plate (Fig. 2F).

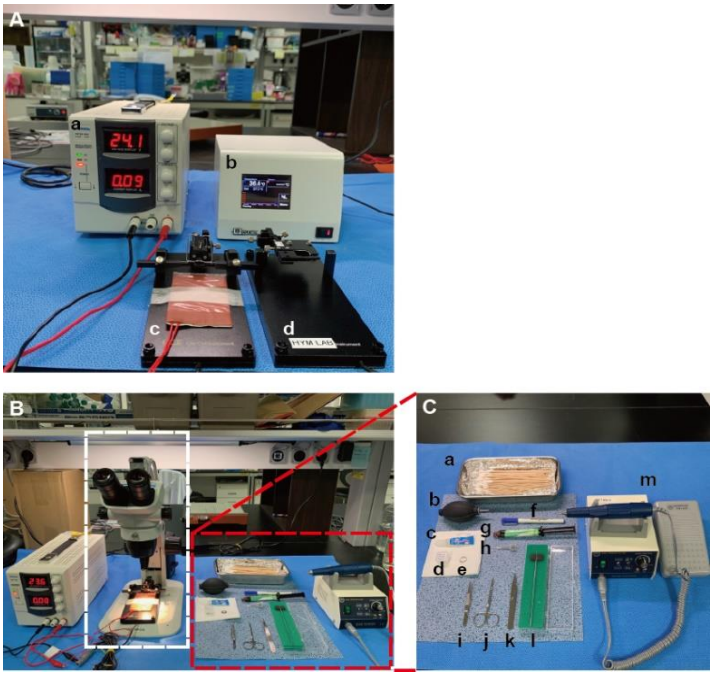


Figure 1. Brain chambers and subsidiary tools for cranial window surgery. **(A)** Brain chambers with heating plates for maintaining body temperature at 37±0.5℃. **a.** Metal and rubber heating plates, **b.** DC temperature controller, **c.** Stereotactic instrument for cranial window surgery, **d.** Head fixation device for imaging. **(B)** Optical microscope (white box) and surgical tools (red box). **(C)** Subsidiary tools for surgery **a.** cotton swab, **b.** air blower, **c.** tissue glue, **d.** cover glass (diameter = 5 mm), **e.** metal frame, **f.** pen, **g.** dental cement, **h.** resin tip, **i.** forceps, **j.** a pair of scissors, **k.** forceps, **l.** micro forceps, **m.** micro drill.

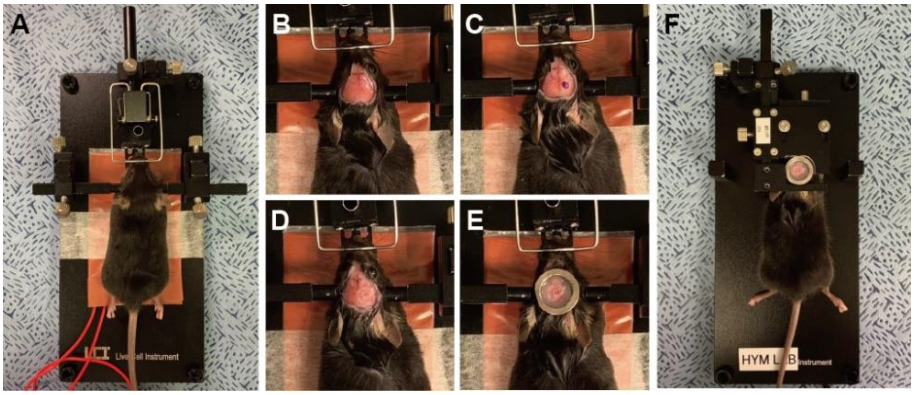


Figure 2. Surgical procedure of cranial window surgery for intravital brain imaging. (A) Fix the mouse in a stereotactic heating plate. **(B)** Remove head skin and the periosteum. **(C)** Coordinate predestinate location carved on right hemisphere. **(D)** Moisturize a circular opening with a drop of isotonic saline solution and seal a circular opening with a 5 mm coverslip. **(E)** Glue and fix the metal frame on the borders of the cranial window and skull area using dental cement. **(F)** Place the mouse on a head fixation device for intravital imaging.

4. Two-photon intravital microscopy

Mice were anesthetized using intraperitoneal injection of Zoletil at a dose of 30 mg/kg and rompun at a dose of 10 mg/kg during imaging. The imaging stage was composed of a XY micro stage and a head fixation device connected to a DC temperature controller (Fig. 3). Two-photon microscopy with W Plan-Apochromat 20x /1.0 water immersion lens (LSM7MP, Carl-Zeiss, Jena, Germany) was used for imaging data generation, and Zen software (Carl-Zeiss, Jena, Germany) was utilized for imaging acquisition. $LysM^{GFP/+}$mice were intravenously injected with 70-kDa Texas red dextran (2.5 mg/kg, Sigma-Aldrich, Darmastadt, Germany) for visualizing blood vessels. $CX_3CR1^{GFP/+}$mice were intravenously injected with CF405M-conjugated Wheat germ agglutinin(WGA) (2.5 mg/kg, Biotium, Fremont, CA, USA) for visualizing blood vessels and PE-conjugated anti-Ly6G antibody (0.1 mg/kg, BioLegend, San Diego, CA, USA) for observing neutrophils. For two-photon excitation, each mouse brain was excited with light of 800 nm and 880 nm wavelength for imaging of green, red, and blue. All images were acquired at a resolution of 512×512 pixels using steps of size 1 μm to a depth of 40-50 μm for 1 min[21].

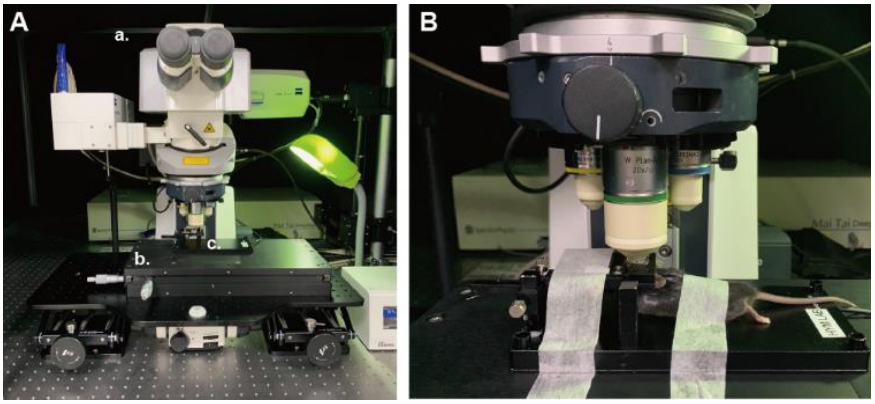


Figure 3. Carl-Zeiss LSM 7 MP and the preparation of intravital imaging.

(A) The view of stage system. **a.** Carl-Zeiss LSM 7 microscope, **b.** The XY micro stage, **c.** The head fixation device connected to DC temperature controller. **(B)** The view of mouse placed on the head fixation device connected to DC temperature controller.

5. LPS-induced neuroinflammatory mouse model

Previous studies established that an LPS-induced inflammation in the periphery can prompt immune responses in the central nervous system[4,22]. To investigate the migratory patterns of neutrophil and microglia during neuroinflammation, mice were given daily intraperitoneal injections of LPS from *Salmonella enterica* serotype enteritidis purified by phenol extraction (1.0 mg/kg, Sigma-Aldrich, Darmastadt, Germany) on two consecutive days. Control mice were intraperitoneally injected with daily PBS injections for two consecutive days. Intravital imaging was performed at 6 hr after LPS injections for two consecutive days.

6. Imaging data analysis

The Volocity software 6.3.1 (PerkinElmer, Waltham, MA, USA), IMRIS ver.7 (Bitplane, Zurich, Swizerland), and Fiji/Image J software were used for 3D and 4D imaging data analysis.

7. Statistical analysis

All experiments were replicated at least three times. Statistical analyses were expressed as mean ± standard error of the mean (S.E.M). Statistical analysis was performed using Prism 7.0 (GraphPad, San Diego, CA, USA). For comparison of two groups, unpaired two-sided Student t-tests were applied. p values less than 0.05 were considered statistically significant.

Ⅲ. RESULTS

1. LPS injection induces weight loss in mice

It has been established that inflammation in the periphery can prompt systemic immune response. Mice were divided into treatment groups receiving two daily intraperitoneal injections of phosphate-buffered saline (PBS) or low-dose lipopolysaccharide (LPS) 1.0 mg/kg. Measurement of mouse weight was performed before the first injection and at 6 hr after second injection for two consecutive days. The LPS group showed 11.75 % more weight loss compared to the control group (Fig. 4). Weight loss in LPS-injected mice may imply signs of sickness, such as prostration, decrease in feeding behavior, and increase of peripheral pro-inflammatory cytokine levels[23].

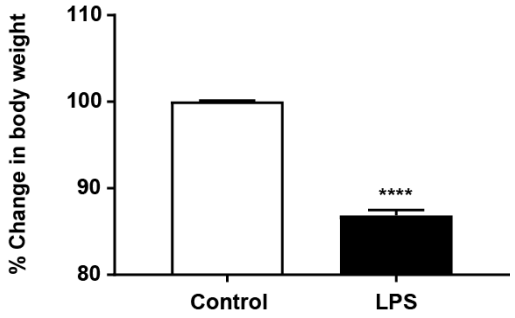


Figure 4. Weight loss of LPS-injected mouse. Representative graph of weight loss in the LPS-treated group compared to the control group. The % Change in body weight indicated (Before LPS injection / After LPS injection) × 100 (n=7 mice per group). Data indicate mean ± SEM, ****$p < 0.0001$

2. Neutrophils infiltrate the brain parenchyma during neuroinflammation

Previous studies have demonstrated that neutrophils are recruited in the brain during LPS-induced systemic inflammation[24,25]. Two-photon intravital time-lapse brain imaging was conducted using LysM$^{GFP/+}$ mice with neutrophils, which display brighter expression of green fluorescent protein (GFP) than monocytes. Since the neutrophil population in the circulatory system is much higher than the monocyte population, most of the GFP-expressing cells are considered to be neutrophils[17,26]. Therefore, LysM$^{GFP/+}$ mice were used for observing neutrophils, which were intravenously injected with Texas red dextran to visualize blood vessels. To investigate migratory patterns of neutrophils, an LPS-induced mouse neuroinflammation model was established. The results showed that the number of infiltrated neutrophils in the LPS group was significantly increased compared to the control group (Fig. 5A, 5B). It was also shown that neutrophils exhibited transendothelial migration (TEM) in brain parenchyma in the LPS group (Fig. 5C).

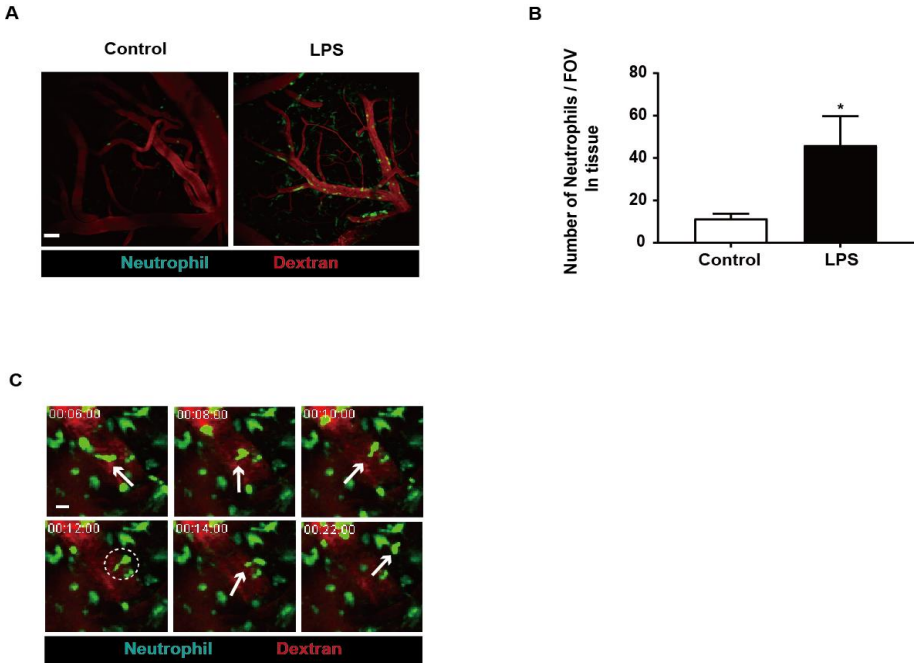
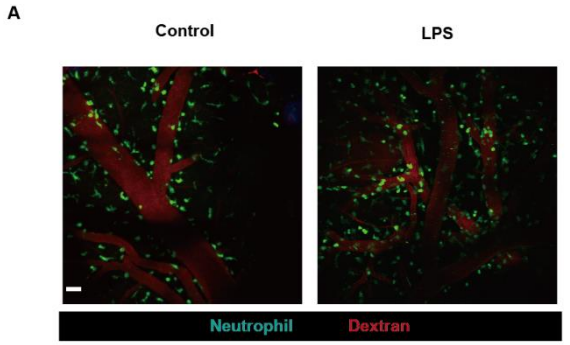
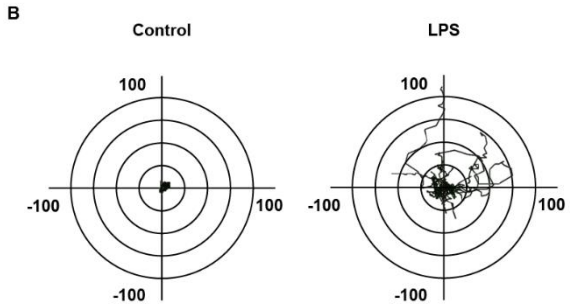


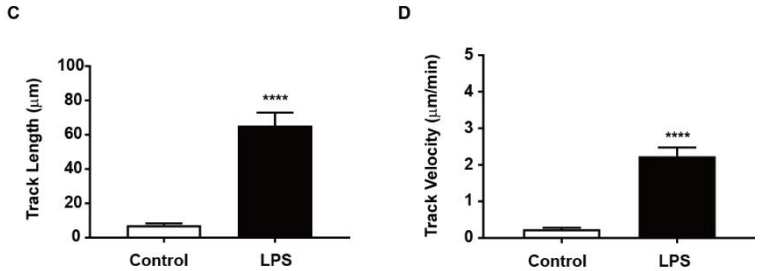
Figure 5. Infiltration of neutrophil from blood vessel into inflamed brain parenchyma. **(A)** Snapshot of intravital images for counting neutrophils (green) using $LysM^{GFP/+}$ mice injected with PBS (control) or LPS and Texas red dextran for visualizing blood vessels (red). Scale bar, 50 μm. **(B)** Representative graph of many infiltrated neutrophils in LPS-treated mice compared to control mice. Data indicate mean ± SEM, $*p < 0.05$, n=3 mice per group of three independent experiments, FOV; Field of view = 2.7 x 10^6 μm^3. **(C)** Transendothelial migration of neutrophil in inflamed brain parenchyma of $LysM^{GFP/+}$ mouse. Scale bar, 20 μm.

3. Neutrophil motility increases in inflamed brain parenchyma

To understand the contribution of neutrophil motility to LPS-induced neuroinflammation, tracking data of neutrophils in brain parenchyma was analyzed in PBS- and LPS-injected mice (Fig. 6A). Migration of neutrophils was displayed in snapshots of intravital images, which were illustrated with tracking graphs (Fig. 6B). The motility of neutrophils was determined by the track length, track velocity, displacement, and meandering index. Track length and track velocity of migrating neutrophils in the brain parenchyma showed statistically significant differences in the LPS group (Fig. 6C, 6D). Additionally, these results demonstrated that neutrophils showed active dynamics such as TEM (Fig. 5C), reverse-TEM, and interstitial migration during neuroinflammation (Fig. 7). Finally, these results especially showed that infiltrated neutrophils persistently migrated within a 20 μm radius for 30 min during neuroinflammatory response (Fig. 6E, 6F). Based on these results, neutrophils were thought to persistently interact with their surroundings such as astrocytes, microglia, and adaptive immune cells during neuroinflammatory response[24,27].







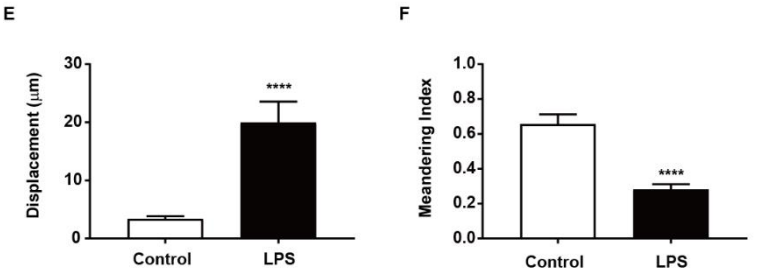


Figure 6. Active motility of migrating neutrophils in inflamed brain parenchyma compared to control group. (A) Snapshot of intravital images for trafficking neutrophils (green) using $LysM^{GFP/+}$ mice injected with PBS (control) or LPS and Texas red dextran for visualizing blood vessels (red). Scale bar, 50 μm. **(B)** Overlay of the track of representative migration tracking of neutrophils in brain parenchyma for 30 min. x, y axis (length), -100~100 μm. n=30 cells per group, PBS or LPS-treated mice of three independent experiments. Migration was quantitatively assessed with the analysis of tracking : **(C)** track length (μm), **(D)** track velocity (μm/min), **(E)** displacement (μm), and **(F)** meandering index (displacement/length) in two different conditions for 30 min. Data indicate mean ± SEM, ****$p < 0.0001$, n=30 cells per group of three independent experiments.

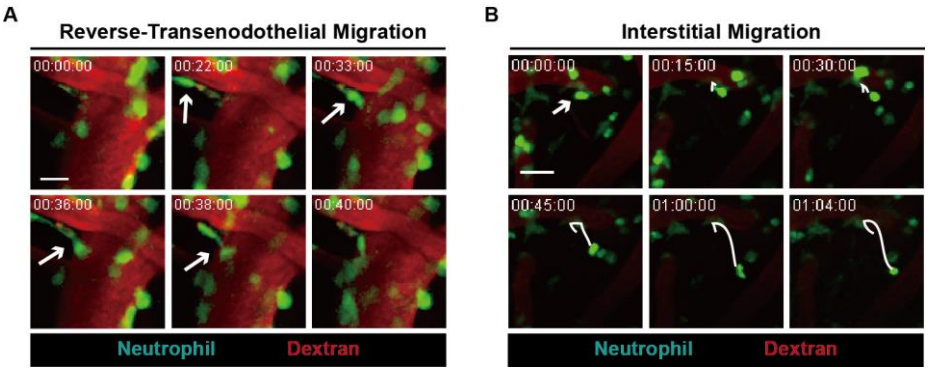


Figure 7. Diverse migratory patterns of neutrophils on the neuroinflammatory response. Snapshot of time-dependent intravital images of neutrophil (green) migration using $LysM^{GFP/+}$ mice injected with Texas red dextran for visualizing blood vessels (red). **(A)** Reverse-transendothelial migration of neutrophil from brain parenchyma into blood vessel. **(B)** Interstitial migration of neutrophil in brain parenchyma. Scale bar, 20 μm.

4. LPS-induced neuroinflammation leads to the loss of microglia and the morphological change of microglia

Previous results showed that infiltrated neutrophils persistently migrated within a 20 μm radius for 30 min (Fig 6). Thereafter, the possibility of neutrophils persistently interacting with their surroundings during neuroinflammation was explored. Specifically, it was possible that neutrophils could interact with microglia, which are the largest population of myeloid cells in the CNS[28]. To investigate the migratory patterns and morphological changes in microglia, two-photon intravital brain imaging was conducted using $CX_3CR1^{GFP/+}$ mice with microglia-specific expression of green fluorescent protein (GFP). After $CX_3CR1^{GFP/+}$ mice were intravenously injected with CF405M-conjugated WGA for visualizing blood vessel structure, time-lapse imaging was performed (Fig 8A). These results showed that the number of microglia in LPS-injected mice decreased compared to the control group (Fig. 8B). In morphological analysis, the morphology of ramified microglia in the control group transformed to that of activated microglia, with large cell bodies and short and thick processes, in the LPS group (Fig. 8C). These results demonstrated that microglia were activated by LPS-induced neuroinflammatory response[10]. Quantification of microglial numbers was done by the presence or lack of GFP expression in $CX_3CR1^{GFP/+}$ mice. According to Fortin M and Steff AM et.al, one possible reason of the

decrease in microglial numbers was suggested to be cell death by a variety of apoptosis-inducing agents[29,30].

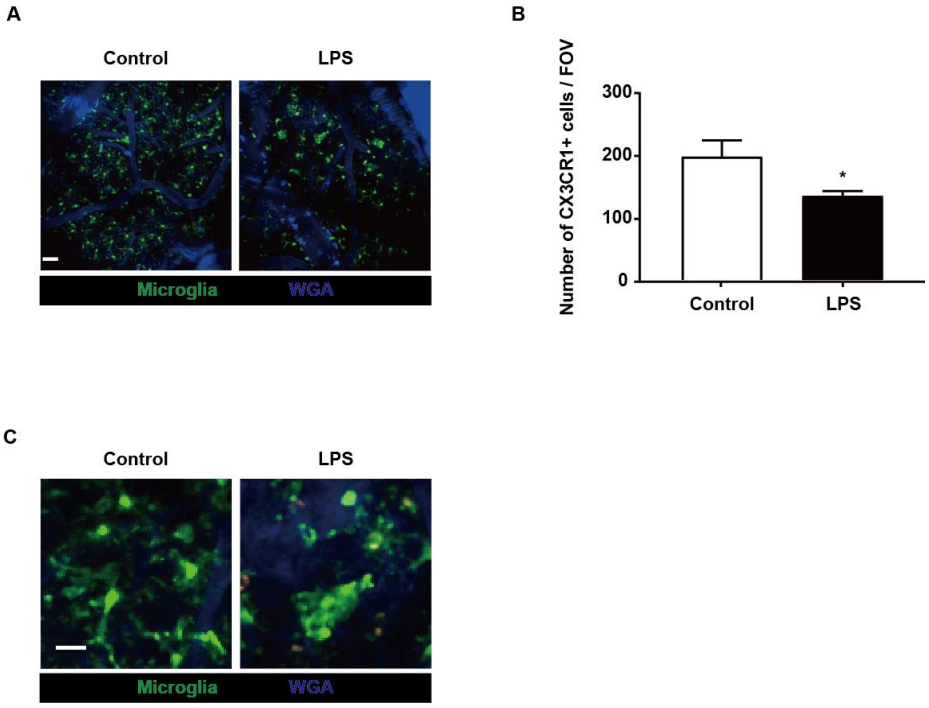


Figure 8. Decreased number and morphological change of microglia. $CX_3CR1^{GFP/+}$ mice were used for observing microglia (green) and injected with PBS (control) or LPS and CF405M-conjugated WGA for visualizing blood vessels (blue). **(A)** Snapshot of intravital image for counting microglia (green) in $CX_3CR1^{GFP/+}$ mice. Scale bar, 50 μm. **(B)** Measurement of microglial number in two different conditions. Data indicated mean ± SEM, $*p < 0.05$ (compared to control), n=3 mice per group of three independent experiments. FOV, 1.84 x 10^7 μm^3. **(C)** Difference of microglial morphology between control and LPS group, Scale bar, 20 μm.

5. Microglia engulfs neutrophils in inflamed brain parenchyma

To observe the interaction between microglia and neutrophil using two-photon microscopy, I used $CX_3CR1^{GFP/+}$ mice, in which are microglia are specifically labeled. The mice were injected with PE-conjugated anti-Ly6G_antibody for the staining of neutrophils in the blood vessel. These results showed that microglial processes directly made contact with neutrophils during neuroinflammatory response (Fig. 9). One phenomenon represented that microglial processes were repeatedly extended and retracted toward an approaching neutrophil, termed “dendrite surveillance extension and retraction cycling habitude” (dSEARCH)[12]. And then microglial processes touched and surrounded that neutrophil as shown in the 3d images (Fig. 9A, B). Another represented that processes of microglia maintained contact with neutrophils for 30 min (Fig. 9C, D). Additionally, other results showed that the intravital snapshots represented colocalization of GFP-microglia and PE-neutrophils (Fig. 10A, C). These results showed that microglial processes were elongated toward microglia-engulfed neutrophil (Fig. 10B, D).

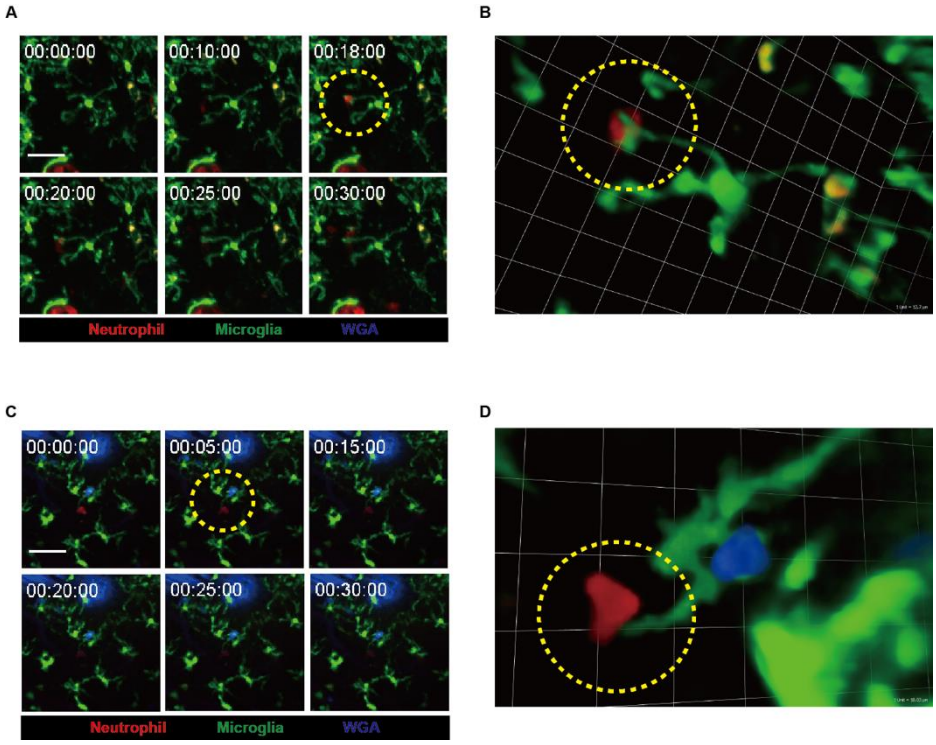


Figure 9. Contact of microglial process and neutrophils in inflamed brain parenchyma. Snapshot of direct contact of microglia and neutrophils in $CX_3CR1^{GFP/+}$ mice injected with the neutrophil antibody PE-conjugated anti-Ly6G and CF405M-conjugated WGA for visualizing blood vessel for 30 min. **(A, C)** Scale bar of Extended view, 40 μm. **(B, D)** Scale bar per 1 unit of 3d view, **(B)** 12.7 μm, **(D)** 18.03 μm

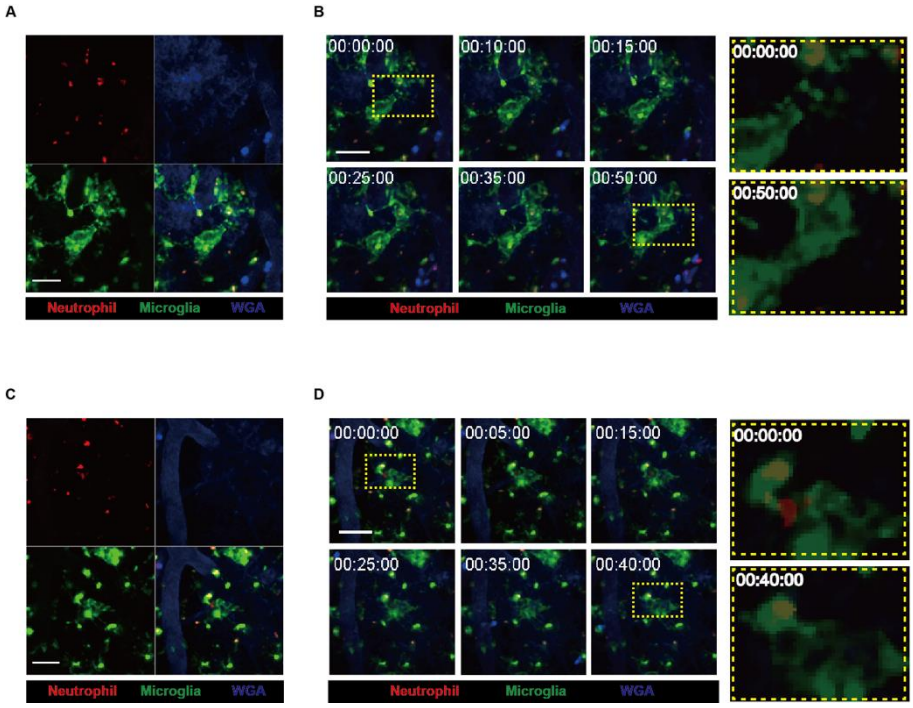


Figure 10. Elongation of microglial processes toward colocalization of microglia and neutrophil in inflamed brain parenchyma. Snapshot of elongation of microglial processes toward colocalized microglia with neutrophils in $CX_3CR1^{GFP/+}$ mice injected with the neutrophil antibody PE-conjugated anti-Ly6G and CF405M-conjugated WGA for visualizing blood vessel co-localization. **(A, C)** Representative colocalization of microglia and neutrophil imaging. Scale bar, 40 μm. **(B, D)** Time-lapse images on elongation of microglial process. Scale bar, 40 μm.

Ⅳ. DISCUSSION

Although many studies have been performed on the relationship of neutrophils and macrophages in the peripheral innate immune system[31-33], studies about the relationship of neutrophils and microglia in the innate immune system of the CNS are scarce, possibly because the brain was originally considered as an immune-privileged organ due to the BBB and the lack of lymphatics[34-37]. However, lately these views have changed over time as evidence of immunological functions within the CNS was revealed[38,39]. Also, more studies showed that blood-derived neutrophils infiltrated into the LPS-inflamed brain parenchyma *ex vivo*[24,40]. This study explored migratory patterns and morphological changes in light of the relationship between neutrophil and microglia on the neuroinflammatory response, especially using two-photon microscopy.

In this study, more neutrophils were found to infiltrate into brain parenchyma of LPS-injected mice compared to control mice. And infiltrated neutrophils in the inflamed brain parenchyma had more active motility than in the brain parenchyma of control mice. Infiltrated neutrophils were thought to possibly interact with other immune cells during neuroinflammatory response. As microglia, which are brain tissue-resident cells, are the first defense line against pathogens in the brain, it was hypothesized that neutrophils might interact with microglia during acute inflammation.

To simultaneously observe microglia and neutrophils, $CX3CR1^{GFP/+}$ mice were injected intravenously with PE-conjugated anti-Ly6G antibodies, which labeled neutrophils in blood vessels. In this study, these results represented that the number of microglia decreased, the morphology of microglia was transformed into the activated form, which is characterized by a large cell body and thick processes[9], and neutrophils frequently interacted with processes of activated microglia in diverse manners after LPS administration. Therefore, the possibility of microglia affecting the infiltration of neutrophils was suggested. This result also represented that microglial cell death occurred by LPS-induced neuroinflammation, which was identified by a presence or lack of GFP expression in $CX_3CR1^{GFP/+}$ mice[29,30]. Previous research established that after LPS administration, microglial death and neutrophil infiltration to the brain parenchyma is observed[41,42]. Additionally, toll-like receptor 4 (TLR4) is expressed on microglia in particular, when the cells are activated by LPS[43]. Microglia that express TLR4 produces pro-inflammatory cytokines such as TNF-α, IL-1β and NO[44]. These cytokines are key mediators of the neuroinflammatory response. Therefore, it is possible that microglial processes directly made contact with neutrophils during neuroinflammation due to such pro-inflammatory cytokines. This is further reinforced by the observation of microglial processes elongating toward colocalized microglia with neutrophils.

In neutrophil recruitment, it is known that endothelial cell (EC)- and pericyte-derived CXCL1 mediates neutrophil crawling, and neutrophil-

secreted CXCL2 is vital for transmigration of neutrophils *in vitro* and *vivo*[24,27]. It was also shown that these chemokine ligands for neutrophil recruitment are mediated by microglia. In addition, it is well established that microglia produces the interleukin IL-1β and CXCL1 for protective recruitment of C-X-C chemokine receptor2 (CXCR2)-expressing neutrophils *in vivo*[24,45], and microglia exploit TLR4 signaling to elicit the production of CXCL2[24,44]. Based on previous research, it was suggested that microglia might mediate neutrophils through these chemokine ligands. However, there was no evidence of microglia-affected infiltration of neutrophils in this study. Further research is necessary to quantify the migratory patterns of neutrophils when microglia are depleted during neuroinflammatory response. Finally, this study proposed that neutrophils contribute to neuroinflammation by making direct contact with microglia. Also, this study, due to its usage of two-photon microscopy, was able to readily observe cellular responses between neutrophils and microglia. Therefore, these results may confirm a variety of research findings about the relationship of neutrophils and microglia.

Ⅴ. CONCLUSION

In this study, I examined the interaction of neutrophils and microglia during neuroinflammation. Two-photon intravital microscopy was mainly used for study. Intraperitoneal injection of low-dose LPS induced sepsis-induced neuroinflammation. 6 hr after LPS injection of two consecutive days, loss of mouse weight, infiltration of neutrophil in brain parenchyma, and microglial activation were significantly shown compared to control groups. Infiltrated neutrophils interacted with microglial process in inflamed brain parenchyma. Whether microglia play a role in neutrophil infiltration during neuroinflammation remains an important question for future investigation.

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ABSTRACT (IN KOREAN)

이광자 생체 이미징기법을 활용한 염증 유발 쥐 뇌에서 선천면역세포의 이동 현상 실시간 분석

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김 유 림

신경염증은 급성·만성 염증을 수반한 알츠하이머병, 파킨슨병, 루게릭병 등의 다양한 발병 기전에서 중요한 역할을 수행한다. 신경염증이 발생한 후, 뇌 조직을 구성하는 세포와 말초 면역세포 사이에는 다양한 상호작용이 발생한다.

본 연구는 중추신경계의 급성염증반응 동안 말초 면역세포의 가장 많은 비율을 차지하는 호중구와 뇌 조직을 구성하는 면역세포인 미세아교세포 간의 발생하는 세포의 이동 양상과 형태학적 변화를 분석하였다.

이광자 생체 이미징기법을 이용하여, 호중구와 미세아교세포를

관찰하기 위해 각각 $LysM^{GFP/+}$와 $CX_3CR1^{GFP/+}$ 마우스를 사용하였다. 특히, $CX_3CR1^{GFP/+}$ 마우스에 PE-conjugated anti-Ly6G 항체를 정맥에 주사하여 호중구와 미세아교세포를 동시에 관찰하였다. 연속 2일 동안 1.0 mg/kg의 LPS를 복강 내 주사하여 뇌의 급성염증반응을 유도하고, 마우스 두개관에 창을 이식하여 뇌에서 보여주는 두 세포의 상호작용을 이광자 생체 이미징기법을 통해 관찰하였다.

LPS를 주사한 마우스 뇌 조직으로 유입한 호중구 수가 대조군보다 급격히 증가하고, 이는 반경 20 μm 내에서 활동적인 운동성을 갖는 것을 확인하였다. 호중구와 미세아교세포의 상호작용하는 가능성을 확인하기 위해, 두 세포를 동시에 관찰하였다. 따라서, 연구 결과는 호중구와 미세아교세포는 다양한 방법으로 상호작용하는 것을 보여준다. 첫 번째 결과는 호중구를 향해 미세아교세포의 돌기가 뻗어 나가고, 호중구를 둘러싸는 모습을 확인하였다. 이후, 미세아교세포의 돌기는 호중구와 약 30분 동안 접촉을 유지하였다. 두 번째 결과는 호중구를 포식한 미세아교세포를 향해 인접한 미세아교세포 돌기가 뻗어 나간다. 이러한 결과들은 두 세포의 직접적인 접촉을 통해 상호작용을 하는 것을 확인하였다.

본 연구 결과는 수행된 이광자 생체 이미징 기법만으로 호중구와 미세아교세포의 상호작용 메커니즘은 분석하는 것이 어렵다. 그러나

세포의 이동 현상을 직접적으로 관찰할 수 있음으로 호중구와 미세아교세포 간의 이동 현상 조절에 대한 이해를 높이는 면역학 기초연구로 이바지할 가능성을 제시한다.

핵심되는 말: 신경염증, 호중구, 미세아교세포, 이광자 생체 이미징기법

PUBLICATION LIST

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