学位論文内容の要旨

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学 位 論 文 題 名

Studies on pathogenic and regulatory mechanisms of immune-related diseases.
(免疫関連疾患の発症および制御機構の解明に関する研究)

Background and Objectives Bronchial asthma, typically recognized as helper T (Th) 2 cell-mediated airway inflammation, is pathogenically characterized by airway hyperresponsiveness (AHR), eosinophilic airway inflammation, mucus hyperproduction in airway epithelium, and elevated serum levels of IgE. Numerous chemicals and medicines against immunological cells have been researched and developed for clinical use in asthma. However, patients suffering from asthma occasionally develop severe neutrophilia in the lung and show steroid resistance, which then results in “severe asthma”. It has been reported that neutrophil infiltration in the lung was not observed in Th2 cell-associated airway inflammation models. Thus, the precise mechanisms of severe asthma are less understood compared with those of Th2 cell-mediated airway inflammation. Previously, Th1- and Th2-mediated airway inflammation models were established by the adoptive cell transfer of ovalbumin (OVA)-specific Th1 or Th2 cells followed by OVA inhalation. In contrast to Th2 cells, Th1 cells induce strong AHR concomitantly with neutrophilia in the lung but without mucus hypersecretion. Therefore, the Th1 cell-mediated airway inflammation model appeared to be suitable for characterizing the pathology of severe asthma, since little has been done to elucidate how Th1 cells induce elevation of AHR.

Tachykinins such as substance P (SP) and neurokinin A (NKA) are located in the excitatory non-adrenergic and non-cholinergic (NANC) nerves of the mammalian respiratory tract. Excitation of these nerves results in the release of tachykinins, which may be involved in the pathogenesis of airway allergy in humans. Because those factors are produced in the airway tissues during inflammatory responses, tachykinin receptor antagonists might become good targets for developing therapeutic drugs for the treatment of allergic inflammation. However, the precise role of NKA/neurokinin-2 receptor (NK2R) signaling has not yet been elucidated, though SP signaling through tachykinin neurokinin-1 receptor was demonstrated to be crucial for the induction of neutrophilia in the lung and AHR elevation. Generally, SP secreted by neurons in response to local tissue damage, is capable of inducing and augmenting many inflammatory responses. Recent papers indicated that SP regulated function of various leukocytes. On the other hand, NKA has known to control various vital responses such as airway contraction, vasodilatation, and vascular permeability in human. However the function of NKA on involvement in immune system has less defined than that of SP.

In the present work, we established a novel AHR induction model by intranasal (i.n.) administration of IFN-γ and investigated the critical role of IFN-γ and NKA in Th1 cell-mediated airway inflammation model. Furthermore, we confirmed the effect of NKA/NK2R signaling on immune system.

Methods In order to elucidate the role of IFN-γ, we examined pathogenesis of the Th1 cell-dependent

airway inflammation after treatment with neutralizing mAb against IFN-γ. To determine the precise role of IFN-γ in the elevation of AHR, we directly administered i.n. IFN-γ to wild-type BALB/c mice consecutive 3 days. At 24 h after the IFN-γ administration, pulmonary function was evaluated. To confirm the direct effects of IFN-γ on the airway component cells such as airway smooth muscle cells (ASMCs), we prepared mouse ASMCs from airway tracheal and examined their mRNA levels with IFN-γ stimulations. To investigate whether NK2R-mediated signaling cascade was involved in the subsequent airway responsiveness in vitro and in vivo, we monitored calcium influx $(Ca^{2+})_i$ in ASMCs after the β-methacholine chloride (Mch) stimulation with or without NK2R antagonist and evaluated whether NK2R antagonist inhibited AHR elevation induced by i.n. IFN-γ administration. Next, CD11c-DTR Tg mice were i.n. injected with diphtheria toxin (DT), and then IFN-γ was further injected into the mice to elucidate whether $CD11c^+$ cells were related with the elevation of NKA level. To address the effect of neuropeptide signaling on dendritic cell (DC)-mediated immune responses, we further investigated expression levels of NKA and NK2R of GM-CSF-induced bone marrow-derived DCs (BMDCs) in Type-1 immune condition and expression level of cytokines in NKA-stimulated DCs. In order to evaluate NK2R-mediated NKA stimulation on DC functions, we transduced NK2R gene into DCs by retrovirus infection system and evaluated surface MHC class II expression level after NKA stimulation. Then, we co-cultured OT-2 $CD4^+$ T cells with Mock- or NK2R-transduced DCs in the presence of OT-2 peptides. To confirm the effect of NKA-NK2R signaling on antigen-specific T cell responses, we cultured spleen cells obtained from OT-2 or OT-1 mice in the presence of NK2R antagonist. Finally, we administrated i.n. NK2R antagonist in Th1 cell-mediated asthma model for evaluating whether inhibition of NK2R signaling reduced IFN-γ-dependent AHR elevation.

Results We found here that Th1 cell-induced AHR elevation was mimicked by i.n. administration of IFN-γ. IFN-γ directly induced NK2R expression and NKA production in the lung. Then, the IFN-γ-induced elevation of AHR was significantly inhibited by specific antagonist of NK2R in our model. We confirmed that one of neurokinin producing cells was $CD11c^+$ cells in lung and IFN-γ stimulated BMDC produced NKA. NKA-NK2R signaling cascade activates DC-mediated immune responses in vitro. Finally, we demonstarated that AHR was significantly inhibited by specific antagonist of NK2R in the Th1 cell-mediated airway inflammation model.

Discussion In the present study, we clearly demonstrated that NKA, one of neuropeptides which were generally located in the sensory nerves of mammalian respiratory tract and produced after excitation of the nerves as well as SP, would be involved in the elevation of AHR induced by severe asthma. It might be possible to consider that severe symptoms of patients suffering from severe asthma are due to excess activation of antigen-specific Th1 immunity and subsequent IFN-γ-mediated augmentation of NKA/NK2R signaling, which causes the increase of Ca^{2+}-dependent contraction of airway tracheal smooth muscle cells. Thus, our established IFN-γ-induced airway inflammation model will become a useful tool to elucidate the pathogenesis of severe asthma and to develop therapeutic drugs for IFN-γ-induced inflammatory diseases via NKA/NK2R signaling.

Conclusion Previous reports demonstrated that IFN-γ secreted by Th1 cells was the critical mediator for the induction of AHR, however, the precise mechanism of AHR induction by IFN-γ remained unclear. In the present study, we first established IFN-γ-mediated AHR elevation model, which was induced only i.n. administration of IFN-γ. Then, we found that IFN-γ activated NKA/NK2R signaling cascade, which was important for IFN-γ-induced AHR elevation. Furthermore, we demonstrated that NK2R-dependent neuropeptide signaling enhanced DC function and subsequent T-cell immune responses. Our present findings suggested that NK2R-mediated neuro-immuno cross-talk would be a promising target for developing novel drugs in Th1 cell-mediated chronic inflammation, including severe asthma.