IL9 leads to airway inflammation by inducing IL13 expression in airway epithelial cells

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Abstract

Constitutive expression of IL9 in the lungs of transgenic (Tg) mice resulted in an asthma-like phenotype consisting of lymphocytic and eosinophilic lung inflammation, mucus hypersecretion and mast cell hyperplasia. Several Th2 cytokines including IL4, IL5 and IL13 were expressed in the lung in response to Tg IL9. IL13 was absolutely necessary for the development of lung pathology. To understand how IL9 induces IL13-dependent lung inflammation and mucus production, we sought the IL13-producing cells. Surprisingly, we found that the absence of T cells and B cells in recombinase-activating gene 1 (RAG1)-deficient IL9 Tg mice enhanced lung inflammation and dramatically enhanced IL13 production. In addition, the lack of mast cells or eosinophils in IL9 Tg mice did not affect IL13 levels in the lung. In situ hybridization for IL13 on lung sections from RAG1−/− IL9 Tg mice revealed that airway epithelial cells were the major IL13-producing cell type. Our results implicate the lung epithelium as a potentially important source of inflammatory cytokines in asthma.

Introduction

Allergic asthma is recognized as a chronic inflammatory disorder of the airways. The complex immune response to antigen in the lung involves various inflammatory cell types including T cells, B cells, eosinophils, macrophages and mast cells (1). There is growing evidence that the predominant cell type involved in the regulation of pathophysiology in asthma is the CD4 T cell of the Th2 subtype characterized by the expression of cytokines like IL4, IL5, IL9, IL10 and IL13 (2). The contribution of these individual cytokines, however, to the development of pathologic and physiologic changes seen in the lungs of asthmatics such as eosinophilia, mucus hypersecretion, mast cell hyperplasia and bronchial hyperresponsiveness are still not completely understood. Recently, interest has been focused on IL9 and its implications in the pathology of asthma (3).

IL9 was originally described as a growth factor for a subset of murine T cell clones (4). Subsequently, more activities of IL9 on various cell types including mast cells, hematopoietic progenitors, B cells, eosinophils, neutrophils and airway epithelial cells have been described (5–10). Most of these inflammatory cells are involved in the pathology of asthma and therefore suggested a potentially important function for IL9. Studies in humans and animals have shown the involvement of IL9 in lung eosinophilia, mucus hypersecretion, Ig production and pulmonary mastocytosis (7, 9–14). These findings have been strongly supported by our studies with transgenic (Tg) mice that constitutively express IL9 selectively within their lungs (15, 16). These mice developed many features that resembled human asthma, including eosinophilic and lymphocytic infiltration of the lung, mucus hypersecretion, sub-epithelial fibrosis, mast cell hyperplasia and bronchial hyperresponsiveness. Further, we showed that the expression of several Th2 cytokines besides IL9, including IL4, IL5 and IL13, was necessary for the same pathology in inducible, lung-specific IL9 Tg mice (16). We demonstrated that IL13, in particular, was essential for the development of lung inflammation and mucus production. To investigate how IL9 induces pathology resulting from Th2 cytokine expression, we sought to identify potential target cells for the action of IL9. Surprisingly, the elimination of T cells did not abolish lung pathology in IL9 Tg mice. We now show that airway epithelial cells are the major producers of IL13 in response to the IL9 transgene expression in the lung, in the absence of inflammatory cells such as T cells.
IL9 induces IL13 in airway epithelial cells

and eosinophils. Therefore, airway epithelial cells might represent a potential target cell for IL9 in the lung and an important source of pathologic cytokines in asthma.

Methods

Mice
Mice (6- to 10-weeks old) were used for the experiments described here. IL9 Tg mice expressing constitutive, lung-specific IL9 under the control of the CC-10 promoter have been previously described (15). Mice deficient for signal transduction of activated T cells 6 (STAT6) (17), IL5 (18) or recombination activating gene 1 (RAG1) (19) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained on C57BL/6 background. IL9 Tg mice (−/−) and IL9 Tg mice (−/+), IL5-deficient (−/−), RAG1-deficient (−/−) and STAT6-deficient (−/−) mice (20) were kindly provided by A.N.J. McKenzie (MRC, Cambridge, UK) and back-crossed onto C57BL/6 background. Genotyping of mice during back-crossing onto IL9 Tg mice was performed by PCR analysis of tail DNA using specific primers for wild-type (WT) and mutant knockout (KO) allele. STAT6: 5′-ATG TCT CGT TGG GGC CTA ATT TCC-3′ and 5′-GCC TCA GGC CAG QAC TTC TC-3′ (WT) and 5′-GAT CTG GAC QAA GAG CAT CAG G-3′ and 5′-GCC TCA GGC CAG QAC TTC TC-3′ (KO). IL5: 5′-CTG GCC TTC AAC TCC TGA TCC T-3′ and 5′-GAA CTC TTG CAG GTA ATC CAG G-3′ (WT) and 5′-CTT GGG TGG AGA GGC TAT GTC TGA ATC CAG G-3′ (KO). IL13: 5′-GGG TCA GTC QAG CCG GGA and 5′-GGT GCT CAG CTC CAC AAT AAG C-3′ (WT) and 5′-CTT GGG TGG AGA QAC TTC TC-3′ and 5′-AGG TCA GAT QAC AGG AGA TC-3′ (KO). IL9: 5′-GGT QAC CTC QCC AGC AAA CCA TGT CC-3′ and 5′-CTT GGG TGG AGA GGC TAT GTC TGA ATC CAG G-3′ (WT) and 5′-AGG TCA GAT QAC AGG AGA TC-3′ (KO). RAG1: 5′-CCG TCA GGC CAG GAT GCT QAC CTC ATT AAT AAG C-3′ and 5′-GCC TCA GGC CAG QAC TTC TC-3′ (WT), 5′-GAG TGT GG-3 and 5′-GGT QAC CTC QCC AGC AAA CCA TGT CC-3′ and 5′-CTT GGG TGG AGA GGC TAT GTC TGA ATC CAG G-3′ (KO).

Lung lavage, tissue fixation and staining
Mice were anesthetized by methoxyflurane inhalation and then sacrificed by carbon dioxide inhalation. Lung lavage and cell enumeration were performed as described previously (15). The lungs were excised completely from the chest, inflated with 1 ml of 10% formalin and immersed in 10% formalin. Tissue processing and histological stainings were performed by the Yale Medical School Research Histology, Department of Pathology.

Cytokine assay
Quantitation of cytokine levels in lung lavage fluid was performed by ELISA according to the manufacturer's instructions. The detection limit for the IL5 ELISA (BD Bioscience, San Diego, CA, USA) was 40 pg ml−1 and for the IL13 ELISA (R&D Systems, Minneapolis, MN, USA) was 39 pg ml−1.

RNase protection assay
RNase protection assay was performed as described previously (16) using the RiboQuant Multi-Probe RNase Protection Assay System according to the manufacturer's instructions (BD Bioscience).

In situ hybridization
Lung tissue was fixed in formaldehyde and processed into paraffin. Sections (5 μ) were cut, deparaffinized, and then treated with proteinase K (10 μg ml−1) for 20 min at 37°C. Subsequently, sections were treated with 0.1 M triethylolamine/0.25% acetic anhydride, pH 8.0, for 10 min at room temperature, and then rinsed in PBS. The IL13 probe, kindly provided by Zhou Zhu (Yale University School of Medicine), was generated as a 615-bp fragment from IL13 cDNA. Restriction sites for Xho and Xba were incorporated in the primers (sense: 5′-GAG TGT GG-3 and anti-sense: 5′-AGC CCC TCT AGA ATA GGC AGC AAA CCA TGT CC-3′) and the fragment

Fig. 1. Characterization of inflammatory cells in lung lavage fluid from IL9 Tg mice. IL9 transgene-negative (IL9 Tg−) or transgene-positive (IL9 Tg+) mice were back-crossed onto IL13−/− or STAT6−/− background. (A) Total lung lavage cell counts. (B) Differential cell counts on lung lavage fluid. Results are expressed as total cell numbers and were obtained from at least 200 cell counts. Data are expressed as mean ± SD. Significant differences in total cell numbers (A) or in the number of eosinophils (B) compared with that of IL9 Tg mice (IL9 Tg+) are indicated with an asterisk.
was sub-cloned into plasmid pBluescript KS (Stratagene, La Jolla, CA, USA). Sense and anti-sense RNA probes were generated using T7 and T3 promoter sites, labeled with a digoxigenin RNA labeling kit (Roche, Indianapolis, IN, USA), and then denatured at 65°C for 10 min. Sense and anti-sense probes were diluted in hybridization buffer (Ambion, Austin, TX, USA) at 30 ng ml⁻¹ and added to sections for overnight incubation at 55°C. The tissues were then washed twice with 4× standard saline citrate (SSC) for 5 min at room temperature, twice with 2× SSC for 10 min at 37°C and incubated with RNase A (10 μg ml⁻¹) for 45 min at 37°C. This was followed by two washes (10 min) in 2× SSC at room temperature and three washes (20 min) in 0.2× SSC at 56°C. After blocking non-specific binding, sections were incubated overnight at 4°C with an anti-digoxigenin antibody conjugated with HRP (Roche). For signal amplification, slides were incubated with biotinyl tyramide (DAKO GenPoint Kit) in the dark for 8 min. The probe was detected by incubation with alkaline phosphatase-conjugated streptavidin (DAKO GenPoint Kit) followed by 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphatase.

Statistical analysis

Values are expressed as means ± SDs. The data were normally distributed, and group means were compared with the Student two-tailed, unpaired t-test using Excel 5 for Apple Macintosh (Microsoft Corporation, Redmond, WA, USA).

Results

Role of STAT6 and IL13 in inflammation in IL9 Tg mice

We have recently shown that the development of lung pathology observed in inducible IL9 Tg mice was dependent on IL13 expression (16). Blockade of IL13 completely abolished lung inflammation, airway epithelial cell hypertrophy and mucus overproduction. We obtained similar results after crossing constitutive IL9 Tg mice (15) onto the IL13−/− background. Total lung lavage cell counts in IL13−/− IL9 Tg mice were significantly decreased compared with IL13+/+ IL9 Tg mice and were comparable to those from non-Tg (WT) control mice (Fig. 1A). Differential cell counts on lung lavage cells confirmed that the increased numbers of macrophages, lymphocytes and especially eosinophils observed in the airways of IL13+/+ IL9 Tg mice were not found in IL13−/− IL9 Tg mice (Fig. 1B). Histologic assessment of lung tissue demonstrated that the accumulation of inflammatory cells around blood vessels and airways and airway epithelial cell hypertrophy with mucus overproduction seen in lung sections of IL13+/+ IL9 Tg mice was absent in IL13−/− IL9 Tg mice (Fig. 2).

To determine if IL13R signals through the STAT pathway were required STAT6−/− mice were examined. The effects of IL13 on the development of lung pathology were dependent on STAT6 signaling since airway inflammation was also totally abolished in IL9 Tg mice which had been...
back-crossed onto the STAT6–/– background (Fig. 1A and B). No increased numbers of inflammatory cells were found in lung lavage fluid from STAT6–/– IL9 Tg mice (Fig. 1A and B). Further, lung sections did not show the epithelial cell hypertrophy with mucus overproduction that was seen in STAT6+/+ IL9 Tg mice (Fig. 2).

The role of lymphocytes in lung pathology of IL9 Tg mice

CD4+ T cells are a major source of IL13. To investigate if T cells are important producers of IL13 in the lungs of IL9 Tg mice, we back-crossed these mice onto a RAG1–/– background eliminating mature T and B cells. Surprisingly, in the absence of T and B cells, lung inflammation was dramatically increased in RAG1–/– IL9 Tg mice compared with RAG1+/+ IL9 Tg mice. Total lung lavage cell counts were significantly increased in RAG1–/– IL9 Tg mice compared with RAG1+/+ IL9 Tg mice. This increase was mostly due to eosinophils (Fig. 3A and B). Also, examination of the lung tissue revealed that epithelial cell hypertrophy with mucus overproduction was still present in the absence of T and B cells in RAG1–/– IL9 Tg mice (Fig. 4). We found that mice on this background developed disease earlier than RAG+/+ mice. Therefore in the data shown, tissue was harvested at 6–7 weeks of age as opposed to the 8- to 10-week old mice shown in Fig. 1.

ELISA performed on lung lavage fluid from RAG1–/– IL9 Tg mice detected high levels of IL13 (84.9 ± 28.4 pg ml⁻¹) and IL5 (57.6 ± 14.5 pg ml⁻¹) while both cytokines were below the detection limit (39 pg ml⁻¹ for IL13 and 40 pg ml⁻¹ for IL5) in lung lavage fluid from RAG1+/+ IL9 Tg mice (data not shown).

RNase protection assay on total RNA from lung tissue confirmed that mRNA levels of IL13, as well as IL5, were clearly elevated in RAG1–/– IL9 Tg mice compared with the mRNA levels in RAG1+/+ IL9 Tg mice (Fig. 5). The mRNA levels of other cytokine genes like IL15, IL2, IL3, IFN-γ, the transgene IL9 and the housekeeping genes were at comparable levels (Fig. 5). Therefore, in the absence of T cells another source of IL13 must exist in the lungs of IL9 Tg mice.

The role of eosinophils and mast cells in pathology of IL9 Tg mice

Eosinophils and mast cells have been shown to be potent producers of several different kinds of cytokines including IL13. Both cell types are represented in the inflammatory response caused by the IL9 transgene expression in the lung (15). To investigate if one of these cell types might be involved in IL13 production, we back-crossed IL9 Tg mice with IL5–/– mice which are not able to generate mature eosinophils, or mast cell-deficient (KitW/KitWv) mice. The lack of eosinophils in IL5–/– IL9 Tg mice resulted in a decreased total lung lavage cell count (Fig. 6A) compared with IL5+/+ IL9 Tg mice but the numbers of macrophages and lymphocytes were still elevated compared with non-Tg control mice (Fig. 6B). Similar results were obtained from IL9 Tg mice which were back-crossed onto the mast cell-deficient (KitW/KitWv) background. Total lung lavage cell counts were decreased in KitW/KitWv IL9 Tg mice compared with WT IL9 Tg mice (Fig. 6A). Surprisingly, the lack of mast cells resulted in the reduction of eosinophils retrieved from the airways (Fig. 6C) which could be an effect of the mixed genetic background of mast cell-deficient (KitW/KitWv) mice or IL5 production by these cells. However, the absence of IL5 and eosinophils (Fig. 7C) or mast cells (Fig. 7D) did not affect the development of epithelial cell hypertrophy and mucus overproduction in IL9 Tg mice as shown in lung sections stained with alcin blue/periodic acid Schiff.

RNase protection assay on total lung RNA from IL5–/– and IL5+/+ IL9 Tg mice revealed that levels of IL13 mRNA, as well as those of all other cytokines mRNAs tested, were...
similar (Fig. 8). Eosinophils were, therefore, excluded as a major source of IL13 in IL9 Tg mice. Also, RNase protection assay using total lung RNA from KitW/KitWv IL9 Tg mice did not show any difference in IL13 mRNA levels (data not shown). This result suggests that mast cells are also not the major source of IL13 in the lungs of IL9 Tg mice.

Role for IL5 in eosinophilia of RAG1-/- mice but not IL13 production

RAG1-/- IL9 Tg mice had extremely high numbers of eosinophils in their airways which correlated with the elevated IL5 and IL13 levels. Since T cells and T cell-derived cytokines might suppress expression of cytokines by eosinophils, we investigated if the absence of T cells in RAG1-/- IL9 Tg mice resulted in cytokine expression, in particular IL13, by eosinophils. We back-crossed RAG1-/- IL9 Tg mice with IL5-/- mice. Even in the absence of eosinophils, IL5-/- RAG1-/- IL9 Tg mice still showed increased total lung lavage cell counts (Fig. 9Aa) which was mostly due to elevated numbers of macrophages compared with IL5-/- RAG1-/- non-Tg mice (Fig. 9Ab). Further, lung sections from IL5-/- RAG1-/- IL9 Tg mice still showed epithelial cell hypertrophy with positive staining for mucus (Fig. 9B).

Fig. 4. Histologic staining for mucin. Lung sections from IL9 transgene-negative (A and C) or IL9 transgene-positive (B and D) mice were stained with alcian blue/periodic acid Schiff for light microscopy. Intense, positive (magenta) staining for mucin was observed not only in hypertrophied airway epithelial cells from IL9 transgene-positive mice (B) but also in IL9 transgene-positive mice that had been back-crossed onto RAG1-/- background (D). Mucin-positive epithelial cells were not detected in IL9 transgene-negative mice (A) or IL9 transgene-negative mice on RAG1-/- background (C). Original magnification × 300.

Fig. 5. Analysis of cytokine expression in total lung tissue. Levels of various mRNAs encoding predominantly T2 cytokines from individual IL9 transgene-negative (lanes 1–4) and -positive (lanes 5–8) mice were compared by RNase protection assay. Representative mice in lanes 3, 4, 7 and 8 had been back-crossed onto RAG1-/- background. The P32-labeled multiprobe template set mCK-1b was used as a size marker. Levels of L32 and GAPDH mRNAs encoding housekeeping genes were used to compare loading of samples.
in IL5−/− RAG−/− IL9 Tg mice, there were still elevated levels of IL13 mRNA detectable (Fig. 10A, lanes 1 and 2). IL13 mRNA was not detected in lung tissue from IL5−/− RAG−/− IL9 transgene-negative mice (Fig. 10A, lanes 3 and 4).

**Cellular source of IL13 mRNA in lung sections from IL5−/− RAG−/− IL9 Tg mice**

Our results indicated that IL13 was still produced even in the absence of major inflammatory cells like T cells, mast cells and eosinophils in IL9 Tg mice. Therefore, we used...
lung tissue from IL5−/− RAG1−/− IL9 Tg mice for in situ hybridization to detect the source of IL13 in these mice. Using an IL13-specific anti-sense probe for hybridization, strong positive staining was observed in the airway epithelium of IL5−/− RAG1−/− IL9 Tg mice (Fig. 10Ba and d). In contrast, hybridization with the sense probe produced no staining in the lung tissue from IL5−/− RAG1−/− IL9 Tg mice (Fig. 10Ba and b).

Discussion

The expression of IL9 selectively within the lungs of Tg mice resulted in pathologic changes similar to that seen in the lungs of human asthmatics including lymphocytic and eosinophilic airway inflammation, mucus hypersecretion and mast cell hyperplasia (15). Previously, we had shown that various T_{h}2 cytokines including IL4, IL5 and IL13 were produced in the lung in response to inducible IL9 expression (16). Although one of them, IL13, was absolutely necessary for the induction of lung inflammation and mucus production, the combined action of all three cytokines was necessary for full pathology to develop. In order to understand how IL9 induces IL13-dependent lung inflammation, we tried to identify potential target cells for the action of IL9 by focusing on lung inflammation, mucus production and, in particular, the expression of IL13.

By crossing constitutive IL9-expressing mice onto the IL13−/− background, we confirmed that IL13 is a critical mediator for the development of pathology in the lung. The effects of IL13 in IL9 Tg mice were STAT6 dependent coinciding with previously published data that this signaling pathway is critical to the contribution of IL13 to phenotypic features of allergic asthma (22, 23), just as in our Tg model.

A major source of IL13 is the CD4+ T cell of the T_{h}2 subtype (24). The importance of these cells for the development of lung pathology has been demonstrated using a murine model of antigen-induced lung inflammation wherein the absence of CD4+ T cells prevents the development of asthmatic features including eosinophilic infiltration of the lung and airway hyperreactivity (25). Assuming that in IL9 Tg mice CD4+ T cells also play a central role in the immune response through the production of cytokines, especially IL13, we investigated if T cells are important target cells for the action of IL9. We crossed IL9 Tg mice with RAG1−/− mice which lack mature T and B cells (19). Studies with RAG1−/− mice had shown before that these mice failed to develop allergen-induced lung inflammation, mucus hypersecretion and airway hyperresponsiveness in murine models of asthma (26). However, reconstitution of RAG1−/− mice with CD4 T cells alone could restore the asthmatic phenotype in response to allergen challenge of the lung (26). Surprisingly, the lack of T and B cells in IL9 Tg mice did not result in abrogation of the asthma-like phenotype but quite the reverse response occurred. Lung inflammation, characterized by extreme high numbers of eosinophils, epithelial cell hypertrophy with mucus accumulation and T_{h}2 cytokine production, in particular IL13, was dramatically increased. We concluded that IL13 production was independent of T and B cells and was sufficient to induce a strong asthma-like phenotype in the lung. Results that IL13 could cause pathology independent of T and B cells by administering IL13 intranasally to RAG1−/− mice had been published previously (27). Our data suggested to us that T cells had more of a regulatory function in our model by keeping lung inflammation under control rather than being the major IL13 producer. We predicted that some other cell type in the lung must therefore be responsible for producing the high levels of IL13 in response to IL9 expression.

Two inflammatory cell types that are present in the lungs of IL9 Tg mice at high numbers are eosinophils and mast cells. For both it has been described that they are able to express IL13 (28–30). The development of mast cells within the airway epithelium is a unique feature of IL9 Tg mice (15). However, from our previous studies on electron-microscopic appearance and histamine release of these cells, we concluded that mast cells are not degranulated or releasing any mediators in our model. We speculated that the effect of IL9 on mast cells was more related to differentiation and proliferation in the airway epithelium rather than to mediator release (15). As predicted, back-crossing of IL9 Tg mice onto a mast cell-deficient background did not abolish lung inflammation or mucus production even though eosinophilia was decreased, suggesting no critical involvement of mast cells in IL13 production. On the other hand, eosinophils seemed to be a major candidate for IL13 production because increased numbers of eosinophils in the lungs of RAG1−/− IL9 Tg mice correlated in our study with increased IL13 expression levels. Evidence that IL9 might directly act on eosinophils was provided before by the detection of IL9Rα expression on human peripheral blood eosinophils and by the IL9-stimulated induction of IL5Rα on eosinophils (11). However, an effect of IL9 on lung eosinophils resulting in IL13 expression could not be confirmed in our study since
the lack of eosinophils in IL5−/− IL9 Tg mice did not decrease IL13 expression levels or lung pathology in particular epithelial cell hypertrophy. Interestingly, we never detected any basophils in the bronchial infiltrates of the IL9 Tg mice and therefore we did not pursue any role for these cells in the associated disease.

To exclude that the presence of T cells might have negatively influenced potential IL13 production by eosinophils, we investigated IL13 production and lung pathology in IL5/RAG1 double-deficient IL9 Tg mice. However, also no role for eosinophils in IL13 production in IL9 Tg mice could be confirmed in the absence of T and B cells. RAG1/IL5 double-deficient IL9 Tg mice had extremely high levels of IL13 expression and lung pathology except eosinophilia was completely preserved. In conclusion, major inflammatory cell types like T cells, mast cells and eosinophils could not be verified as a major source for IL13 in IL9 Tg mice.

We finally decided to use in situ hybridization to detect the IL13-expressing cells in IL5/RAG1 double-deficient IL9 Tg mice. These mice had the highest IL13 expression levels and we were able to identify airway epithelial cells as the main IL13 producer. The airway epithelium is believed to play an important role during asthmatic inflammation in the lung by not only secreting mucus but also by producing a variety of chemokines and cytokines. Several recent observations have demonstrated that IL9 might directly influence airway epithelial cell function since IL9Rα had been detected on human and murine epithelial cells (31, 32). An involvement of IL9 in regulating mucus expression in the airways had been shown in vivo and in vitro (10, 15, 16, 31). Further, the IL9-induced release of chemokines in vitro by human and murine lung epithelial cells was previously reported (13, 32). The IL9 stimulation of human bronchial epithelial cells resulted in release of two T cell chemotactic factors, IL16 and regulated on activation, normal T cells expressed and secreted (RANTES) (13). In mice, addition of IL9 to primary lung epithelial cultures and cell lines induced the expression of the CC-chemokines eotaxin and MCP-5 (27). The direct
expression of IL13 by epithelial cells has not been demonstrated so far. One study has suggested the production of IL4 by immunohistochemistry (33). Therefore, our observation that IL9 might induce directly or indirectly the expression of IL13 in airway epithelial cells represents a novel and important finding. There is evidence in humans for expression of IL13 in airway epithelium using immunohistochemistry on biopsies of bronchial epithelium (34). However, IL13 expression has never been demonstrated via in situ hybridization to show cellular source of message for IL13. Furthermore, there is no previous evidence for epithelial production of IL13 in a well-defined murine system. Production of IL13 by human alveolar macrophages has been reported (35). We cannot exclude, therefore, that macrophages contribute to IL13 production in addition to the epithelial cells described herein. In conclusion, we have shown that IL13 production in IL9 Tg mice occurred independent of major inflammatory cell types like T cells, B cells, mast cells and eosinophils. In IL5/RAG1 double-deficient IL9 Tg mice, we have identified airway epithelial cells as the major location of IL13 expression and lymphocytes as inhibitory of its expression. In future experiments, we will have to elucidate if epithelial cells represent a direct target cell for IL9 in the lung. However, our findings presented here might implicate a new role for IL9 in airway epithelial cell function by inducing cytokine production. In addition, our data add new interesting aspects to the contribution of airway epithelial cells to bronchial inflammation in asthma.

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Abbreviations
KO knockout
RAG recombinase-activating gene
SSC standard saline citrate
STAT signal transduction of activated T cell
Tg transgenic
WT wild type

References
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Kopf, M., Brombacher, F., Hodgkin, P. D. et al. 1996. IL5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 4:15.


