

Murine Th Clones Confer Late Asthmatic Response upon Antigen Challenge

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Key Words

Airflow limitation • Bronchial asthma • Helper T cell • Late asthmatic response

Abstract

Background: Helper T (Th) cells are deeply involved in the pathophysiology of bronchial asthma, such as eosinophilic inflammation, bronchial hyperresponsiveness and remodeling. However, it is still unclear how Th cells contribute to airflow limitation, another cardinal feature of bronchial asthma. **Method:** Unprimed BALB/c mice were transferred with ovalbumin (OVA)-reactive Th clones. Pulmonary function was monitored using a Buxco BioSystem Plethysmograph before and after OVA challenge. **Results:** When Th-transferred mice were challenged with OVA, enhanced pause (P_{enh}), an indicator of airflow limitation was significantly increased 6 and 24 h after challenge, while no response was observed 30 min after challenge. Neither bovine serum albumin, an irrelevant antigen, challenge on Th-transferred mice nor OVA challenge on Th-non-transferred mice caused airway responses. **Conclusion:** Th cells conferred antigen-induced airflow limitation to unprimed mice.

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Introduction

Two types of asthmatic responses are induced upon antigen inhalation in atopic asthmatics, immediate asthmatic response (IAR) and late asthmatic response (LAR) [1, 2]. IAR is mediated by IgE-dependent mast cell release of mediators such as histamine, prostaglandins and cysteinyl-leukotrienes (CysLTs) [3–5]. LAR develops 3–8 h after allergen inhalation. LAR is associated with the development of airway inflammation and airway hyperresponsiveness (AHR). The inflammation is mainly driven by activated helper T (Th) cells and eosinophils [6–8], and leads to mucosal injury, excessive mucus production and AHR, thus resulting in bronchoconstriction [9–13]. Some investigators reported that late allergic response might be dependent on immediate allergic response, as it was sometimes inducible by IgE crosslinking [14, 15]. On the contrary, others reported that LAR is associated with activation of Th cells [13, 16–18]. We and others provided evidence that adoptive transfer of Th cells conferred airway eosinophilia and AHR to unprimed recipients [19–22]. However, it is still unclear whether Th cells may contribute to airflow limitation. So, the present study examined whether transfer of Th cells confer airflow limitation (LAR) or not, employing Th clone transfer model.

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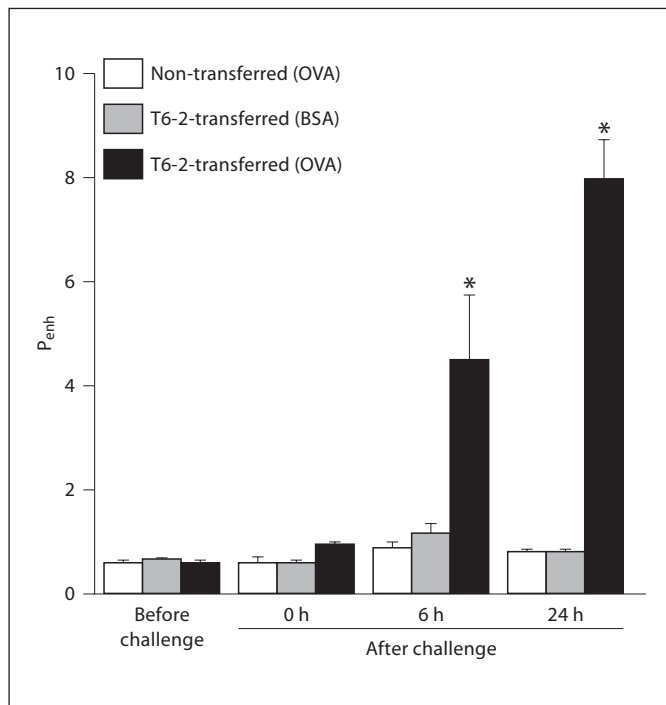


Fig. 1. Transfer of Th clone conferred airflow limitation after antigen challenge. An OVA-reactive Th clone, T6-2, was established from splenic T cells of DO11.10 mice. Unprimed BALB/c mice were transferred intravenously with T6-2 (1×10^7 cells) and challenged with OVA. P_{enh} was recorded for individual animals before challenge, 30 min, 6 and 24 h after challenge. As a control, T6-2-transferred mice were challenged with BSA, and non-transferred mice were challenged with OVA. P_{enh} values of T6-2-transferred mice challenged with OVA, T6-2-transferred mice challenged with BSA and non-transferred mice challenged with OVA were 0.59, 0.66 and 0.59 before challenge, 0.95, 0.62, 0.61 30 min after challenge, 4.50, 1.42, 0.91 6 h after challenge, 7.97, 0.80, 0.81 24 h after challenge, respectively. Data are expressed as the mean $\pm$ SEM. Each experimental group consists of >3 animals. * $p < 0.05$, compared with before challenge.

Animals and Methods

Animals

BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan). DO11.10 transgenic (Tg) mice (BALB/c background) were kindly provided by Dr. Dennis Y. Loh (Nippon Roche Research Center, Kamakura, Japan) [23]. Mice were housed under specific pathogen-free conditions in an environmentally controlled clean room. All animal experiments were performed under protocols approved by the Tokyo Metropolitan Institute of Medical Science Animal Care and Use Committee.

Establishment of Ovalbumin-Reactive T-Cell Clones

Splenic T cells (2×10^6) were prepared by passage through a nylon fiber column (Wako Pure Chemical, Osaka, Japan) accord-

ing to the method of Julius et al. [24], and cultured for 6–8 days in the presence of irradiated syngeneic spleen cells as antigen-presenting cells (APC, 6×10^6) and ovalbumin (OVA, 100 μ g/ml). Cloning by limiting dilution was performed with remaining viable cells in the presence of irradiated APC, OVA and interleukin (IL)-2 as described previously [20]. After 2–3 weeks, positive wells were transferred to macrowells along with irradiated APC, OVA and IL-2.

Cell Transfer and Challenge Procedures

Th clones (1×10^7) in 0.3 ml Hank's balanced salt solution (HBSS) were injected into the tail vein of unprimed BALB/c mice. 24 h after the cell transfer, mice were challenged intranasally with OVA (100 μ g in 50 μ l PBS). As a control, Th clone-transferred mice were challenged with bovine serum albumin (BSA), or non-transferred mice were challenged with OVA.

Measurement of Airflow Limitation

24 h after the transfer of Th clones, pulmonary function of mice was monitored using a Buxco BioSystem Plethysmograph (Buxco Research Systems, Wilmington, N.C., USA). Enhanced pause (P_{enh}) was recorded before (baseline), 30 min, 6 and 24 h after OVA challenge.

Bronchoalveolar Lavage Procedures

Bronchoalveolar lavage (BAL) was performed as reported previously [25]. Briefly, the trachea was cannulated with a polyethylene tube through which the lungs were gently lavaged with 0.4 ml HBSS 5 times (2 ml total). On average, 1.9 ml BALF was recovered and then centrifuged. Differential cell counts were made by microscopic examination of centrifuged preparations stained with May-Giemsa, counting 200 cells in each animal.

Statistical Analysis

Results are presented as the mean $\pm$ SEM. Statistical analysis was performed with ANOVA. To test differences between individual groups, we performed Student's t-test, and a $p < 0.05$ was considered statistically significant.

Results and Discussion

To investigate a possible role of Th cells on airflow limitation, unprimed BALB/c mice were transferred with an OVA-reactive Th clone, T6-2, and monitored using a Buxco BioSystem Plethysmograph. Airflow limitation was determined by a dimensionless parameter: P_{enh} , which is believed a suitable parameter for the continuous measurement of airflow limitation [26, 27]. P_{enh} value of T6-2-transferred mice before challenge (baseline) was not statistically different from that obtained before cell transfer (data not shown), or 30 min after OVA challenge (fig. 1), when IAR should be detectable. P_{enh} value was increased approximately 8- and 14-fold 6 and 24 h after challenge, respectively. Both values were significantly elevated compared to that obtained before challenge ($p <$

0.05). The response subsided 48 h after challenge (data not shown). To determine the antigen specificity of the response, T6-2-transferred mice were challenged with BSA, an irrelevant antigen, as a control. P_{enh} was not significantly changed 30 min, 6 and 24 h after the challenge (■). In addition, P_{enh} value was not increased in OVA-challenged mice that were not transferred with Th clones (□). These findings clearly indicated that the response was caused by antigen-specific activation of Th clones. Our present result clearly demonstrated for the first time that transfer of Th cells conferred airflow limitation upon antigen challenge.

Bronchial asthma is characterized by persistent airway inflammation with marked eosinophilia, AHR, airway remodeling and airflow limitation. Th2 cytokines play critical roles in these processes [28–32]. As Th2 response is characterized with marked IgE production, there is continued debate as to the relative contribution of IgE-dependent versus T-cell-dependent mechanisms in the pathogenesis of asthma [14, 15, 33]. Cell transfer model has been employed to investigate the role of Th cells on allergic responses in vivo in the absence of IgA, IgE and IgG antibodies, and it was previously found that eosinophilia [20], AHR [21] and airway remodeling [34] were induced upon antigen challenge in Th2-transferred mice independently of IgE-mediated response. Our present study clearly demonstrated that Th cells conferred airflow limitation to unprimed recipients as well. The number of eosinophils and neutrophils in BALF of T6-2-transferred mice was significantly increased 6 h after OVA challenge (data not shown), consistent with our previous report [20, 35]. The number of lymphocytes and monocytes/macrophages was not significantly changed. It is pretty intriguing to determine whether the response would be dependent upon CysLTs derived from eosinophils by administering CysLT antagonists. Depletion of neutrophils by administering anti-Gr-1 mAb might be another possibility.

Transfer of three other clones (T5-1, T6-4 and T6-7) also caused LAR upon antigen challenge, while 16 clones (B5-6, BT1, BT2, BT3, BF3, BF3.1, BF4, BF7, T5-2, T5-4, T5-6, T6-1, T6-3, T6-8, T6-9 and T6-10) did not, indicating that only part of Th cells can cause airflow limitation upon antigen challenge. Identification of phenotypes or markers of Th cells that confer airflow limitation is another intriguing question. Transfer of T6-2 caused eosinophilia and AHR besides airflow limitation, but that of T6-7 caused eosinophilia and airflow limitation but not AHR. Transfer of BF4 and T6-8 conferred both eosinophilia and AHR, but not airflow limitation (data not

shown). The key mediators of airflow limitation may be different from Th2 cytokines, as all these Th clones robustly produced Th2 cytokines, IL-4, IL-5 and IL-13 in vitro. IL-4, IL-5, IL-13 and IFN- γ concentration in BALF of T6-2-transferred mice 6 h after challenge were measured by specific ELISAs, but not significantly elevated (data not shown). IFN- γ , a Th1 cytokine, downmodulated Th2-driven AHR and eosinophilia [36], but on the other hand, induced CysLT receptor (CysLTR) 1 expression on smooth muscle cells [37] and CysLTR2 on endothelial cells [38], T cells and eosinophils [39, 40], and enhanced CysLT-mediated inflammation. It was recently reported that IL-17 plays an important role in allergen-specific T-cell-mediated immune responses [41], and Th17 cells are associated with airway inflammation and AHR [42, 43]. These findings collectively indicate that not only Th2 but also Th1 and Th17 might be involved in the pathophysiology of asthma. In addition, adhesion molecules on T cells, such as intercellular adhesion molecule-1 (ICAM-1) and very late antigen-4 (VLA-4) might be important for airflow limitation, as the blockade of these molecules inhibited LAR in guinea pig [44] and sheep asthma model [45]. Identification of key molecules involved in T-cell-dependent airflow limitation warrants further characterization of Th clones employing cytokine array, DNA microarray and two-dimensional electrophoresis.

Unrestrained plethysmography is ideally suited for long-term measurements of conscious animals, and P_{enh} value was employed to evaluate both IAR and LAR in murine models [26, 27]. There is a controversy regarding the validity of P_{enh} , because it is not a direct measurement of lower respiratory tract [46]. On the other hand, the alternation in P_{enh} value was well correlated with that in pulmonary resistance [47–49]. T6-2-transferred mice exhibited eosinophilic inflammation in the lower airway, consistent with the view that activation of Th cells resulted in LAR. Further measurements of lung mechanics using tracheotomized and mechanically ventilated mice are under way to differentiate airway resistance between upper and lower airways.

In conclusion, our study clearly demonstrated that airflow limitation was induced by Th cells in the absence of IgE-mediated reaction. Th-targeted therapy would be of considerable interest in controlling asthma.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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