Unique and overlapping gene expression patterns driven by IL-4 and IL-13 in the mouse lung

Christina C. Lewis, PhD,[a] Bruce Aronow, PhD,[b] John Hutton, MD,[b] Joanna Santeliz, PhD,[a] Krista Dienger, BS,[a] Nancy Herman, BS,[a] Fred D. Finkelman, MD,[a,c,d] and Marsha Wills-Karp, PhD[a] *Cincinnati, Ohio*

Background: Allergic asthma results from inappropriate T_H2-mediated inflammation. Both IL-4 and IL-13 contribute to asthma pathogenesis, but IL-4 predominantly drives T_H2 induction, whereas IL-13 is necessary and sufficient for allergen-induced airway hyperresponsiveness and goblet cell hyperplasia. Although these 2 cytokines share signaling components, the molecular mechanisms by which they mediate different phases of the allergic asthmatic response remain elusive.

Objective: We sought to clarify the role or roles of IL-4 and IL-13 in asthma-pathogenesis.

Methods: We used DNA Affymetrix microarrays to profile pulmonary gene expression in BALB/c mice inoculated intratracheally with ragweed pollen, house dust mite, IL-4, IL-13, or both cytokines. IL-13 dependence was confirmed by comparing pulmonary gene expression in house dust mite–inoculated wild-type and IL-13 knockout mice.

Results: A signature gene expression profile consisting of 23 genes was commonly induced by means of inoculation with house dust mite, ragweed pollen, or IL-4 plus IL-13. Although rIL-4 and rIL-13 treatment induced an overlapping set of genes, IL-4 uniquely induced 21 genes, half of which were interferon response genes and half of which were genes important in immunoregulation. IL-13 uniquely induced 8 genes, most of which encode proteins produced by epithelial cells.

Conclusions: IL-4 and IL-13 together account for most allergen-induced pulmonary genes. Selective IL-4 induction of IFN-γ response genes and other genes that might negatively regulate allergic inflammation could partially explain the greater importance of IL-13 in the effector phase of allergic airway disease. (J Allergy Clin Immunol 2009;123:795-804.)

Key words: *T_H2 cytokines, microarrays, allergic asthma, mouse*

Abbreviations used
AHR: Airway hyperresponsiveness
GTPase: Guanosine triphosphatase
HDM: House dust mite
IL-4R: IL-4 receptor
IL-13R: IL-13 receptor
RWP: Ragweed pollen
STAT: Signal transducer and activator of transcription

The dramatic increase in asthma incidence lends urgency to the quest for new therapeutic targets.[1] Although asthma's cause is multifactorial, it is thought to arise largely from maladaptive inflammatory responses in genetically susceptible individuals to common aeroallergens. More specifically, allergic asthma is mediated by T_H2-polarized, $CD4^+$ T-lymphocyte secretion of IL-4, IL-5, and IL-13, which stimulate airway hyperresponsiveness (AHR), pulmonary eosinophilia, increased serum IgE levels, subepithelial fibrosis, and goblet cell metaplasia.[2,3] However, the precise molecular mechanisms by which T_H2 cytokines mediate allergic responses are still poorly understood.

Although numerous studies support a role for IL-4 in the initiation of the immune responses that lead to asthma,[4,5] IL-4 is not required for AHR or goblet cell metaplasia.[6-8] However, components of the IL-4 receptor (IL-4R) signaling cascade that are also activated by IL-13 (IL-4Rα, signal transducer and activator of transcription [STAT] 6, and IL-13 receptor [IL-13R] α1) are essential for both disease development and maintenance,[9-11] and the importance of IL-13 in the effector phase of pulmonary allergy has been demonstrated in several ways. Specific blockade of IL-13 in allergen-challenged mice reverses AHR and mucus production.[12,13] Acute IL-13 administration and transgenic pulmonary IL-13 overexpression stimulate many features of the allergic phenotype.[12-14] Allergen-immunized IL-13–deficient mice do not have AHR and goblet cell metaplasia, and adoptive transfer of antigen-specific T_H2 cells generated from IL-13–deficient mice fails to elicit AHR in recipient mice, despite considerable production of IL-4 and IL-5 and significant airway inflammation.[15] Thus, collectively, the current literature suggests that although IL-4 is essential for the initial development and expansion of T_H2 responses, IL-13 is essential for the effector phase of the response.

The present study seeks to clarify why IL-13 contributes uniquely to the effector phase of airway allergy, even though IL-4 and IL-13 both signal by binding to the type 2 IL-4R complex, which is composed of the IL-4Rα and IL-13Rα1 chains. To this end, we conducted a comprehensive gene-

From the Divisions of [a]Immunobiology and [b]Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati; [c]the Department of Medicine, University of Cincinnati College of Medicine; and [d]the Cincinnati Veterans Affairs Medical Center.

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Reprint requests: Marsha Wills-Karp, PhD, Division of Immunobiology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, MLC 7038, Cincinnati, OH 45229. E-mail: wildc7@cchmc.org.

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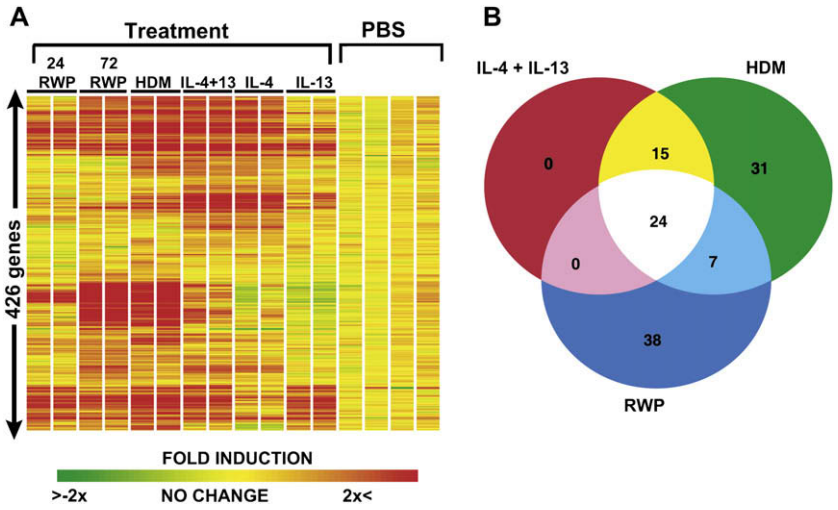

FIG 1. Gene expression changes in the lungs of allergen- and cytokine-treated mice. **A**, Hierarchic clustering of gene expression data from HDM or RWP allergen-exposed, rIL-4– and rIL-13–treated, and PBS-exposed BALB/c wild-type mice revealed that 426 genes were significantly different ($P < .05$) between the treatment and PBS control groups. Data shown are for 2 independent samples per treatment **B**, Venn analysis of gene expression changes showed that 23 genes were shared after all 3 exposures.

profiling experiment to (1) define the gene expression patterns associated with allergen challenge in the mouse lung and (2) to define the overlapping or unique pathways regulated by IL-4 and IL-13. Our studies demonstrate that IL-4 and IL-13 together induce most pulmonary genes that are activated by inhaled allergens and show that most genes activated by one of these cytokines are also activated by the other. However, they also identify sets of genes that are uniquely activated by IL-4 or IL-13 and provide a possible basis for the dominance of IL-13 in the effector phase of airway allergy by suggesting that some genes that are uniquely activated by IL-4 might inhibit allergic airway inflammation.

METHODS

Animals

Four-week-old female BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, Me). All mice were housed under laminar flow hoods in an environmentally controlled specific pathogen-free animal facility. The studies reported conformed to the principles for laboratory animal research, as outlined by the Animal Care and Use Committee of Cincinnati Children's Hospital Medical Center.

Allergen and cytokine treatment protocols

Mice were sensitized by means of intraperitoneal injection of 150 μg of endotoxin-free ragweed pollen (RWP) protein or house dust mite (HDM; Greer Laboratories, Lenoir, NC) plus alum or PBS on days 0 and 3. On days 10 and 17, mice were anesthetized with ketamine and xylazine (45 and 8 mg/kg body weight, respectively) and challenged intratracheally with 40 μL of PBS (control) or PBS that contained 200 μg of either RWP or HDM. Lungs were harvested at 72 hours (RWP and HDM) after the last allergen challenge. The timing and doses of rIL-4 and rIL-13 administration were those that induced allergic phenotypic changes similar to those observed with allergen challenges *in vivo*.[16] Thus mice were inoculated daily by means of intratracheal challenge with either PBS for 10 days, IL-4 (2 μg) for 10 days, IL-4 for 10 days with IL-13 during the last 3 days, or PBS for 7 days, followed by IL-13 for 3 days, as previously described.[16] Lungs from cytokine-treated mice were harvested at 72 hours.

Microarray assays

RNA was isolated from whole lungs of mice and hybridized to Affymetrix U74v2 GeneChips (Affymetrix, Santa Clara, Calif), as previously described (for details, see the Methods section in this article's Online Repository at www.jacionline.org).[16,17]

Quantitative real-time RT-PCR

IL-13–specific genes were validated in a separate set of mice by means of RT-PCR, as previously described (for details, see this article's Online Repository at www.jacionline.org).[18]

RESULTS

Comparison of allergen and T_H2 cytokine–induced gene expression patterns

Initial experiments compared gene expression in whole lungs isolated from mice challenged with PBS, RWP, or HDM or with rIL-4, rIL-13, or rIL-4 plus rIL-13. Of the approximate 19,207 unique genes and 7,600 expressed sequence tags represented by the 45,000 probes on the array set, expression of 1,813 gene transcripts was found to be significantly different in the lungs of allergen- or cytokine-treated lungs compared with that seen in their corresponding control groups. Hierarchic cluster representation of a subset of these genes (426 genes) revealed both similarities and differences in gene expression patterns between the allergen-sensitized and allergen-challenged and cytokine-treated mice (Fig 1, *A*). Venn analysis was performed on a set of 115 unique genes that represented the compilation of differentially expressed genes (≥2-fold change) from each of these 3 treatments (Fig 1, *B*). *Zbtb16* was the only gene downregulated by a factor of greater than 2 in the allergen- and cytokine-treated groups. Comparison of the two 72-hour allergen-treated groups with the rIL-4 plus rIL-13–treated group revealed significant overlap among the 3 treatment groups, with 24 genes being induced by more than 2-fold by each treatment (Table I). Not surprisingly, 24 of the 39 genes induced by more than 2-fold by the