Allergen-induced Th2-independent production of IL-4 in the airways of mice

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Abstract
It is thought that release of cytokines from allergen-damaged epithelial cells induces production of an innate source of IL-4 and IL-13 that are important in initiating adaptive T-helper type II (Th2)-mediated allergic responses. However, detecting innate production of IL-4 or IL-13 in vivo is difficult due to high levels of adaptive production of IL-4 and IL-13 by Th2 cells. The IL-4 receptor (IL-4R) and the IL-4/13 co-receptor (IL-13R) share a common receptor subunit (IL-4Ra). Consequently, both IL-4 and IL-13 signal via the IL-4Ra-associated signaling molecule, signal transducer and activator of transcription factor 6 (STAT6). STAT6 signaling in T-cells, B-cells, and airway epithelial cells is essential for Th2 differentiation, isotype class switching to IgE, and mucus production, respectively. Therefore, Epi-STAT6 mice (STAT6-/- mice with airway epithelial-specific transgenic STAT6 expression) are defective in Th2 immune responses but can produce mucus in response to IL-4 and/or IL-13 in their airways. As compared to wildtype mice, allergen-challenged Epi-STAT6 mice were deficient in IgE production and the levels of IL-13 expressed were not above appropriate controls. However, significant levels of IL-4 and mucin gene expression were detected in their lungs. These observations support the existence of an allergen-responsive, non-Th2-derived source of IL-4 in the airways of mice.

Introduction
The innate immune mechanisms that link allergen exposures to allergic adaptive immune responses are not well understood. One idea is that proteolytic allergens, including those produced by Aspergillus fumigatus, can damage epithelial cells [1]. Damaged epithelial cells release cytokines (e.g., IL-33, IL-25, and thymic stromal lymphopoietin) which are thought to contribute to the initiation of allergic immune responses by inducing the production of an innate source of IL-4 and IL-13 [2]. However, Th2 cells produce high levels of IL-4 and IL-13, making it difficult to determine in vivo if there exists an allergen-induced non-Th2-derived source of IL-4 and IL-13 in the airways.

We used wildtype and Epi-STAT6 mice. Epi-STAT6 mice are mSTAT6-/- and express transgenic hSTAT6 under control of the epithelial-specific rat CC10 promoter. Airway epithelial STAT6 signaling causes mucus production and transcription of the mucin gene, Muc5ac [3]. T-cell STAT6 signaling is essential for Th2 differentiation [4] and B-cell STAT6 signaling is essential for class switching to IgE [5]. Therefore, Epi-STAT6 mice do not develop Th2 immune responses and as such provide a convenient way to investigate innate production of IL-4 and/or IL-13 in the airways.

Objective
To test the hypothesis that an innate source of IL-4 and/or IL-13 exists in the airways of mice.
Allergen-induced Th2-independent production of IL-4 in the airways of mice

![Graph a: Serum IgE levels](image)

![Graph b: IL-4 levels](image)

![Graph c: Muc5ac levels](image)
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Figure Legend

Figure 1. Allergen-induced IL-4, IL-13, and Muc5ac gene expression in Epi-STAT6 mice.

(A) Total IgE in serum of wildtype (WT) and Epi-STAT6 (Epi) mice exposed to either PBS or Aspergillus fumigatis extract (Ag) directly to their airways.

(B-D) Gene expression of IL-4, IL-13, and Muc5ac in lungs of WT and Epi mice exposed to either PBS or Ag directly to their airways. Results are mean ± SEM (n = 6 mice/group).

*p<0.05.

Protocols

For antigen studies, we anesthetized mice with an i.p. injection of a mixture of ketamine and xylazine (45 and 8 mg/kg, respectively). The mice were hung from their incisors using a rubber band stretched across a foam pad, and a small spatula was used to gently extend their lower jaws and tongues. Aspergillus fumigatus extract (Greer Labs) 100 ug in 50 ul PBS or 50 ul PBS (controls) was pipetted into the oropharynx, causing aspiration. Airway challenges occurred on days 1, 3, 5, and 7. Measurements were made on day 8.

Serum IgE

A sandwich ELISA kit for mouse total IgE (PharMingen) was used according to manufacturer’s instructions. Serum was analyzed in duplicate at 1/50 dilution in 10% FBS in PBS. OD readings were converted to ng/ml using values obtained from standard curves and multiplied by the dilution factor.

Gene expression

Lungs were homogenized in Trizol (Sigma), RNA was isolated and converted to cDNA by reverse transcription. Preverified Taqman assays (Applied Biosystems) were used to detect IL-4, IL-13, and the Muc5ac by real-time PCR. Copy numbers were normalized to those of the housekeeping gene, GAPDH.

Statistics

ANOVA and Tukey-Kramer post test were used. Means and SEMs are shown. P-values less than 0.05 were considered statistically significant vs. appropriate PBS controls.

Results & Discussion

We compared allergen-induced airway responses in wildtype and Epi-STAT6 mice. Repeated Aspergillus fumigatus extract challenges to the airways of wildtype mice resulted in significant induction of serum IgE and gene expression of IL-4, IL-13, and Muc5ac. Muc5ac is a mucin gene recognized as an excellent biomarker for airway epithelial mucus production [6]. The results were as expected for Th2-competent wildtype mice. However, allergen challenges to the airways of Epi-STAT6 mice resulted in complete lack of induction of IgE but still statistically significant levels of IL-4 gene expression and moderate levels of Muc5ac gene expression. IL-13 levels in Epi-STAT6 mice were not induced above Epi-STAT6-PBS controls. Thus, the small induction of IL-4 and moderate induction of Muc5ac detected in Epi-STAT6 mice indicates the existence of an allergen-induced non-Th2 cell-derived source of IL-4 in the airways of mice.
Conclusions
This observation supports the existence an allergen-induced innate source of IL-4 in the airways of mice.

Limitations
IL-4-induced STAT6 signaling in T-cells is essential for Th2 differentiation in vitro, and STAT6/-/- mice are deficient in Th2 immune responses. However, these studies cannot rule out the possibility of a STAT6-independent mechanism of Th2 cell development in vivo.

Additional Information

Methods

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Supplementary Material
Please see https://sciencematters.io/articles/201602000022.

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Ethics Statement
The experiments involving mice were approved by the Northwestern University Animal Care and Use Committee and complied with the “Guide for the care and use of laboratory animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996).

Citations

[2] Rohit Divekar and Hirohito Kita. "Recent advances in epithelium-derived cytokines (IL-33, IL-25, and thymic stromal lymphopoietin) and allergic inflammation". In: Current Opinion in Allergy and Clinical Immunology 15.1 (Feb. 2015), pp. 98–103. DOI: 10.1097/aci.0000000000000133. URL: http://dx.doi.org/10.1097/aci.0000000000000133.

