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The effect of tension on the physical characteristics and migration of monocytes

장력이 단핵구의 이동성 및 물리적 특성에 미치는 영향

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Major in Immunology

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Abstract

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Monocyte plays an important role in the early stages of the innate immune response. Monocytes are generated in hematopoietic organs and actively migrate to inflamed site. Surface adhesion molecules on the monocyte are known to participate migration and extravasation through its interactions with various ligands. Recently, studies showed that the tension caused by receptor-ligand interaction

functionally regulates immune cells. I hypothesized that inflammation caused by infection alters the physical characteristics of monocyte and consequently affects early innate immune responses. Using a tension gauge tether platform, I confirmed altered tension in monocytes isolated from *Streptococcus pneumoniae* (SP) infected mice compare to one that from mock infected mice. To determined global gene profile change of monocyte from infected mice, expression of transcription factors, cell adhesion molecules, cytoskeleton and signaling related proteins were analyzed. Finally, migration chip assay was conducted to show the actual change in cell mobility after SP infection. Increased cell mobility will contribute to accelerated monocyte emigration from the bone marrow ensuring sufficient cell supply to infected tissue.

Keyword: Monocytes, Cellular adhesion molecule, Migration, Immune

response, Tension, Receptor−ligand interaction, Physical properties

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Table of Contents

List of figures

Introduction

Inflammation is caused by stressed and damaged condition, such as infection [1]. Innate immune cells, including monocytes, play a key role in initiating inflammation and pathogen clearance by recognizing pathogen-associated molecular patterns [2]. Monocytes also move to the site of infection and differentiates into macrophages during inflammation [3, 4]. Monocytes and monocyte-derived macrophages play an important role in both innate and adaptive immune response, including inflammation, tissue remodeling, vascular homeostasis and pathogen clearance [5, 6].

Monocytes are originated from the bone marrow (BM) where most of immune cells develop. Stromal cell, endothelial cells and red blood

cells are also present in BM cavity [7]. Infection can induce alteration of physical characteristics including adhesion molecules, surface proteins in many cell populations. This changes are related to mobilization of monocytes [3, 4, 8–10]. Integrins expressed on most of BM cells are seminal in cell migration after infection. Integrin is transmembrane receptor composed of alpha and beta subunit that link immune cells with ECM (extracellular matrix) or mediate cell–cell interactions [11, 12]. Integrins also transduce mechanical information that generated from the ECM into attached cells [13]. Integrins are subdivided into various classes [14] and monocytes express adhesion molecules, such as $\alpha L\beta 2$, $\alpha M\beta 2$, and $\alpha V\beta 3$ [8].

Mechanical tension caused by integrin-mediated cell-cell interaction functionally regulates immune responses by various immune cells, including macrophages and T cells [15-18]. Whereas, mechanobiological property of monocyte which potentially contributes to cell migration and functional regulation has not been studied.

Tension gauge tether (TGT) platform is fine mechanical sensor which is composed of two complementary DNA strands. TGT allows to measure the single molecule forces generated by receptor-ligand binding in cellular level [13]. Typically, one strand has a ligand which binds a target receptor on the cell surface and the other strand is biotinylated for immobilization on the substrate [19]. I applied TGT

platform to confirm mechanical property of monocyte depends on different strength of mechanical tension. As the infection triggers the production of regulatory factors in BM, monocytes generated during the infection are functionally different compare to one that from healthy condition. I hypothesized that infection shift mechanical tension between monocyte integrin and their receptor, which can affect immune response. I confirmed that monocyte from infected animal has weakened integrin−ligand interaction.

Material and Method

Mice

Female C57BL/6 wild-type (WT) mice aged 6-8 weeks were purchased from Orient (Seongnam, South Korea). These mice were maintained under specific pathogen free conditions at the Asan Institute for life science. This study was approved by the Institutional Animal Care and Use Committee (2018-12-302). All animal experiments were conducted in accordance with the approved guidelines.

Infection

Streptococcus pneumonia (SP) and *Salmonella* Typhimurium (Sal) were provided by Dr. Gabriel Nunez (University of Michigan). Influenza (Flu) was provided by Dr. Baik Lin Seong (Yonsei University). Mice were anaesthetized by injecting 100 μl of

ketamine (25 mg/ml) and xylazine (2 mg/ml) mixture and intranasally inoculated with 1×10^7 CFU of SP, 5×10^7 CFU of Sal and 1×10^5 PFU of Flu, respectively. Control mice were treated same volume of phosphate-buffered saline (PBS; Welgene, LB001-02) instead of pathogen.

BM monocyte extraction

Three days after infection, femur and tibias were removed and cut both edges of the bones to expose the diaphysis. The BM was obtained by flushing the bone with PBS using a 26G needle and 10 ml syringe. To isolate monocytes, I used mouse monocyte isolation kit (EasySep™, Stem Cell, 19861), following the manufacturer's procedures. Cells were labeled with antibody cocktail and magnetic particles and applied for negative selection of monocytes using the magnet (BioLegend).

Monocyte extraction from lung tissue

Lung of PBS and infected mouse was taken off and chopped with surgical scissor. Then, diverse cell types were extracted from the lung through incubation with lung isolation buffer (RPMI 1640 medium (Hyclone, SH30027.01) containing 3% heat-inactivated fetal bovine serum (FBS) (Hyclone, SH30919.03), collagenase Ⅳ (Sigma, C5138-1g) and DNase- I (Roche, 11284932001). 75% percoll (GE Healthcare, 17-0891-01) was placed at the bottom of a 15 ml conical tube and mixture of 40% percoll and cell suspension was stacked above the 75% percoll solution. Then, the tube was centrifuged and pure monocyte layer was appeared at the middle of the mixed solution. Only the monocyte layer was isolated to 15 ml conical tube with transfer pipette and centrifuged again with full of lung isolation buffer. After the centrifugation, removed the supernatant and add isolation buffer. then cells were isolated with

Monocytes Isolation Kit.

Flow cytometry

Flow cytometry was performed using an FACS canto (BD Biosciences). Cells were blocked with BD Fc Block Receptor Binding Inhibitor (BD Bioscience) for 15 min on ice, followed by staining with fluorochrome-conjugated antibodies for 15 min on ice in the dark. Monocytes ($CD11b^{+}Siglec-F^{-}Ly6C^{+}Ly6G^{-}$) were separated using monoclonal antibodies conjugated with fluorochrome: CD11b (M1/70, FITC), Siglec-F (S17007L, PE), Ly6C (AL21, APC), Ly6G (1A8, PE-Cy7), all purchased from BioLegend.

Flow cytometry sorting

Flow cytometry sorting was performed using an FACS Aria Sorter (BD Biosciences). Single cell solutions were blocked with BD Fc

Block Receptor Binding Inhibitor (BD Bioscience) for 15 min on ice, followed by staining with fluorochrome-conjugated antibodies for 15 min on ice in the dark. For the sorting of monocytes, the following antibodies were used Table 1.

Table 1.

Antibodies used for FACS sorting.

Antibody	Specificity	Clone	Fluorochrome	Company / Cat No.
CD11b	Monocytes/ Neutrophils	M1/70	FITC	BioLegend / 101205
Siglec-F	Eosinophils/ Neutrophils	E50-2440	PE	BD Biosciences / 552126
Ly6C	Monocytes/ Neutrophils	AL-21	APC	BD Biosciences / 560595
Ly6G	Neutrophils/ Granulocytes	1A8	PE-Cy7	BioLegend / 127618

RNA sequencing

RNA of sorted monocytes were extracted with the RNeasy mini Kit (Qiagen, Cat No. 74106), according to the manufacturer’ s protocol. RNA quality and quantity was confirmed using a Nano-drop. RNA sequencing was performed at Macrogen Inc. (www.macrogen.com)

TGT test

TGT platform was designed by co-worker from Incheon University. Primary monocytes were seeded on each well of the 8 well chamber and incubated at 200 ul density on TGT surfaces at 37℃ and 5% for 30 min, 1hr and 2 hr. After the incubation, cells were fixed with 4% paraformaldehyde for 10 min and washed 3 times by PBS. Bright field and fluorescent images were acquired using 60x objective of Nikon Ti2.

Immune cell migration assay

After ECM coating, M-CSF containing media and serum-free media were introduced with constant flow rate (40 ul/hr) into the upper and the lower flow channel through the tubing connected to the inlet of the upper and the lower flow channel, respectively. monocytes were injected into the central flow channel. M-CSF gradient was generated throughout from the upper to the lower channels by diffusion of the attractant.

Microscopy and Imaging

Time-lapse images were acquired on a Ti2 (Nikon) inverted microscope equipped with an on-stage incubation chamber which maintained the temperature at 37℃. And the mixed gas was injected to the incubation chamber that maintaining the concentration at 5%. To maintain humidity inside of the chamber, deionized water

container was placed in the chamber. Time-lapse phase-contrast images were acquired by a camera (Andor, iXon Life) using NIS elements software.

Results

To determine the changes of monocytes number in BM and lung following SP infection, C57BL/6J mice were treated with PBS or infected with *Streptococcus pneumoniae* (SP). Mice were then sacrificed 3 days after infection and monocytes were isolated using magnetic-based negative isolation method. The number of isolated monocytes per mice was measured. Although mean bone marrow (BM) monocytes number of SP-infected mice were higher than that of PBS group, it was not statistically significant. Whereas, the lung had significantly increased monocyte after infection (Fig. 1).

Based on previous studies that suggesting the role of cellular tension on adhesion, migration, and proliferation of immune cells [11, 13, 16, 19], I hypothesized that the tension caused by receptor-ligand interaction would affect the immunological character of monocytes. To test this hypothesis, I used tension gauge tether

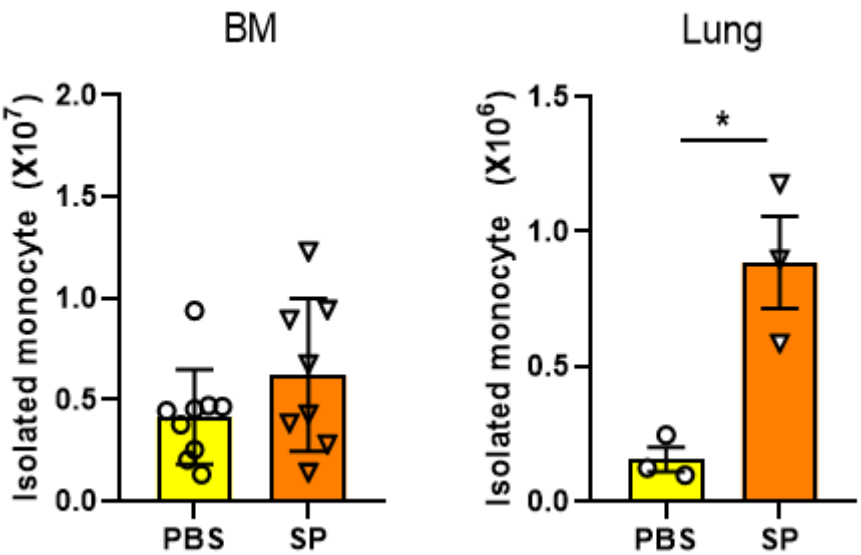

Fig. 1. The comparison of cells in PBS and SP infected mice.

C57BL/6J mice were treated for 3days with PBS and *Streptococcus pneumoniae* (SP). Monocytes were isolated by negative selection kit and measured. The number of isolated monocytes in BM and lung from PBS and SP-infected group. Monocytes from SP group has more than PBS group in lung.

(TGT) platform, which can measure tension and mobility of cells (Fig. 2). To confirm the migration of monocytes, BM and lung monocytes from naive and SP-infected mice were seed on the TGT. Digital image correlation (DIC) and fluorescence images were obtained and used to classify morphology of monocytes in terms of adhesion, spreading, and migration states (Fig. 3). When tension of integrin-ligand bond is larger than tension tolerance, cells form several types of rupture on TGT [11, 20, 21]. I classified the rupture pattern into partial rupture, entire rupture and dragging rupture and counted the number of rupture depending on each criterion.

Initially, I expected to see increase adhesion and the number of ruptures with monocyte isolated from SP-infected mice because monocytes undergo an extravasation process to migrate inflamed tissue through a receptor-ligand interaction with high affinity during infection [3-6]. However, the number of ruptures was reduced with

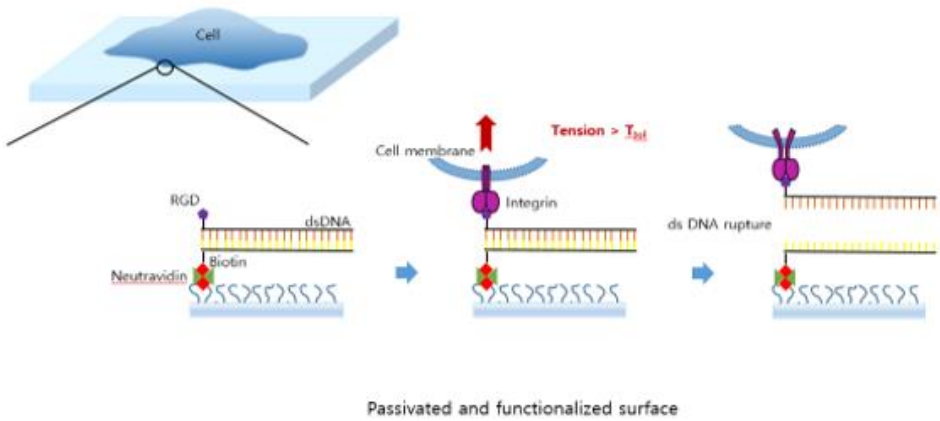

Fig. 2. Schematics of TGT tension sensor

The schematic show that a ligand for integrin receptor is immobilized on the TGT surface. Ligand-TGT constructs were immobilized by Biotin. Ruptures occur when the tension applied by the cell through the receptor is larger than its T_{tol}.

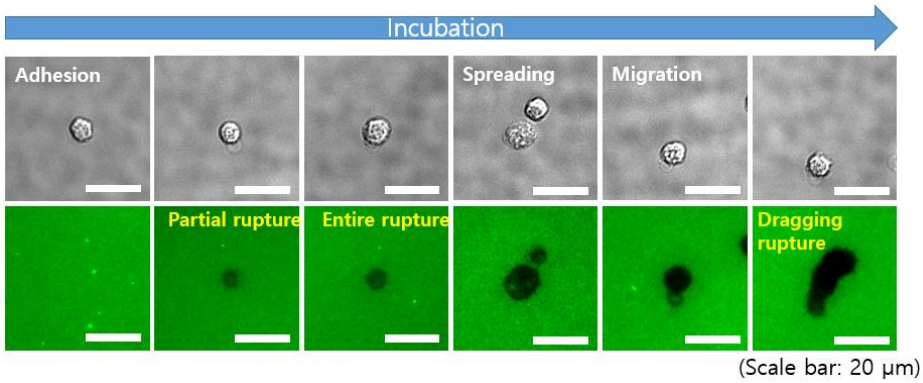

Fig. 3. Adhesion and migration of monocyte on TGT surfaces

Analysis of TGT rupture pattern. Initially, single cell was adhered on TGT surface and spread over time. Adhesive cell and spreading cell could be confirmed by partial rupture and entire rupture on TGT. If cell spread on TGT, cell can migrate on TGT surface. Migration could be confirmed by rupture as referred to dragging rupture on TGT.

monocyte isolated from SP-infected mice as well as *Salmonella* Typhimurium (Sal)-infected mice (Fig. 4). Monocytes isolated from influenza (Flu) virus infected mice showed comparable total rupture with that of PBS-infected mice but exhibited significantly reduced dragging rupture formation (Fig. 4). The ability to form dragging rupture reflecting the mobility of cells was reduced to significant level in monocytes isolated from infected mice. My results suggested that induced tension by receptor-ligand interaction affects cell mobility during infection. In later experiment, I used SP infection model as it showed the most significant difference among Sal, Flu, and SP models.

Isolated monocytes from PBS and SP-infected mice were applied on the TGTs with T_{tol} = 43 pN, 54 pN and 100 pN for 30 min, 1 hr and 2 hr. Integrins expressed on monocytes was bound to the top DNA strand of TGT. Monocytes can pull ligands through cellular

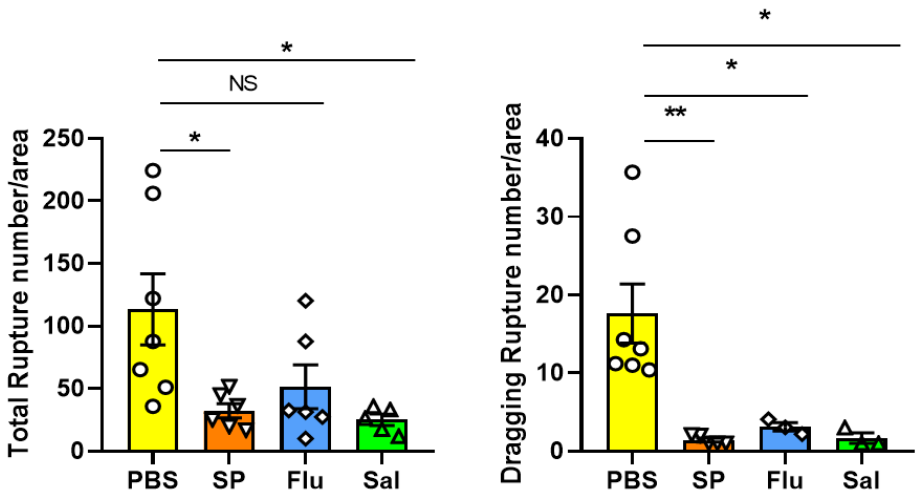

Fig. 4. The comparison of ruptures pattern in PBS, SP, Flu and Sal infected mice after 1 hr incubation at 54 pN

Comparison of the number of total rupture and dragging rupture for PBS, *Streptococcus pneumonia* (SP), *Influenza* (Flu), *Salmonella* Typhimurium (Sal)-treated group. ruptures analyzed based on two variables: total rupture and dragging rupture. The number of rupture after 1 hr incubation was counted using Image J.

forces transmitted by integrins, which lead to rupture on TGT surface. Although no significant difference could be identified at 100 pN (Fig. 5). Total and dragging ruptures on 54 pN had significant differences at 30 min, 1 hr, excluding 2 hr, while at 43 pN, significant differences were observed at 30 min, 1 hr, and 2 hr (Fig. 5). Hereafter, I used 1hr for further experiment.

When TGT test was performed at different tolerance for 1 hr, less rupture was produced on all TGT condition in SP group than PBS group (Fig. 6). In monocytes isolated from BM, total rupture and dragging rupture did not show significant difference on TGT having 100 pN tolerance, but that of 54 pN and 43 pN showed significant decrease in the SP group (Fig. 7A). In the lung, it also was showed that the number of ruptures in the SP group decreased, having similar tendency with BM (Fig. 7B). Since there were slight differences on each pN, the migration ability of monocytes separated from BM in

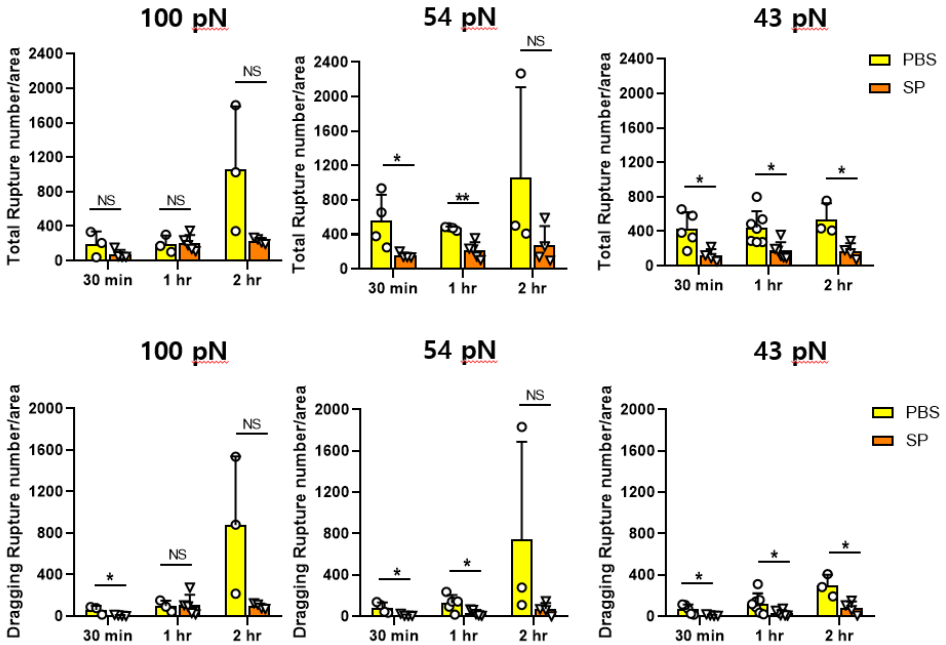

Fig. 5. The comparison of ruptures pattern in PBS and SP infected mice after 30 min, 1 hr and 2 hr incubation

Comparison of the number of total rupture and dragging rupture in BM from PBS and SP infected group. The number of rupture after 30 min, 1 hr, 2 hr incubation was counted. Ruptures analyzed based on two variables: total rupture and dragging rupture. The number of rupture was counted using Image J on 54 pN. The most significant result was obtained when incubated at 54 pN for 1 hr incubation.

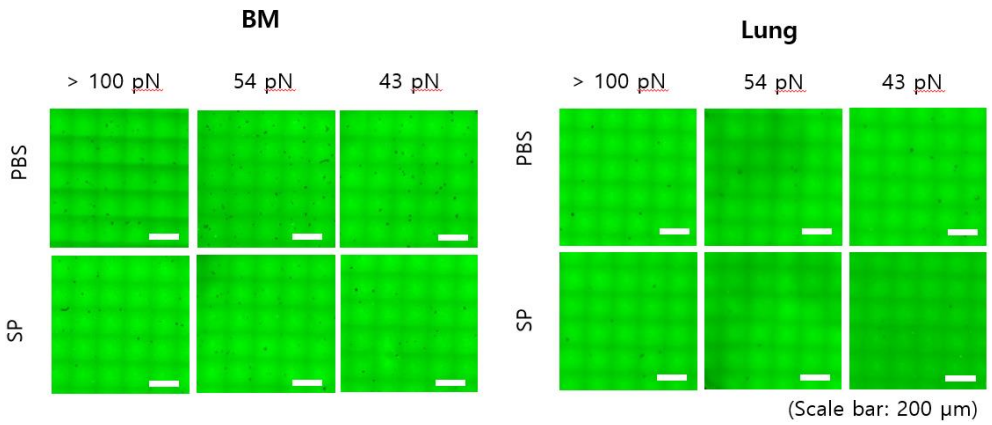

Fig. 6. Image of integrin mediated adhesion of monocytes derived from BM and lung in PBS and SP infected mice on TGT surfaces after 1 hr incubation

The image show rupture on TGT surfaces (incubation time, t = 1 hr). Cells adhere on 100, 54 and 43 pN TGT that we made. On all tolerance, the number of ruptures of naïve group is more than SP group in BM and lung. The TGT assay in each condition was repeated at least three times.

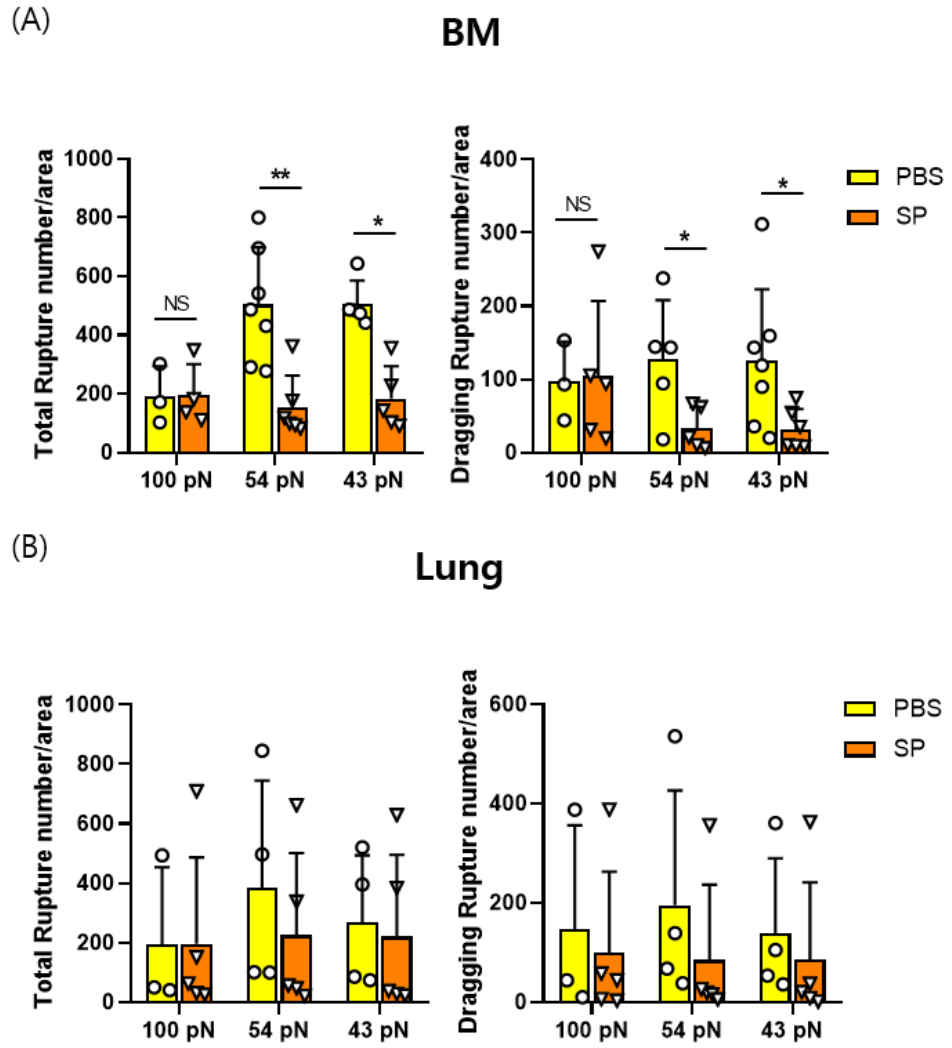

Fig. 7. The comparison of ruptures pattern from BM and lung in PBS and SP infected mice after 1 hr incubation

Comparison of the number of total rupture and dragging rupture in BM (A) and lung (B) from PBS and SP-infected group. The number

of rupture after 2 hr incubation was counted. Ruptures analyzed based on two variables: total rupture and dragging rupture. The number of rupture after 1 hr incubation was counted using Image J.

PBS and SP group was confirmed via TGT according to pN and time (Fig. 8).

To link activation of monocyte by infection with their altered migratory phenotype, I analyzed gene expression profile RNA sequencing. To obtain maximum purity, monocytes were isolated by FACS sorting. $CD11b^{+}Siglec-F^{-}Ly6C^{+}Ly6G^{-}$ cells were gated to sort monocytes (Fig. 9). Forces exerted by integrin receptor-ligand are transmitted to cell membrane and alters expression of surface protein [22].

Heatmap was prepared for three different criteria: (a) transcription factors that related to cell adhesion molecules (CAMs), focal adhesion, (b) cytoskeletal regulation, (c) signaling molecules (Fig. 10). Transcription changes were considered significant when expression number were different more or less than doubled fold compared to the control group. RNA sequencing data revealed that

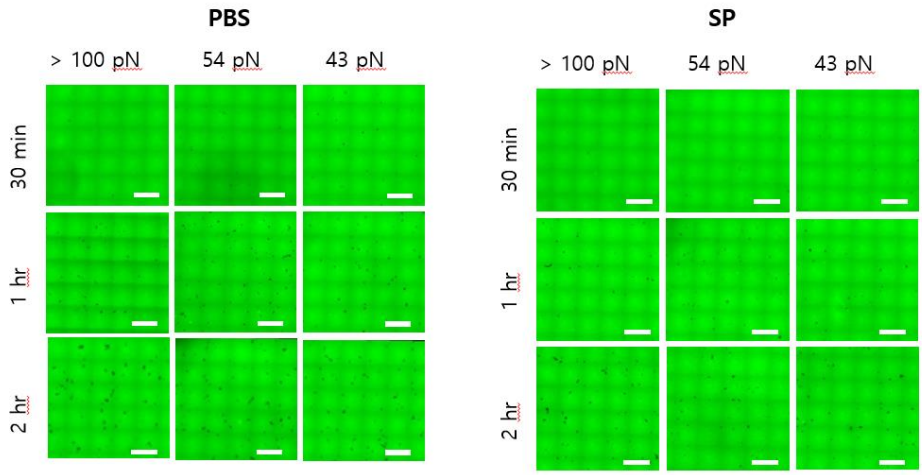

Fig. 8. Image of integrin mediated adhesion of monocytes derived from BM in PBS and SP infected mice on TGT surfaces after 30 min, 1 hr and 2 hr incubation.

The image show rupture on TGT surfaces in BM from PBS and SP treated group (incubation time, t = 30 min, 1 hr, 2 hr). Cells adhere on 100, 54 and 43 pN TGT that we made. The number of ruptures increased over time. The number of rupture in naïve group is more than SP group.

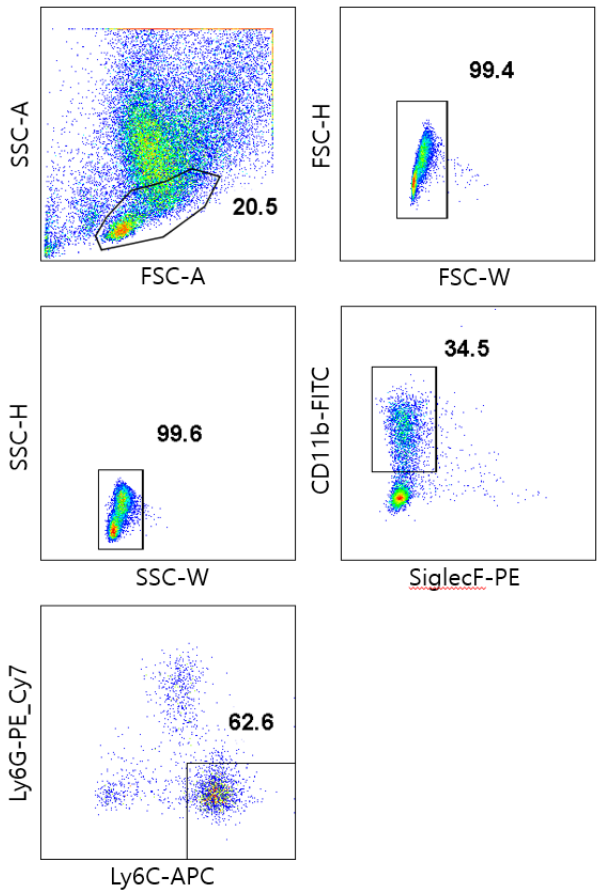

Fig. 9. Strategy of gating for FACS sorting

FACS sorting strategy for purification of monocytes population from BM. Monocytes were sorted on the makers as known monocytes expressed. To monocyte isolation, $CD11b^{+}$, $Siglec-F^{-}$, $Ly6C^{+}$, $Ly6G^{-}$ gating used. The sorted populations were used for RNA sequencing.

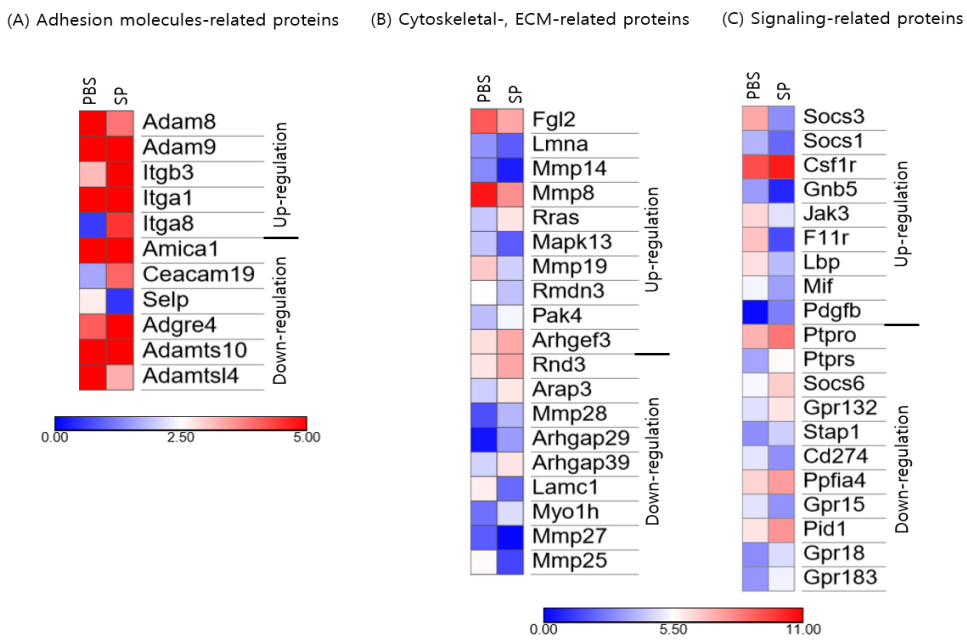

Fig. 10. Heatmap for comparison of transcription factors in PBS and SP infected group

Heatmap of the differentially expressed transcription factors from RNA sequencing data in PBS and SP treated group. Data represents P-value (log10). Up-regulated genes and down-regulated genes are shown in color.

disintegrin and metalloproteinase (ADAM) subtype, a factor that interferes with the function of integrin, was up-regulated. Meanwhile, Selectin and Ceacams related to cell adhesion were down-regulated. Among the factors related to adhesion molecules, several types of Rho GTPase were downregulated in the SP group. Recent studies have focused on how the tension between these integrins and receptors affects multiple immune cells. I hypothesized that many genes expressed in cells change during infection, and as a result, tension caused by changing receptor ligand interactions will affect the immune response. Although TGT test and cDNA microarray analysis were performed to determine the changes in the gene expression pattern after infection. Because TGT test use fixed cell at specific time points, it could not be compared exactly. Instead, a migration chip that can see kinetics of a single cell was additionally executed. The M-CSF containing media and serum-free media were

added with constant flow rate (40 ul/hr) into the upper and the lower flow channel, respectively (Fig. 11). Isolated monocytes were added into the central flow channel. It can be compare cell motility between PBS and SP group.

(A)

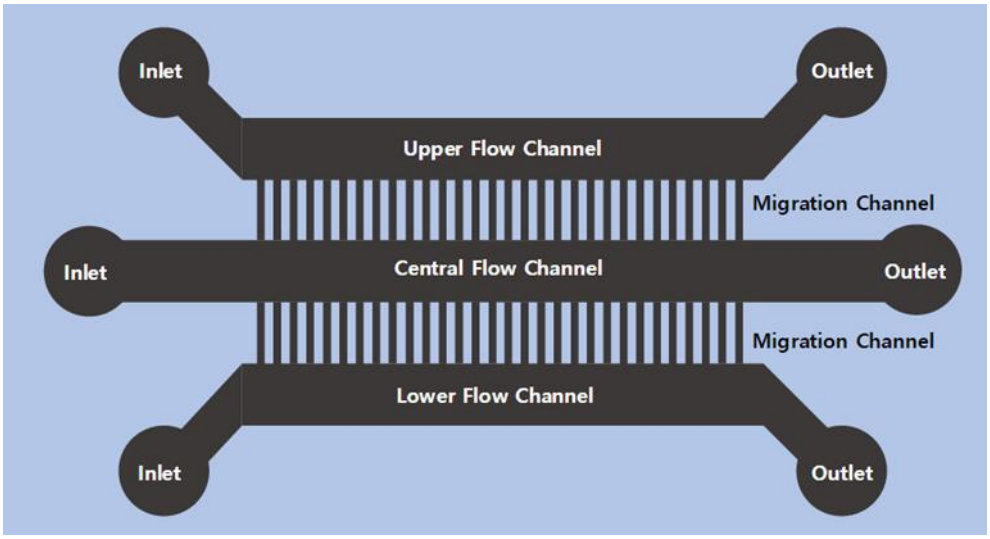

Fig. 11. Designed microfluidic chip for single-cell migration. (A) Schematic of the chip.

The schematic show that chip for cell migration. The Migration chip is useful to confirm the cell’ s kinetics. It can also be confirmed that the result according to TGT data. In order to inducing gradient inside the migration chip, Upper flow channel was added RPMI medium containing chemoattractant M-CSF and lower flow channel was added pure RPMI medium. Monocytes were added to central flow

channel.

Discussion

Despite a long history of immunological studies, it was not that long ago when researches start to investigate the mechanobiological involvement in functional characterization of immune cells. Mechanical force that occurs during receptor-ligand interaction has critical roles in cellular biological process including differentiation, proliferation, survival, and activation of various cell types [23-30]. Integrins are especially critical to cope with bacterial and viral infection in terms of microbial pathogenesis and functional maturation [23, 24, 31-34]. *M. tuberculosis* infection increases expression of integrin $\alpha V\beta 3$, which promotes monocyte adhesion to ECM [8]. RNA-sequencing data from my research also showed that integrin $\alpha V\beta 3$ was up-regulated in SP-infected monocyte. However, overall receptor-ligand interaction between ligand and SP-infected monocyte integrin was weaker when it compared to interaction with

control monocyte isolated from PBS group. The reason for weaker cellular adhesion by SP-infected monocyte, even with higher expression of $\alpha V\beta 3$, may be ascribed to less efficient integrin clustering. Indeed, previous study showed that expression level of integrins are similar between immune cells isolated from infected and non-infected animals [34]. But infected monocyte showed disrupted integrin clustering which affects integrin activation, strength of binding, and phosphorylation [34]. I also confirmed that expression level of $\alpha L\beta 2$ and $\alpha M\beta 2$ on monocyte are not changed after SP infection using flow cytometry. Further researches are needed to elucidate factors related to control of focal adhesion after infection.

T. gondii-infected monocyte has defects in cell spreading, clustering of integrin, and focal adhesion kinase. These defects allows monocyte migrate at a higher speeds than uninfected monocytes [35]. However, lipopolysaccharide or *Escherichia coli*

infection did not induce impairment of integrin clustering, indicating mechanobiological property of monocyte may vary depending of the pathogen they encounter [35]. Furthermore, *N. gonorrhoeae* can bind to Ceacams, molecule that involved in bacteria–cell adhesion [32, 36]. In contrast, nonpathogenic *Neisseria cinerea* can lead to adhesion of Ceacams–expressing cells. In my setting, Ceacams were down–regulated suggesting receptor–ligand interaction is weakened in SP–infected mice. The result also suggests that infection–triggered alteration of signaling and gene expression can modulate cell adhesion and migration following infection. It seems that SP infection makes focal adhesion weaker or disrupts integrin clustering formation.

Integrin is critical mediators of viral and bacterial infections, and integrin–mediated adhesion as well as pathogenic processes [32]. Other study confirmed that monocytes and endothelial cells are

activated by infection, which lead to enhancing expression of adhesion molecules, including E-selectin, fibronectin-1 [9]. To know exactly how the monocyte react upon infection, it is important to understand which integrin subtypes are changed in their expression levels and whether these changes affect cellular processes during infection. Other study suggested that the *T. gondii*-induced disruption in integrin-mediated adhesion is linked to a dysregulation of components of the focal adhesion complex. My results also indicate that SP infection weakens receptor-ligand interaction. Based on previous studies and my results, I speculated that adhesion of SP-infected monocyte is weaker maybe since focal adhesion could not made well.

Several studies have shown that physical pressure on the cell stimulate cell to activate pro-inflammatory responses. The PIEZO ion channel can induce a pro-inflammatory response by sensing

cyclical pressure in myeloid cells [37]. It is similar to the content that a proper cellular reaction occurs by recognizing stimuli such as pressure and tension in surrounding environment of the cell. Although a pro-inflammatory response occurs through pressure, I thought that low tension is important for the migration of cells from bone marrow to peripheral blood based on the results of my experiment.

Previous studies mainly used TGT test to analyze cells in a stationary state after incubation [13, 19]. Although TGT test was efficient to measure tension in single cell level, the study did not show whether the change in tension alters migration profile of live monocytes. As the migration pattern and speed are closely related to immune response, I used system to confirm the mobility of live cells utilizing migration chip. This technique was efficient to monitor the live monocyte migration and kinetics using microscopy time lapse. In addition, I coated the chip with cyclic RGD which were used for TGT

test to assess the cellular tension is involved in mobile characteristics of infected monocyte. By combining the fields of immunology and mechanobiology, the inter-relation between altered mechanical characteristic and cell migration under host's infection can be studied extensively. As I used live imaging with migration chip to show leukocyte behavior, such as crawling and swarming, further efforts on introducing new mechanobiological techniques will allow researchers in immunology field to understand cellular communication and in vivo characteristics better in the future.

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국문 초록

장력이 단핵구의 이동성 및 물리적 특성에 미치는 영향

단핵구들은 일반적으로 혈류 내에서 순환하지만, 병원체가 침입하거나 염증이 발생하면 표적 부위로 이동하여 대식세포로 분화하여 선천 면역 반응의 초기에 중요한 역할을 한다. 단핵구들이 표적 부위로 이동할 때 혈관 내벽 세포와 단핵구가 발현하고 있는 세포부착 분자들을 이용한다고 알려져있다. 수용체-리간드의 상호작용에서 발생하는 장력, 리간드 밀도 등이 대식세포의 기능 조절에 미치는 영향은 비교적 잘 연구되어 있지만, 단핵구에서는 분화에 미치는 영향 외에는 연구가 잘 되어있지 않다. 또한, 세포 주변 환경으로부터의 물리적 자극이 대식세포와 T세포를 비롯한 일부 면역 세포의 기능 조절에 미치는 영향이 잘 연구되어 있기때문에, 단핵구에서 또한 주변 환경이 면역기능에 영향을 미칠 것이라 가설을 세웠다. TGT라는 플랫폼을 이용하여 리간드-수용체 상호작용시

발생하는 장력을 조절하고 세포의 이동성을 확인하였다. 마우스에 Streptococcus pneumoniae (SP) 박테리아를 감염시켰을 때, 세포부착 분자들이 증가하고 그에 의해 세포의 이동성이 확연하게 증가할 것이라고 생각했었다. 하지만 예상한 바와 달리 감염군에서 대조군에 비해 세포의 이동성이 감소하였다. SP 뿐만 아니라, 살모넬라와 인플루엔자 감염에서도 유사한 경향성을 보였다. 이와 같은 결과를 토대로 감염시 발생하는 리간드-수용체 상호작용 장력이 세포의 이동성에 영향을 준다는 것을 확인하였다. 감염시 세포에 일어나는 물리적 변화를 확인하고, 리간드-수용체 상호작용에 의해 세포에 전달되는 장력이 단핵구의 혈관외 유출 및 세포의 기능에 어떠한 영향을 주는지 밝히기 위해 RNA sequencing을 통해 대조군과 감염군에서 발현하고 있는 세포부착 분자 간의 차이를 확인하였고 이후 migration chip 연구를 통해 세포 이동성의 변화를 확인하고자 한다.

주요어 : 단핵구, 세포부착분자, 리간드-수용체 상호작용, 세포의 이동성,

면역반응, 장력, 물리적 특성

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