Allergology International. 2014;63(Suppl 1):59-61
DOI: 10.2332/allergolint.13-LE-0634



Dear Editor

## Antigen-Induced Mixed and Separated Inflammation in Murine Upper and Lower Airways

Allergic rhinitis (AR) and bronchial asthma (BA) have been recognized as manifestations of a single entity allergic respiratory disease. It has been shown that many patients with BA also suffer nasal symptoms, even though not all the asthmatic patients develop AR.[1] In addition, AR is regarded as a risk factor for the development of BA. Previous studies have reported that AR patients show increased onset of bronchial hyperresponsiveness (BHR) even without any clinical evidence of BA.[2] Therefore, in order to investigate the relationship between AR and BA, it is important to compare the inflammatory responses developed in the upper and lower airways. However, analysis models capable of separately inducing allergic responses in the upper and lower airways have scarcely been reported.

Numerous murine models of allergic airway inflammation have been used to study the pathogenesis of AR and BA.[3,4] In most of them, antigen was challenged *via* the upper airway by provocation with a nebulized antigen or by intranasal (i.n.) instillation of an aqueous antigen.[3,5] Then, inflammatory responses were evidently observed in the lower airways. However, even though challenged antigen exactly passes through the nasal cavity, most of these models have not investigated the nasal responses, and therefore it has not been fully clarified whether nasal and bronchoalveolar inflammation is occurred at the same time after antigen challenge *via* the nasal route.

In order to develop murine models individually displaying upper and lower airway inflammation, we compared the nasal and bronchoalveolar responses in ovalbumin (OVA)-immunized mice upon three different antigen challenge procedures, high-volume (a single shot of 25 μL antigen) i.n. challenge (IN/HV), low-volume (four shots of 5 μL antigen) i.n. challenge (IN/LV), and intratracheal (i.t.) challenge (IT) (Supplementary Fig. 1 and SUPPLEMENTARY METHODS).

OVA-IT induced infiltration of inflammatory cells into the lower airways without affecting the nose. Thus, large numbers of eosinophils and neutrophils were recovered in bronchoalveolar lavage fluid (BALF) of OVA-IT mice, though only the change in eosinophils was statistically significant (Fig. 1a, b). On the other hand, the nasal lavage fluid (NALF) cells were hardly observed in OVA- or saline-IT mice (Fig. 1c, d).

On the contrary, OVA-IN/LV caused infiltration of inflammatory cells into the nasal cavity without affecting the lower airway. The numbers of eosinophils and neutrophils in NALF significantly increased in response to IN/LV with OVA (Fig. 1c, d), though the BALF cells were hardly observed in OVA- or saline-IN/LV mice (Fig. 1a, b).

Upon OVA-IN/HV, inflammatory responses were induced in both the lungs and nasal cavity. The numbers of eosinophils and neutrophils in BALF (Fig. 1a, b) as well as NALF (Fig. 1c, d) clearly increased, though the change in the NALF cells was not statistically significant.

These inflammatory features were confirmed by upper and lower airway-specific pathophysiological responses. As shown in Figure 1e, the sneezing response, a common symptom of AR, was significantly induced in OVA-IN/LV mice, in comparison with saline-challenged control mice. The same level sneezing response was also observed in OVA-IN/HV mice, though it was not statistically significant in comparison with saline-IN/HV mice. This might be because, at least in part, a relatively large number of sneezing was induced by IN/HV with saline. These findings suggest that antigen challenge through the nasal cavity exactly induces the nasal response in immunized mice even though instillation of high-volume aqueous solution into the nose also triggers non-specific sneezing response. In contrast, sneezing was hardly observed in OVA- or saline-IT mice (Fig. 1e).

On the other hand, the bronchial responsiveness of OVA-IT and OVA-IN/HV but not OVA-IN/LV mice was significantly augmented in comparison with that of saline-challenged mice (Fig. 1f).

Consequently, in our models, OVA-IT enabled to induce lower airway-specific inflammatory responses. In contrast, upper airway-specific responses and simultaneous upper and lower airway responses were successfully induced by OVA-IN/LV and -IN/HV, respectively.

Recently, the relationship between allergic upper and lower airway diseases has become widely recognized. Indeed, several mechanisms of lower airway dysfunction in patients with AR such as altered breathing pattern have been suggested.[6,7] These clinical observations seem to be reproduced in our mouse model in which both upper and lower airway responses were obtained by IN/HV procedure.

On the other hand, in order to investigate the mechanisms of allergic upper and lower airway inflammation distinctively, several attempts to develop corresponding murine models have been made so far. Prior to the successful establishment of separated upper and lower airway inflammation models in our present study, Li *et al.* have also tried to create such models.[8] Even though certain levels of upper and lower airway-specific inflammation were observed in their models, they rinsed the nasal cavity of mice with saline after the nasal antigen application in the

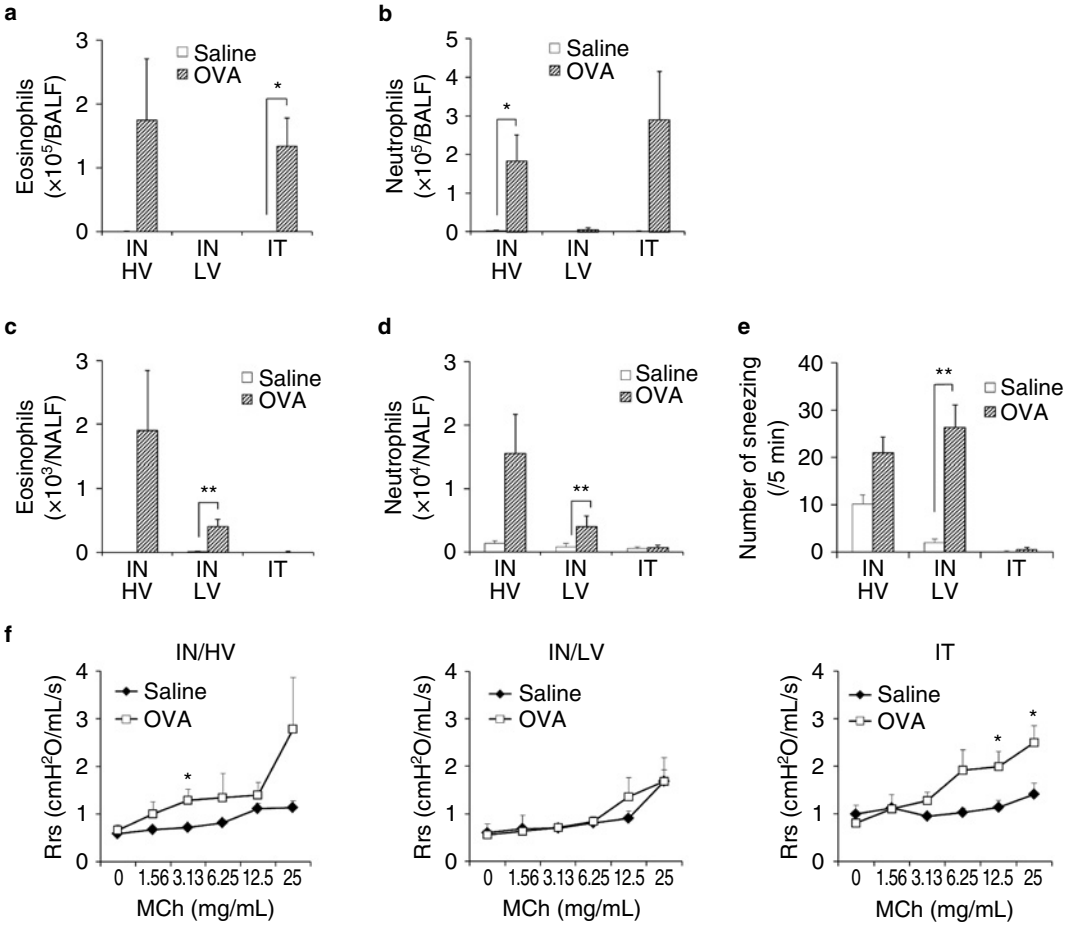


**Fig. 1** Intranasal or intratracheal antigen challenge-induced inflammatory responses. The number of eosinophils and neutrophils in BALF (**a** and **b**) and NALF (**c** and **d**), sneezing response (**e**) and bronchial responsiveness (**f**) in IN/HV, IN/LV, and IT mice were evaluated. Detailed protocols are described in the Supplementary Materials. *$p < 0.05$, **$p < 0.01$ (n = 3-7). BALF, bronchoalveolar lavage fluid; HV, high-volume challenge; IN, intranasal; IT, intratracheal challenge; LV, low-volume challenge; Mch, methacholine; NALF, nasal lavage fluid; OVA, ovalbumin; Rrs, respiratory system resistance.

case of antigen challenge to the lower airways. In our present study, lower airway-specific inflammation was induced by the direct i.t. administration of antigen. Therefore, the experimental system we used for inducing bronchoalveolar responses is most likely to less physical irritating to the nasal cavity and more selective to the lower airways than that used by Li *et al*.

In apparent contradiction with our findings that upper airway-specific responses without affecting bronchial responsiveness were obtained in IN/LV experiment, Hens *et al*. reported that a selective nasal antigen provocation induced significant BHR in mice.[9] The reason for the discrepancy is unclear, though mice were challenged with inhaled antigen for 8 consecutive days before the nose-specific antigen provocation in their models. Therefore, the lung function might already be affected during the antigen inhalation period. In order to elucidate the exact relationship between allergic upper and lower airway diseases, it may be helpful to study whether long-term IN/LV in our experimental system affects lower airway function.

In conclusion, we developed the comparative murine models of mixed and separated upper and lower airway inflammation by employing i.n. and i.t. challenge procedures. They may be useful for understanding the mechanisms, progression and relationship of allergic airway diseases, such as AR and BA.

## ACKNOWLEDGEMENTS

This work was supported by Grant-in-Aid for Agrihealth Project from the Ministry of Agriculture, Forestry and Fisheries of Japan to T. Hiroi, and JSPS KAKENHI to M. Saeki (Grant Number 24791817) and to O. Kaminuma (Grant Number 24500501).

## SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS and Supplementary Figure 1 are available online.

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Conflict of interest: No potential conflict of interest was disclosed.

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