Role of IL-4 receptor α–positive $CD4^+$ T cells in chronic airway hyperresponsiveness

Frank Kirstein, PhD, Natalie E. Nieuwenhuizen, PhD,* Jaisubash Jayakumar, PhD, William G. C. Horsnell, PhD, and Frank Brombacher, PhD *Cape Town, South Africa, and Berlin, Germany*

Background: T_H2 cells and their cytokines are associated with allergic asthma in human subjects and with mouse models of allergic airway disease. IL-4 signaling through the IL-4 receptor α (IL-4Rα) chain on $CD4^+$ T cells leads to T_H2 cell differentiation *in vitro*, implying that IL-4Rα–responsive $CD4^+$ T cells are critical for the induction of allergic asthma. However, mechanisms regulating acute and chronic allergen-specific T_H2 responses *in vivo* remain incompletely understood.

Objective: This study defines the requirements for IL-4Rα–responsive $CD4^+$ T cells and the IL-4Rα ligands IL-4 and IL-13 in the development of allergen-specific T_H2 responses during the onset and chronic phase of experimental allergic airway disease.

Methods: Development of acute and chronic ovalbumin (OVA)–induced allergic asthma was assessed weekly in $CD4^+$ T cell–specific IL-4Rα–deficient BALB/c mice (Lck^{cre}IL-4R$\alpha^{-/lox}$) and respective control mice in the presence or absence of IL-4 or IL-13.

Results: During acute allergic airway disease, IL-4 deficiency did not prevent the onset of T_H2 immune responses and OVA-induced airway hyperresponsiveness or goblet cell hyperplasia, irrespective of the presence or absence of IL-4Rα–responsive $CD4^+$ T cells. In contrast, deficiency of IL-13 prevented allergic asthma, irrespective of the presence or absence of IL-4Rα–responsive $CD4^+$ T cells. Importantly, chronic allergic inflammation and airway hyperresponsiveness were dependent on IL-4Rα–responsive $CD4^+$ T cells. Deficiency in IL-4Rα–responsive $CD4^+$ T cells resulted in increased numbers of IL-17–producing T cells and, consequently, increased airway neutrophilia.

Conclusion: IL-4–responsive T helper cells are dispensable for acute OVA-induced airway disease but crucial in maintaining chronic asthmatic pathology. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *T_H2 cell, acute allergic airway disease, chronic asthma, cytokine receptors, IL-4, IL-13, gene-deficient mice*

Allergic asthma is a chronic inflammatory disease of the airways characterized by an inappropriate immune response to harmless environmental antigens. T_H2 cells regulate adaptive immune responses to allergens, and their presence correlates with disease symptoms in human subjects and mice.[1] IL-4 plays a crucial role in the *in vitro* and *in vivo* differentiation of T_H2 cells, suggesting major contributions for IL-4 and its receptor, IL-4 receptor α (IL-4Rα), in regulating allergic T_H2 responses.[2] However, the initiation and maintenance of *in vivo* T_H2 differentiation is incompletely understood. The IL-4/IL-4Rα pathway is not essential for T_H2 polarization in certain *in vivo* settings, and important roles for the cytokines IL-25 and IL-33 and type 2 innate lymphoid cells (ILC2s) have been suggested.[2] Several studies described IL-4–independent T_H2 differentiation in response to allergens or helminth infection.[3,4] Other studies suggested that IL-4 signaling is required for the expansion and maintenance of T_H2 responses after *Nippostrongylus brasiliensis* infection or allergen treatment but not for the initial T_H2 differentiation.[5,6]

Although we and others have described IL-4Rα–independent airway hyperresponsiveness (AHR),[7,8] mouse models of allergic asthma generally highlight the importance of IL-4, IL-5, IL-13, and the IL-4Rα/signal transducer and activator of transcription 6 signaling pathway for the development of allergic pathology, including airway eosinophilia, goblet cell hyperplasia, and AHR. Mice deficient in IL-4Rα showed abrogated T_H2 cell differentiation and were protected from ovalbumin (OVA)–induced allergic airway disease.[9-11] IL-4Rα is ubiquitously expressed, and we and others have successfully used mice with cell type–specific disruptions of the *Il4ra* gene to identify IL-4Rα–dependent disease symptoms mediated by airway epithelial cells,[12] smooth muscle cells,[11,13] and macrophages.[10] The *in vivo* requirements for IL-4Rα signaling on $CD4^+$ T cells in the initiation of allergen-specific T_H2 responses remain incompletely understood. Furthermore, it has been shown that $CD4^+$ cells and IL-4 are required for chronic lung inflammation and AHR but might not be necessary for chronic pathogenesis, including AHR, after acute inflammatory responses have resolved.[14-17]

In the present study we used previously described Lck^{cre}IL-4R$\alpha^{-/lox}$ mice with a $CD4^+$ T cell–specific IL-4Rα disruption[18] to define whether IL-4Rα–responsive $CD4^+$ T cells and the IL-4Rα ligands IL-4 and IL-13 are required for the initial *in vivo* differentiation of an allergen-specific T_H2 response and the subsequent onset of OVA-induced allergic airway disease.

From the International Centre for Genetic Engineering and Biotechnology (ICGEB) and Division of Immunology, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Science, University of Cape Town.

*Natalie E. Nieuwenhuizen, PhD, is currently affiliated with the Department of Immunology, Max Planck Institut für Infektionsbiologie, Berlin.

Supported by grants from the National Research Foundation (NRF; South Africa; grant no. 80747), the South African Research Chairs Initiative (SARChI; grant no. 64761), the South African Medical Research Council (SAMRC; Immunology of Infectious Disease), and the International Centre for Genetic Engineering and Biotechnology (ICGEB; Cytokines and Diseases).

Disclosure of potential conflict of interest: F. Kirstein, N. E. Nieuwenhuizen, J. Jayakumar, and F. Brombacher have received research support from the National Research Foundation (NRF) South Africa, the South African Medical Research Council (SAMRC), and International Centre for Genetic Engineering and Biotechnology. W. G. C. Horsnell has received research support from NRF South Africa and the SAMRC.

Received for publication November 25, 2014; revised October 23, 2015; accepted for publication October 26, 2015.

Corresponding author: Frank Brombacher, PhD, Program Coordinator, ICGEB, Level 1 Werner and Beit South, Faculty of Health Science, University of Cape Town Campus, Anzio Road, Observatory, 7925 Cape Town, South Africa. E-mail: frank.brombacher@icgeb.org. Or: frank.brombacher@uct.ac.za.

0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2015.10.036

Abbreviations used
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
FITC: Fluorescein isothiocyanate
ICOS: Inducible costimulator
ILC2: Type 2 innate lymphoid cell
IL-4Rα: IL-4 receptor α
OVA: Ovalbumin
PE: Phycoerythrin

Furthermore, we investigated whether $CD4^+$ T cells require IL-4Rα signals to sustain a T_H2-type phenotype and to promote disease pathology during chronic allergic airway inflammation. We found that acute allergic airway disease and T_H2 differentiation are independent of IL-4 signaling and that IL-13 drives airway pathology. Loss of IL-4Rα signaling on $CD4^+$ T cells resulted in increased IL-17 production and airway neutrophilia. Importantly, IL-4–responsive $CD4^+$ T cells promoted chronic asthmatic disease. In conclusion, IL-4–responsive T helper cells are dispensable for acute OVA-induced airway disease but required to maintain chronic asthmatic pathology.

METHODS

Mice

Six- to 8-week-old female mice were used in the experiments. Generation of $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ (C.Cg-$Il4ra^{tm1Fbb}$/$Il4ra^{tm2Fbb\text{-}Tg[Lck\text{-}Cre]/J}$) mice and $IL\text{-}4R^{-/lox}$ littermates on a BALB/c background was described previously.[18] $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ mice and $IL\text{-}4R\alpha^{-/lox}$ littermate control mice were intercrossed with IL-4–deficient[19] or IL-13–deficient[20] BALB/c mice for 9 generations. Animal procedures were approved by the University of Cape Town Animal Ethics Committee.

Models of allergic airway disease

Acute OVA-induced allergic airway disease. Mice were sensitized intraperitoneally and challenged intranasally with OVA (Sigma-Aldrich, Aston Manor, South Africa), as previously described.[10] In some experiments mice received 0.2 mg/mouse anti–IL-17A antibody[21] (clone MM17F3, kind gift of C. Uyttenhove) or isotype-matched control antibody by means of intraperitoneal injection on days 20 and 23.

Chronic OVA-induced allergic airway disease. Mice were sensitized subcutaneously with 20 μg of OVA adsorbed to 0.65% alum on days 0, 7, 14, and 21. On days 27 and 29 and then twice weekly for 4 weeks, mice were challenged intranasally with 20 μg of OVA in 50 μL of PBS after achievement of anesthesia.[10]

AHR

Respiratory resistance and compliance of the whole respiratory system and Newtonian resistance of the central airways were determined by means of forced oscillation measurements with a flexiVent system (SCIREQ, Montreal, Quebec, Canada) by using the single-compartment (“snapshot”) perturbation or constant phase model, as previously described.[11]

Evaluation of goblet cell hyperplasia

Periodic acid–Schiff reagent–positive goblet cells and total epithelial cell nuclei in 5 to 8 bronchioles per lung section containing 80 to 220 epithelial cells were counted, and the ratio was converted to a percentage.[13]

Measurement of serum antibody levels

Total IgE and allergen-specific IgG antibody levels were measured by means of ELISA, as described previously.[11]

Analysis of airway and lung cell populations

Single-cell suspensions were prepared from lungs of individual mice and stained for intracellular cytokines, as previously described.[10] Bronchoalveolar lavage (BAL)[11] and lung cell composition of individual mice was determined by means of cell-surface staining with Siglec-F–phycoerythrin (PE), CD11c-allophycocyanin, and GR-1–fluorescein isothiocyanate (FITC) for eosinophils, neutrophils, and alveolar macrophages; CD3-PE, CD4–peridinin-chlorophyll-protein complex, and T1/ST2-FITC for T helper cells; and lineage–peridinin-chlorophyll-protein complex–Cy5.5, inducible costimulator (ICOS)–allophycocyanin, and T1/ST2-FITC for ILC2s. Flow cytometry was performed on a FACSCalibur flow cytometer (BD Biosciences, Erembodegem, Belgium), and data were analyzed with FlowJo version 10 software (TreeStar, Ashland, Ore). All antibodies were purchased from BD Biosciences or eBioscience (San Diego, Calif), except T1/ST2 (MD Bioproducts, St Paul, Minn). In some experiments BAL cells were stained with the Rapi-Diff Stain Set (Clinical Diagnostics CC, Johannesburg, South Africa) and analyzed based on differential cell counts, as described previously.[11]

Analysis of lung cytokine responses

Cytokine concentrations in lung tissue homogenate (1 mg/mL protein concentration) were determined by means of ELISA, according to the manufacturer's protocols (IL-4, IL-5, and IFN-γ: BD Biosciences; IL-13, IL-17, IL-25, and IL-33: R&D Systems, Minneapolis, Minn).

Statistical analysis

P values were calculated with GraphPad Prism 4 software (GraphPad Software, San Diego, Calif) by using the nonparametric Mann-Whitney test or Kruskal-Wallis test with the Dunn posttest. Respiratory resistance and compliance data were analyzed with repeated-measures ANOVA with the Bonferroni posttest. *P* values of .05 or less were considered significant.

RESULTS

Development of OVA-induced allergic airway disease in $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ mice

The role of IL-4Rα–responsive $CD4^+$ T cells in acute OVA-induced allergic airway disease was investigated by using $CD4^+$ T cell–specific IL-4Rα–deficient mice ($Lck^{cre}IL\text{-}4R\alpha^{-/lox}$) and IL-4Rα–responsive $IL\text{-}4R\alpha^{-/lox}$ littermate control mice (Fig 1, *A*). IL-4Rα was efficiently disrupted on the cell surfaces of $CD3^+CD4^+$ T cells but present on $CD3^+CD8^+$ T cells from lungs of OVA-treated $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ mice, confirming previous results (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org).[18] Development of AHR, airway eosinophilia, and goblet cell hyperplasia are the main characteristics of allergic airway disease and were absent in saline-treated $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ mice and $IL\text{-}4R\alpha^{-/lox}$ littermate control animals (see Fig E1, *B-D*). In OVA-treated mice respiratory resistance and compliance of the whole respiratory system and Newtonian resistance of the central airways were measured in response to increasing doses of inhaled methacholine as indicators of AHR. Importantly, AHR was not affected by the loss of IL-4Rα expression on $CD4^+$ T cells. OVA treatment induced increased respiratory and Newtonian resistance and a decrease in respiratory compliance in $CD4^+$ T cell–specific IL-4Rα–deficient mice ($Lck^{cre}IL\text{-}4R\alpha^{-/lox}$) and IL-4Rα–expressing $IL\text{-}4R\alpha^{-/lox}$ littermate control mice, with no significant differences between these groups (Fig 1, *B*). Furthermore, efficient goblet cell hyperplasia was observed after OVA treatment in $Lck^{cre}IL\text{-}4R\alpha^{-/lox}$ mice and littermate control

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