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学位の種類	博士（　人間生物学　）
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学位論文題目	Inflammasome in respiratory epithelial cells restricts influenza virus infection (インフルエンザウイルス感染抑制に関与する気道上皮由来インフラマソーム活性化機構の解析)

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論文の要旨
Abstract of thesis

Purpose

The respiratory epithelium is exposed to the external environment and functions as a sensor of infectious agents to initiate inflammatory response. Influenza A virus is a critical human respiratory pathogen that infects millions of people worldwide in seasonal epidemics. Influenza virus induces inflammatory responses as a result of respiratory epithelial cell death. The pro-inflammatory response from respiratory epithelial cells triggers recruitment of immune cells including macrophages and neutrophils to remove infectious agents. These migrated immune cells contribute to excessive inflammation. Thus, the response mechanism of inflammation in respiratory epithelium is crucial for understanding the pathogenesis of influenza A virus. However, the exact mechanism responsible for inflammatory responses, which may determine the pathogenesis of influenza A virus, is still unclear. This study aimed to clarify the mechanism of Influenza A virus-induced inflammation.

Methods

To address this issue, the applicant examined the number of respiratory epithelial cell death by trypan blue dye exclusion assays and DNA fragmentation assays upon influenza virus infection. The applicant also measured the secretion level of pro-inflammatory cytokine IL-1β by ELISA and detected apoptotic and pyroptotic cell death using

anti-cleaved caspase-3 and anti-ASC antibodies, respectively, by indirect immunofluorescence assays. To identify a novel inflammasome receptor, the applicant examined the proteomic approach of ASC interacting proteins and the high-content screening using genome-wide human shRNA library. The interaction between MxA and endogenous ASC was confirmed by immunoprecipitation using anti-ASC antibody and MxA-ASC co-localization was confirmed by immunofluorescence in infected PL16T cells at 36 h post infection. To determine a viral ligand recognized by MxA inflammasome, PL16T cells were transfected with viral polymerase subunits and NP, and then treated with poly I:C at 24 h post transfection to express MxA. To analyze the extent of ASC oligomerization, 0.5% Triton X-100-treated PL16T cells were crosslinked with 2 mM bis(sulfosuccinimidyl) suberate and analyzed by Western blotting with anti-ASC antibody. To examine *in vivo* function of MxA inflammasome, the applicant used a transgenic mouse carrying the entire human *Mx* locus except *MxB* gene (hMx-Tg). Bronchoalveolar lavage fluid (BAL) was collected by washing the trachea and lungs twice by injecting a total of 2 ml PBS containing 0.1% BSA, and measured IL-1β secretion by ELISA and $CD45.2^{+}$ total BAL leukocytes by flow cytometry. To assess whether antiviral activity of MxA in hMx-tg mice is dependent on the inflammasome, the applicant generated hMx-Tg × *Casp1/11*$^{-/-}$ mice and monitored their survival against influenza virus infection. To investigate single effects of the MxA inflammasome in respiratory epithelium against influenza virus infection, the applicant generated chimeric mice by bone marrow transplantation from *Nlrp3*$^{-/-}$ mice to hMx-Tg recipient mice to exclude the inflammasome response in immune cells, and measured IL-1β secretion and $CD45.2^{+}$ total BAL leukocytes.

Results and Discussion

The applicant observed that Influenza A virus infection induced apoptosis and pyroptosis in normal or precancerous human bronchial epithelial cells. In PL16T human precancerous respiratory epithelial cells, the number of apoptotic cells increased at early phases of infection, but pyroptotic cells were observed at late phases of infection. These findings suggest that apoptosis is induced at early phases of viral infection, but the cell death pathway is shifted to pyroptosis at late phases of viral infection. The applicant also found that the type I IFN-mediated JAK-STAT signaling pathway promotes the switch from apoptosis to pyroptosis by inhibiting apoptosis possibly through the induced expression of *Bcl-xL* anti-apoptotic gene. Further, the inhibition of JAK-STAT signaling repressed pyroptosis, but enhanced apoptosis in infected PL16T cells, suggesting type I IFN signaling pathway triggers pyroptosis but not apoptosis in the respiratory epithelial cells in a mutually exclusive manner to initiate pro-inflammatory responses against influenza virus infection.

Using influenza virus infection as a model, the study by the applicant revealed the role of MxA on respiratory epithelial inflammasome activation. Not only the MxA expression through poly I:C treatment but also NP viral protein as a pathogen-associated ligand is required to trigger the inflammasome activation. This suggests that IL-1β secretion is tightly regulated by under the control of type I/III IFN signaling and the pathogen recognition by MxA. This may guarantee the local pro-inflammatory response in peripheral tissues specific for pathogenic infectious agents. The study by the applicant also provided the first evidence *in vivo* on the significance of inflammatory response in respiratory epithelial cells against influenza virus infection. Further pathological studies on the epithelial inflammatory response may lead to design of new therapeutic strategies for IAV-induced pneumonia.

Pyroptosis is a caspase-1-dependent inflammatory cell death controlled by inflammasomes, multiprotein complexes consisting of caspase-1, apoptosis-associated speck like protein containing a CARD (ASC), and cytoplasmic pathogen recognition receptors (PRRs) such as NOD-like receptor (NLR) family proteins. NLR family PYD-containing 3 (NLRP3) inflammasome is crucial for influenza A virus pathogenesis through pro-inflammatory cytokine secretion from macrophages. However, the exact mechanism of inflammasome activation in respiratory epithelial cells, especially a sensor molecule for influenza virus infection, is largely unknown.

Using the high-content shRNA library screening and the LC-MS proteomic analysis, the applicant identified a human myxovirus resistance gene 1 (MxA) as a novel inflammasome receptor in respiratory epithelial cells. MxA recognizes NP viral protein as a pathogen-associated ligand, and oligomerization of MxA is required for the inflammasome formation in respiratory epithelial cells but not macrophages. The expression of human MxA *in vivo* activated the inflammasome formation in bronchiolar epithelial cells and protected mice from influenza virus infection by repressing virus spread from the bronchioles to distal alveolar regions.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant identified a human MxA as a novel inflammasome receptor in respiratory epithelial cells. The expression of human MxA *in vivo* activated the inflammasome formation in bronchiolar epithelial cells and protected mice from influenza virus infection by repressing virus spread from the bronchioles to distal alveolar regions.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 03rd July, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.