

Ragweed-induced expression of GATA-3, IL-4, and IL-5 by eosinophils in the lungs of allergic C57BL/6J mice

J. PAUL JUSTICE,^{1,2} M. T. BORCHERS,² J. J. LEE,²
W. H. ROWAN,¹ Y. SHIBATA,¹ AND M. R. VAN SCOTT¹

¹Department of Physiology, Brody School of Medicine at East Carolina University, Greenville, North Carolina 27858; and ²Department of Biochemistry and Molecular Biology, Mayo Clinic, Scottsdale, Arizona 85259

Received 7 May 2001; accepted in final form 1 October 2001

Justice, J. Paul, M. T. Borchers, J. J. Lee, W. H. Rowan, Y. Shibata, and M. R. Van Scott. Ragweed-induced expression of GATA-3, IL-4, and IL-5 by eosinophils in the lungs of allergic C57BL/6J mice. *Am J Physiol Lung Cell Mol Physiol* 282: L302–L309, 2002; 10.1152/ajplung.00158.2001.—Allergen-induced recruitment of T lymphocytes and eosinophils to the airways is associated with increased expression of the transcription factor GATA-3. In this study, the relationship between airway inflammation and GATA-3 expression in the lungs was investigated using ragweed-sensitized C57BL/6J mice. Intratracheal ragweed challenge increased both the number of GATA-3-expressing cells in the perivascular and peribronchial regions and the amount of expression per cell. Interleukin (IL)-4 and IL-5 levels in bronchoalveolar lavage fluid were upregulated in parallel with GATA-3 expression. GATA-3 mRNA and protein colocalized to eosinophils. Eosinophils isolated from the lungs and stimulated with phorbol 12-myristate 13-acetate and/or A-23187 released IL-5. The release was inhibited by actinomycin D, which indicates that de novo synthesis of the cytokine was involved. Western blot analysis of proteins from isolated eosinophils demonstrated expression of the p50 subunit of nuclear factor- κ B, a transcription factor that is implicated in control of GATA-3 expression. These data provide evidence that allergen challenge increases GATA-3 and proinflammatory cytokine expression by pulmonary eosinophils, which could provide positive feedback for the inflammatory response.

asthma; airway inflammation; major basic protein; transcription; interleukin

ASTHMA IS CHARACTERIZED by pulmonary inflammation, reversible airflow obstruction, and airway hyperresponsiveness (1, 2, 7, 10). The asthma phenotype evolves from a complex cascade of immunological responses to aeroallergens, which leads to leukocyte recruitment to the airways and subsequent remodeling of the inflamed tissue (11, 14, 18). The recruitment of eosinophils is associated with increased production of the Th2 cytokines [interleukin (IL)-4 and IL-5], and ectopic expression within the airways of these two factors has been shown to induce eosinophil recruitment (21, 31). The biological role of IL-5 is limited to

control of eosinophil proliferation, differentiation, activation, and survival (6, 9, 23, 34, 37, 43). In contrast, the biological significance of IL-4 is broader and includes stimulation of CD4⁺ T-cell differentiation and proliferation (40), B-cell class switching (25), and the induction of eosinophil-specific chemokine secretion (38). Thus IL-4 and IL-5 are key factors in asthma pathogenesis.

Studies suggest that CD4⁺ T cells are the primary source of IL-4 and IL-5 in allergic inflammation (1, 15, 17, 32). However, there is evidence that eosinophils are capable of providing positive feedback to the inflammatory response via production and secretion of these cytokines (16). In murine lungs infected with *Shistosoma mansoni*, eosinophils are reported to be the dominant source of IL-4 and IL-5 and to cause prolonged eosinophil recruitment and continued granuloma formation (33). To date, a similar role for eosinophils in the development of allergic asthma has not been established.

Transcription of both the IL-4 and IL-5 genes is regulated in part by the helix-loop-helix transcription factor GATA-3 (3, 30, 46). In murine T lymphocytes, GATA-3 is a major positive regulatory factor for IL-4 production (46, 47), and GATA-3 is required for IL-5 expression in T cells (45, 46). Ouyang and colleagues (27) recently established that GATA-3 is part of a master switch for Th2 commitment in T cells. This response to GATA-3 is independent of STAT-6, which indicates that the effect is not mediated by the IL-4/STAT-6 signaling pathway.

Evidence supporting a role for GATA-3 in the development of asthma is accumulating. Biopsies from human asthmatic patients have demonstrated a significant increase in the number of GATA-3 mRNA-positive cells in the airways compared with tissue from non-asthmatic individuals (26). Using a murine model of asthma, Zhang and colleagues (45) demonstrated that T-cell-specific expression of a GATA-3 dominant-negative mutant during the sensitization and challenge processes attenuated allergen-induced airway eosino-

Address for reprint requests and other correspondence: M. R. Van Scott, Dept. of Physiology, Brody School of Medicine at East Carolina Univ., 6N98 Brody Bldg., Greenville, NC 27858 (E-mail: vancottm@mail.ecu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

philia. These observations together with studies correlating eosinophilic inflammation with Th2 cytokine production (24) suggest that GATA-3 plays a key role in eosinophilic inflammation of the airways.

In the current study, ragweed (RW)-sensitized C57BL/6J mice were used to define the pattern of GATA-3 expression in the lungs over a 72-h period after allergen challenge. Maximum expression of GATA-3 was observed 12–24 h after RW challenge and corresponded with the appearance of IL-4 and IL-5 in bronchoalveolar lavage (BAL) fluid. Greater than 95% of the GATA-3 mRNA expressing cells in the lungs were eosinophils. Furthermore, *in vitro* stimulation of RW-primed lung eosinophils demonstrated transcriptional dependence for Th2 cytokine production.

MATERIALS AND METHODS

Animals. Male 6–10-wk-old C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). NJ.1638 mice were obtained from J. Lee and N. Lee (Mayo Clinic, Scottsdale, AZ). All mice were maintained by the Department of Comparative Medicine at the Brody School of Medicine at East Carolina University. Animals were fed Purina laboratory chow and sterilized tap water *ad libitum*. All studies were approved by the Animal Welfare and Use Committee at the Brody School of Medicine.

Sensitization and challenge of animals. A 14-day protocol was used. Mice were sensitized by intraperitoneal injection of RW on days 0 and 4 (80 µg/injection, lot 56-29; endotoxin content <2.3 ng/mg RW; Greer Laboratories, Lenoir, NC). Sensitization solution consisted of 1 mg of RW in 1 ml of 0.9% NaCl (Baxter, Deerfield, IL) plus 333 µl of Inject alum (Pierce, Rockford, IL). On day 11, animals were anesthetized with ketamine (90 mg/kg body wt) and xylazine (10 mg/kg body wt) and challenged by intratracheal administration of RW (10 µg of RW in 0.1 ml of 0.9% NaCl).

Lung lavage, tissue fixation, and sectioning. Lungs were lavaged with a single 1.0-ml aliquot of PBS (GIBCO-BRL, Grand Island, NY). Samples were centrifuged at 2,000 rpm

for 5 min, and the supernatants were removed and stored at –80°C. Lungs were removed, infused with 4% paraformaldehyde (in PBS) for 30 min, rinsed with PBS, and immersed in 0.5 M sucrose (in PBS) overnight at 4°C. Lungs were inflated with and embedded in optimal cutting temperature compound (Sakura FineTek, Torrance, CA) and stored at –80°C.

Cytokine determination. Concentrations of IL-4 and IL-5 were determined using two-site immunoenzymetric assay kits (Endogen, Cambridge, MA) according to the manufacturer's instructions. The lower limits of detection were 1 pg/ml for both IL-4 and IL-5.

In situ hybridization. *In situ* hybridization was performed as described by Helton and colleagues (13). Lung sections (10–20 µm) were placed on Superfrost Plus microscope slides (VWR Scientific, West Chester, PA), acetylated using 0.25% acetic acid anhydride, and dehydrated in ethanol. Sections were hybridized using synthetic DNA oligonucleotides with the following sequences: GATA-3 antisense (5'-CTCGGCTGTGCTCGCGCCT-3') and GATA-3 sense (5'-AGGGCGCGGAGCACAGCCGAG-3'). Oligonucleotides were synthesized by GIBCO-BRL and labeled on the 3' end using α-³⁵S-labeled dATP (1,075 Ci/mmol, New England Nuclear, Boston, MA) and terminal deoxynucleotidyl transferase (Boehringer Mannheim, Indianapolis, IN) to a specific activity of ~0.9–1.8 µCi/mmol. Sections were incubated with 1 × 10⁶ counts/min of α-³⁵S-labeled dATP per 25 µl of hybridization buffer for 12 h at 37°C. Slides were dipped in Kodak NTB-3 emulsion (Sigma Chemical, St. Louis, MO), exposed for 72 h, and processed using Dektol and Kodak fixer (Sigma). Quantification of the mRNA hybridization signal was performed using NIH Image 1.60 software (W. Rasband, National Institute of Mental Health) and a Macintosh G3 computer. Integrated density for GATA-3 mRNA expression in the lung sections was determined using the NIH imager program. The area of the cell being scanned and the mean density of the scanned region were multiplied to yield the integrated density. Numbers of GATA-3 mRNA-expressing cells were normalized per millimeter of basement membrane for airways and blood vessels. Enumeration of GATA-3 mRNA values for the alveolar region were normalized per square millimeter of distal lung area.

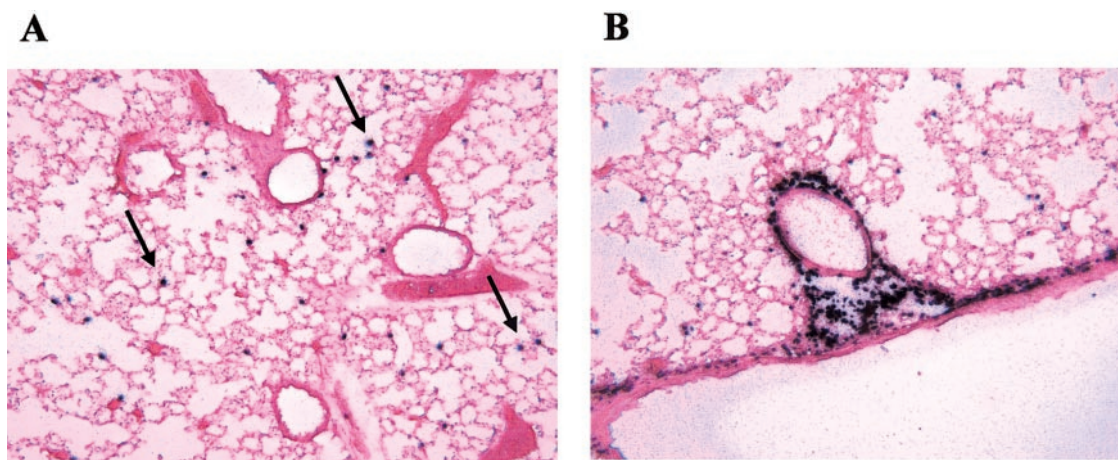


Fig. 1. *In situ* hybridization for GATA-3 mRNA in murine lung tissue. GATA-3 antisense RNA probe was hybridized to lung-tissue sections from C57BL/6J mice that were sensitized to ragweed (RW) and challenged with either PBS (A) or RW (B). Intense black deposits represent GATA-3-positive cells visualized by nuclear tract bulk staining of ³⁵S-labeled antisense probes. Arrows in the PBS-challenged group indicate GATA-3 mRNA-positive cells in the alveolar region. Sections from 8–12 mice per group were analyzed; representative photographs from each group are shown.

Table 1. Time course of GATA-3 mRNA positive cell infiltration of the lungs

Time After Challenge, h	Airway, cells/mm basement membrane		Blood Vessel, cells/mm basement membrane		Alveoli, cells/mm ²	
	PBS	Ragweed	PBS	Ragweed	PBS	Ragweed
0	ND	ND	ND	ND	7 ± 2	10 ± 3
12	ND	27 ± 6*†	ND	49 ± 10*†	9 ± 4	8 ± 5
24	ND	39 ± 4*†	3 ± 2†	61 ± 4*	12 ± 5	32 ± 6*†

Values are means ± SE ($n = 3$ mice for PBS groups and $n = 5$ mice for ragweed-challenged group). Lungs were removed and fixed in paraformaldehyde at indicated times after ragweed challenge. Lung sections were hybridized with ³⁵S-labelled GATA-3 antisense oligonucleotide probe. Hybridization of probe was visualized by autoradiography and numbers of GATA-3 positive cells were quantified by microscopy. PBS, phosphate-buffered saline; ND, not detectable. * $P < 0.05$, significant difference between PBS- and ragweed-challenged groups at that time; † $P < 0.05$, significant difference between time points within a treatment group for a given tissue type.

Immunofluorescence and immunocytochemistry. Frozen lung sections were analyzed for eosinophil major basic protein (MBP) and GATA-3 protein expression using anti-MBP monoclonal antibody and anti-GATA-3 polyclonal antibody (Santa Cruz Biotech, San Diego, CA). Briefly, endogenous peroxidase activity was quenched with 3% H₂O₂ (Sigma) and 80% methanol (J. T. Baxter, Phillipsburg, NJ) in PBS. Lung sections were permeabilized with pepsin (Zymed Laboratories, San Francisco, CA) and 0.01% Triton X (Sigma). Sections were blocked with 10% mouse serum (Vector Laboratories, Burlingame, CA) and incubated with anti-GATA-3 Ab (1:50 dilution; 200 µg/ml) that was biotinylated with the DAKO-ARK biotinylation kit (DAKO, Carpinteria, CA). GATA-3 antibody staining was visualized by the streptavidin-diaminobenzidine system (DAKO) as described by the manufacturer. Subsequently, cellular autofluorescence was blocked by incubation of sections with 1% Chromotrope-R (Sigma), and slides were incubated in rat anti-MBP Ab (1:3,000 dilution; 1.4 µg/ml) before the addition of Alexa-488 conjugated goat anti-rat IgG antibody (1:5,000 dilution; 0.5 µg/ml; Molecular Probes, Eugene, OR).

Isolation of peripheral blood and airway eosinophils. Isolation of peripheral blood eosinophils was performed by collecting 0.5 ml of peripheral blood from the tail vein of NJ.1638 (T-cell-specific and IL-5 transgenic) mice (22). White

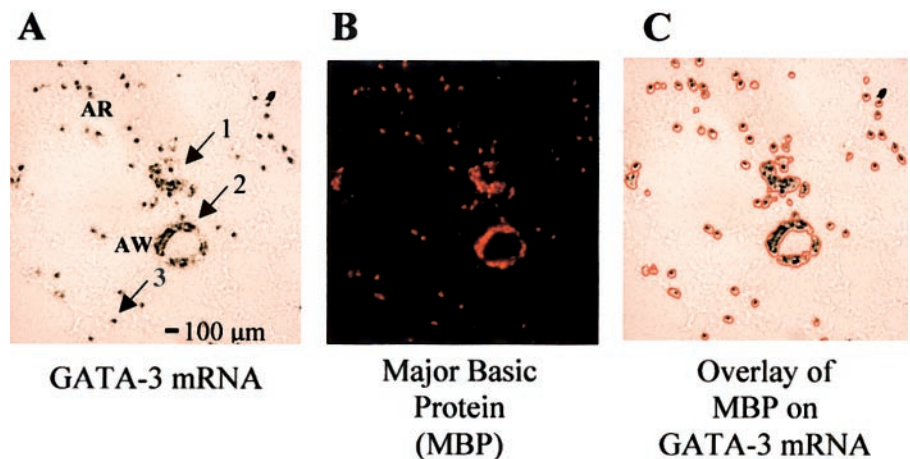
blood cells were isolated by centrifugation on a discontinuous Percoll gradient with subsequent hypotonic lysis of remaining red blood cells. Lung eosinophils were isolated from RW-challenged C57BL/6J mice. The lungs were perfused with ice-cold PBS introduced through the right ventricle and were excised, minced, digested in collagenase (1% collagenase in PBS; Sigma), and filtered through a 100-µm mesh. White blood cells were isolated on a discontinuous Percoll gradient. Eosinophils were enriched from both populations by the negative-selection process using anti-CD90 and anti-CD45R antibodies to deplete the B- and T-cell populations using the MACS magnetic bead separation method per the manufacturer's suggested protocol (Miltenyi Biotechnical, Auburn, CA). Eosinophil fractions were routinely enriched to <98%.

In vitro stimulation of eosinophils. Purified peripheral blood and lung eosinophils were resuspended in RPMI-1640 (GIBCO-BRL) and 5% fetal calf serum (GIBCO-BRL) at a cell density of 1×10^6 cells/ml for both peripheral blood and lung eosinophils. The cells were stimulated with 10^{-7} M phorbol 12-myristate 13-acetate (PMA) and 10^{-7} M A-23187 (Sigma). Actinomycin D was added at a concentration of 10^{-6} M when indicated. Samples were stimulated in 96-well plates at 37°C for 30 min, 1 h, and 16 h.

Western blot analysis of GATA-3 and nuclear factor-κB. Purified peripheral blood eosinophils from NJ.1638 mice were collected by centrifugation and resuspended in extraction buffer that contained 10 mM Tris·HCl, pH 7.4, 0.32 M sucrose, 2 mM EDTA, 1 mM EGTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 2 µg/ml pepstatin A. The cells were disrupted by sonication on ice twice for 20 s. Lysates were centrifuged for 1 h at 100,000 g. The supernatants were collected and protein concentrations were determined (Bio-Rad). Proteins were separated by 12% SDS-PAGE and transferred to 0.45-µm polyvinylidene difluoride membranes (Immobilon). GATA-3 and the p50 subunit of nuclear factor (NF)-κB were identified by immunoblotting with goat anti-GATA-3 Ab and goat anti-NF-κB p50 Ab (both from Santa Cruz Biotechnology), respectively, followed by horseradish peroxidase-conjugated donkey anti-goat IgG Ab. Immunoreactive protein complexes were detected by using enhanced chemiluminescence detection reagents.

Statistical analysis. All experimental values within the individual data sets were compared by ANOVA and Tukey's HSD post hoc test ($P < 0.05$). Values presented in this report represent means ± SE.

Fig. 2. GATA-3 mRNA expression colocalized to major basic protein (MBP)-expressing eosinophils. A GATA-3 antisense RNA probe was hybridized to lung-tissue sections from RW-challenged C57BL/6J mice (A), and immunocytochemistry was subsequently performed on the same tissue sections to probe for MBP expression using a Cy3-conjugated secondary antibody (B). GATA-3 mRNA expression was visualized by nuclear tract bulk staining of ³⁵S-labeled antisense probes. Overlaying images of dual-stained sections demonstrated colocalization of GATA-3 mRNA and MBP (C). Photographs are representative of sections from 6 mice. AW, airway; AR, alveolar region. Arrow 1, GATA-3 mRNA⁺ airway eosinophil; arrow 2, GATA-3 mRNA⁺ blood vessel eosinophil; and arrow 3, GATA-3 mRNA⁺ alveolar eosinophils.



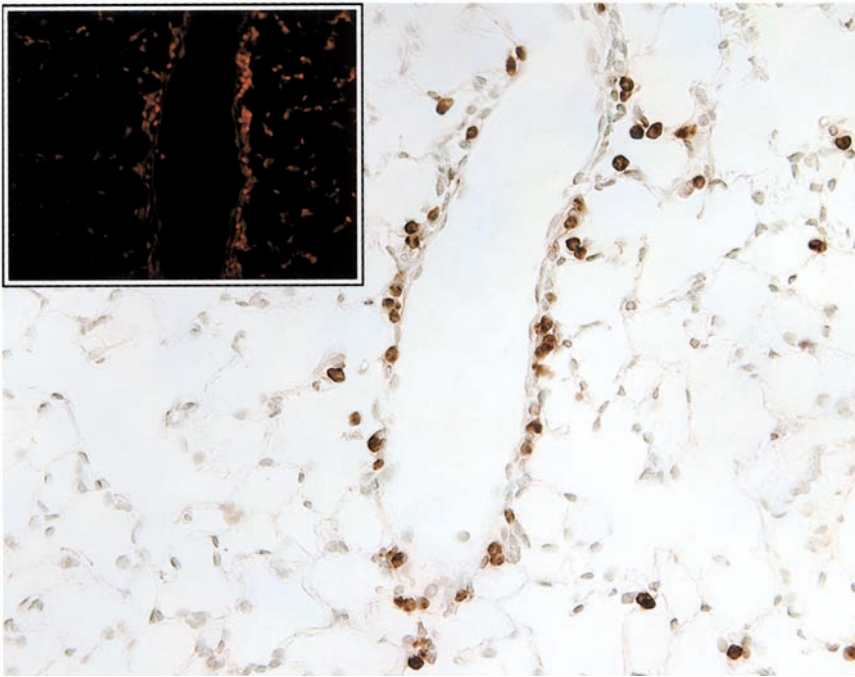


Fig. 3. Colocalization of GATA-3 protein and MBP in lung tissue after RW challenge. Expression of GATA-3 and MBP was analyzed by dual immunocytochemistry. GATA-3 protein was visualized by diaminobenzidine staining, and MBP expression was visualized using a Cy5-labeled secondary antibody (*inset*). Figure is representative of 4 animals.

RESULTS

GATA-3 mRNA-positive cells are recruited to the airways after RW allergen challenge. In situ hybridization revealed that GATA-3 mRNA expression increased over the 24 h after RW challenge. The GATA-3-positive cells were located along the basement membrane of the airways and blood vessels and were broadly dispersed in the alveolar region (Fig. 1). A significant increase in the number of GATA-3-positive cells was observed in the airways and blood vessels 12 h after RW challenge (Table 1). In contrast, an increase in expression in the alveolar region was delayed. In sensitized mice, a significant increase in the number of GATA-3 mRNA-positive cells was not observed until 24 h after RW challenge. Interestingly, recruitment to the airways and blood vessels was only observed after the airway challenge, whereas sensitization alone elicited expression of GATA-3 in the alveolar region. Kinetics of the GATA-3 mRNA-positive cell distribution reflected the pattern of eosinophil recruitment beginning with extravasation through the blood vessels at 12 h, localization around vessels and large airways by 24 h, and a

minor increase in alveolar eosinophilia at these time points.

Expression of GATA-3 mRNA and GATA-3 protein colocalizes with MBP-positive cells. Combined in situ hybridization for GATA-3 mRNA and immunofluorescence for MBP protein demonstrated that GATA-3 mRNA colocalized with MBP in the airways (Fig. 2). Of the GATA-3 mRNA-positive cells, >95% costained for MBP. Colocalization was also observed at the protein level (Fig. 3). These results indicate that the majority of cells expressing GATA-3 in the airways after allergen challenge are eosinophils. To confirm this finding, peripheral blood eosinophils from nonsensitized and nonchallenged NJ.1638 mice were isolated, and Western blot analysis was performed (Fig. 4). Expression of GATA-3 and the p50 subunit of NF- κ B, the upstream modulator of GATA-3, were detected.

Changes in GATA-3 mRNA expression at the cellular level were assessed by measuring the integrated density of silver grains over individual cells in autoradiographs of lung sections after in situ hybridization. The integrated density was significantly elevated 24 h

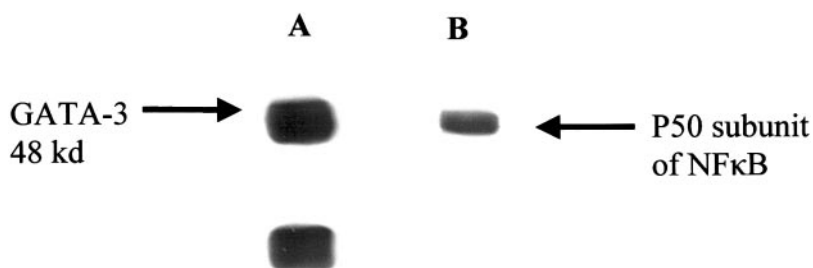


Fig. 4. Expression of GATA-3 and nuclear factor- κ B (NF- κ B) proteins by peripheral blood eosinophils. Peripheral blood eosinophils from NJ.1638 mice were isolated and the cytosol fraction was assessed for GATA-3 (A) and NF- κ B (B) expression by Western blot analysis. Procedure was repeated on three separate occasions, and a representative Western blot is shown.

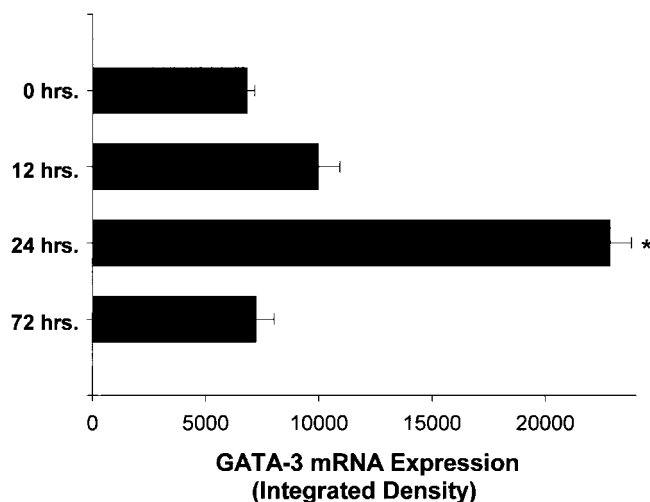


Fig. 5. Increased cellular expression of GATA-3 mRNA 24 h after RW challenge. Amount of GATA-3 mRNA expression per cell was analyzed by determining the integrated density for individual cells within the submucosa of the airways. At least 100 cells per section were assessed. Only sections containing ≥ 3 airways were analyzed; 3–6 mice were evaluated at each time point. Values represent means $\pm$ SE; * $P < 0.05$.

after RW challenge (Fig. 5). By 72 h postchallenge, GATA-3 expression returned to baseline levels. The increased integrated density was indicative of either increased synthesis or decreased degradation of GATA-3 mRNA after the allergen challenge.

IL-4 and IL-5 levels in BAL fluid increase after RW challenge. IL-4 and IL-5 concentrations in the BAL fluid of RW-challenged mice were increased significantly 24 h postchallenge (Table 2). Comparison of the IL-4 and IL-5 levels with the expression of GATA-3 (shown in Fig. 5) indicates that the three parameters changed in parallel, and that eosinophils were a probable source of IL-4 and IL-5 in the lungs after allergen challenge.

Nonspecific activation of airway and peripheral blood eosinophils in vitro leads to de novo production of IL-4 and IL-5. The ability of eosinophils to produce the Th2 cytokines IL-4 and IL-5 was assessed in vitro using cells isolated from peripheral blood of NJ.1638 mice and the lungs of RW-challenged wild-type mice.

Table 2. Effect of ragweed challenge on Th2 cytokine concentration in bronchoalveolar lavage fluid

	n, no. of mice	[IL-4], pg/ml	[IL-5], pg/ml
Intraperitoneal ragweed, intratracheal PBS	5	2.0 $\pm$ 0.6	1.8 $\pm$ 0.4
Intraperitoneal ragweed, intratracheal ragweed	8	69.0 $\pm$ 7.0*	49.0 $\pm$ 10.0*

Values are means $\pm$ SE. Lungs were lavaged with 1.5 ml of ice-cold PBS 24 h after intratracheal ragweed challenge and were stored at -80°C . Lavage fluid was not concentrated. Concentrations of interleukin-4 and -5 ([IL-4] and [IL-5], respectively) were analyzed by sandwich ELISA. * $P < 0.05$, significant difference from PBS-challenged mice.

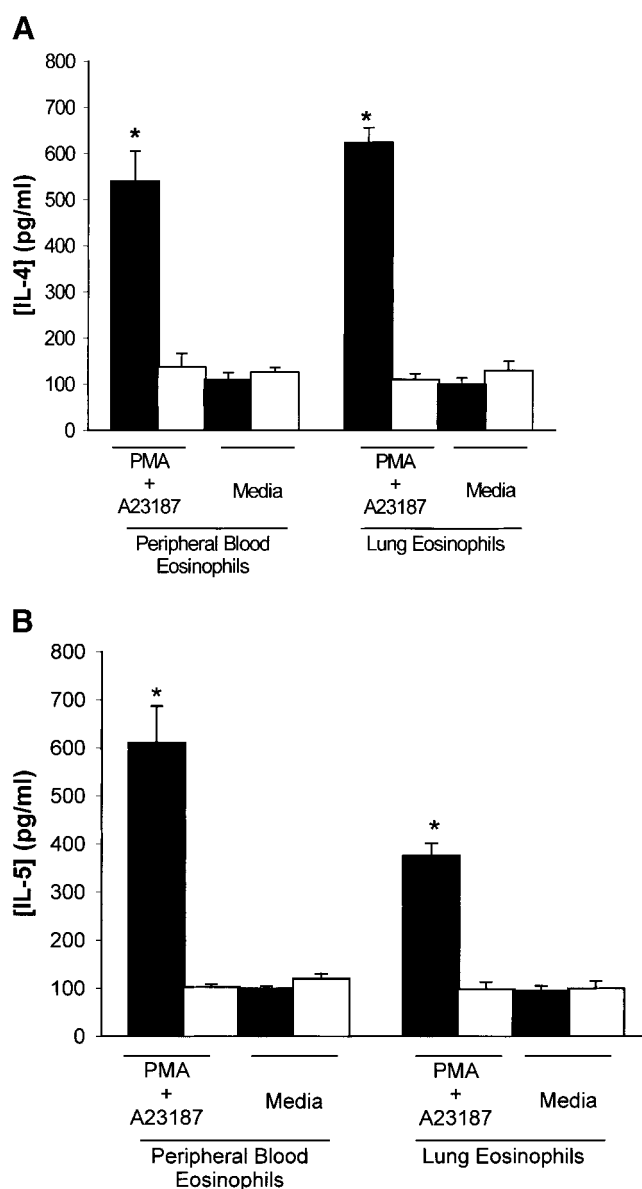


Fig. 6. Production of interleukin (IL-4) and IL-5 by peripheral blood and lung eosinophils in vitro. Peripheral blood and lung eosinophils were purified and stimulated in vitro with the combination of phorbol 12-myristate 13-acetate (PMA; 10^{-7} M) and A-23187 (10^{-7} M) for 18 h in the absence (solid bars) and presence (open bars) of actinomycin D. Concentrations of IL-4 ([IL-4], A) and IL-5 ([IL-5], B) in the media were analyzed by sandwich ELISA. Procedure was repeated on 3 independent occasions, 12 samples per experiment were taken, and cells were pooled from 6–10 mice per experiment. Data are means $\pm$ SE; * $P < 0.05$, significant difference from all other treatment groups within an eosinophil population.

Incubation with PMA and A-23187 for 18 h induced the release of both IL-4 (Fig. 6A) and IL-5 (Fig. 6B). Release of preformed IL-4 and IL-5 was unlikely, because exposure of the cells to PMA or A-23187 for 30–120 min did not lead to the release of these cytokines (data not shown). In addition, incubation of the cells with the RNA polymerase II inhibitor actinomycin D abolished IL-4 and IL-5 release, which suggests that cytokine

production is a function of transcriptional activation of these Th2 cytokine genes.

DISCUSSION

The results of this study provide evidence that allergen challenge induces expression of GATA-3 and GATA-3-responsive genes in pulmonary eosinophils. A role for GATA-3 in the development of asthma was initially demonstrated by Nakamura and colleagues (26), who reported a significant elevation of GATA-3 mRNA in lung tissue from asthma patients compared with lung tissue from nonasthmatic subjects. Further evidence of a role for GATA-3 in allergen-induced lung inflammation was provided by Zhang and colleagues (45), who found a dominant negative mutant expressed for GATA-3 in mice and demonstrated that the pulmonary response to allergen challenge was attenuated. Expression of the GATA-3 mutant was restricted to T cells and was induced during both the sensitization and airway-challenge periods. The results of these studies were therefore consistent with the known importance of Th2 lymphocytes in asthma and the role of GATA-3 in the growth of this cell type. The results of the present study indicate that GATA-3 plays a broader role in allergic inflammation. Expression by pulmonary eosinophils and the capacity of that cell type to produce IL-5 provide a mechanism for positive feedback in the inflammatory response at the site of the allergen challenge.

There is considerable evidence that GATA-3 regulates IL-5 gene expression in human and murine T cells. For example, expression of IL-5 in EL-4 and myeloid cells requires the presence of GATA-3 (3, 36, 44), mutation of the GATA-3 binding site in EL-4 cells abolishes antigen or cAMP-induced IL-5 production (44), GATA-3 binds to the IL-5C promoter site in EL-4 cells (20), and retroviral infection with GATA-3 cDNA enhances IL-5 production in developing Th1 T-cell clones (8). The role of GATA-3 in the regulation of IL-4 expression is more controversial. In contrast to IL-5, early studies of IL-4 expression demonstrated that ectopic expression of GATA-3 did not induce IL-4 production (46), and GATA-3 antisense oligonucleotides did not inhibit IL-4 promoter activity (30). However, recent studies suggest that GATA-3 plays a more direct role in IL-4 transcription. Retroviral expression of GATA-3 in committed Th1 cells enhances IL-4 production (8), and expression of a dominant negative mutant for GATA-3 inhibits allergen-induced IL-4 production (45). In addition, introduction of GATA-3 into STAT-6-deficient T cells induces DNase I hypersensitive sites in the IL-4 locus and triggers STAT-6 independent IL-4 production, which is a novel concept in the paradigm of IL-4 gene regulation (27). Taken together, the evidence indicates that GATA-3 is an important factor in the regulation of both IL-4 and IL-5.

The molecular mechanism by which GATA-3 enables the polarization of T-cell subsets into the Th2 category has also been described by Ouyang and colleagues (28),

who observed that developing Th1 cells could inhibit GATA-3 expression through an IL-12-dependent mechanism. Conversely, GATA-3 expression was found to inhibit IL-12 signaling in T cells. These findings have been further substantiated by the demonstration that ectopic expression of GATA-3 in developing Th1 cells inhibits interferon- γ production and commitment to the Th1 phenotype (8). Collectively, these findings indicate that the role of GATA-3 in both the upregulation and downregulation of gene expression is critical to the development of a Th2 immune response.

The present study demonstrates that murine tissue eosinophils express GATA-3 after allergic sensitization and challenge. A previous report by Zon and colleagues (48) demonstrated GATA-3 expression in the HL-60-C15 and 3+C-5 sublines of the human eosinophil-like cell line, HL-60. GATA-3 has also been shown to downregulate the expression of genes in HL-60 cells (35). The finding that eosinophils express GATA-3 in vivo is therefore a logical outcome of the present study.

The observation that GATA-3 is expressed and is functionally active in eosinophils has important implications in the progression and self-perpetuation of allergic reactions that lead to asthma. Production of IL-5 by tissue eosinophils should contribute to a microenvironment that supports the localization, longevity, activation, and proliferation of these cells, perhaps independent of Th2 cells. However, its importance in human asthma is unknown. The acute sensitization and challenge protocol used in this study results in a high degree of eosinophil accumulation in the airways with minimal recruitment of lymphocytes. Therefore, the role that eosinophil GATA-3 expression plays in allergic airway inflammation is likely to be amplified in this model. In contrast, chronic allergic airway inflammation such as is observed in human asthma is characterized by significant accumulation of lymphocytes in the airways. Nakamura and colleagues (26) observed that 60–90% of the GATA-3-positive cells in human lung tissue were CD3-positive T cells; however, <15% of the GATA-3-positive cells were identified as eosinophils. Thus in human asthma, eosinophil expression of GATA-3-responsive genes is not likely to be the primary source of proinflammatory cytokines leading to airway inflammation but rather may provide a redundant source of Th2 cytokine to support the chronic inflammatory process.

REFERENCES

1. Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, Durham SR, and Kay AB. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. *Am Rev Respir Dis* 146: 500–506, 1992.
2. Bjornsdottir US and Cypcar DM. Asthma: an inflammatory mediator soup. *Allergy* 54 Suppl 49: 55–61, 1999.
3. Blumenthal SG, Aichele G, Wirth T, Czernilofsky AP, Nordheim A, and Dittmer J. Regulation of the human interleukin-5 promoter by Ets transcription factors. Ets1 and Ets2, but not Elf-1, cooperate with GATA3 and HTLV-I Tax1. *J Biol Chem* 274: 12910–12916, 1999.

4. Boushey HA and Fahy JV. Targeting cytokines in asthma therapy: round one. *Lancet* 356: 2114–2116, 2000.
5. Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, Warrington SJ, Renzetti L, Rames A, Bock JA, Boyce MJ, Hansel TT, Holgate ST, and Barnes PJ. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356: 2149–2153, 2000.
6. Coffman RL, Seymour BW, Hudak S, Jackson J, and Rennick D. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245: 308–310, 1989.
7. Eum SY, Haile S, Lefort J, Huerre M, and Vargaftig BB. Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. *Proc Natl Acad Sci USA* 92: 12290–12294, 1995.
8. Ferber IA, Lee HJ, Zonin F, Heath V, Mui A, Arai N, and O'Garra A. GATA-3 significantly downregulates IFN- γ production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. *Clin Immunol Immunopathol* 91: 134–144, 1999.
9. Finkelman FD, Katona IM, Urban JFJ, Holmes J, Ohara J, Tung AS, Sample JV, and Paul WE. IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol* 141: 2335–2341, 1988.
10. Frigas E, Loegering DA, Solley GO, Farrow GM, and Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin Proc* 56: 345–353, 1981.
11. Fujita-Yamaguchi Y, Sacks DB, McDonald JM, Sahal D, and Kathuria S. Effect of basic polycations and proteins on purified insulin receptor. *Biochem J* 263: 813–822, 1989.
12. Grimaldi JC, Yu NX, Grunig G, Seymour BW, Cottrez F, Robinson DS, Hosken N, Ferlin WG, Wu X, Soto H, O'Garra A, Howard MC, and Coffman RL. Depletion of eosinophils in mice through the use of antibodies specific for C-C chemokine receptor 3 (CCR3). *J Leukoc Biol* 65: 846–853, 1999.
13. Helton TE, Daunais JB, and McGinty JF. Convulsant doses of cocaine alter immediate early gene and opioid peptide expression in rat limbic forebrain. *Brain Res Mol Brain Res* 20: 285–288, 1993.
14. Hogan SP and Foster PS. Cytokines as targets for the inhibition of eosinophilic inflammation. *Pharmacol Ther* 74: 259–283, 1997.
15. Kapsenberg ML, Hilkens CM, Jansen HM, Bos JD, Snijders A, and Wierenga EA. Production and modulation of T-cell cytokines in atopic allergy. *Int Arch Allergy Immunol* 110: 107–113, 1996.
16. Kay AB, Barata L, Meng Q, Durham SR, and Ying S. Eosinophils and eosinophil-associated cytokines in allergic inflammation. *Int Arch Allergy Immunol* 113: 196–199, 1997.
17. Krakauer T. IL-10 inhibits the adhesion of leukocytic cells to IL-1-activated human endothelial cells. *Immunol Lett* 45: 61–65, 1995.
18. Kung TT, Jones H, Adams GK, Umland SP, Kreutner W, Egan RW, Chapman RW, and Watnick AS. Characterization of a murine model of allergic pulmonary inflammation. *Int Arch Allergy Immunol* 105: 83–90, 1994.
19. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, and Barnes PJ. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356: 2144–2148, 2000.
20. Lee HJ, O'Garra A, Arai K, and Arai N. Characterization of cis-regulatory elements and nuclear factors conferring Th2-specific expression of the IL-5 gene: a role for a GATA-binding protein. *J Immunol* 160: 2343–2352, 1998.
21. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, Brenneise IE, Horton MA, Haczku A, Gelfand EW, Leikauf GD, and Lee NA. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 185: 2143–2156, 1997.
22. Lee NA, McGarry MP, Larson KA, Horton MA, Kristensen AB, and Lee JJ. Expression of IL-5 in thymocytes/T cells leads to the development of a massive eosinophilia, extramedullary eosinophilopoiesis, and unique histopathologies. *J Immunol* 158: 1332–1344, 1997.
23. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, and Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 167: 219–224, 1988.
24. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, and Djukanovic R. The relationship between airway inflammation and asthma severity. *Am J Respir Crit Care Med* 161: 9–16, 2000.
25. Lutzker S, Rothman P, Pollock R, Coffman R, and Alt FW. Mitogen- and IL-4-regulated expression of germ-line Ig- γ -2b transcripts: evidence for directed heavy chain class switching. *Cell* 53: 177–184, 1988.
26. Nakamura Y, Ghaffar O, Olivenstein R, Taha RA, Soussi-Gounni A, Zhang DH, Ray A, and Hamid Q. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 103: 215–222, 1999.
27. Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, and Murphy KM. STAT6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 12: 27–37, 2000.
28. Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, and Murphy KM. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* 9: 745–755, 1998.
29. Persson CG, Erjefalt JS, Korsgren M, and Sundler F. The mouse trap. *Trends Pharmacol Sci* 18: 465–467, 1997.
30. Ranganath S, Ouyang W, Bhattacharya D, Sha WC, Grupe A, Peltz G, and Murphy KM. GATA-3-dependent enhancer activity in IL-4 gene regulation. *J Immunol* 161: 3822–3826, 1998.
31. Rankin JA, Picarella DE, Geba GP, Temann UA, Prasad B, DiCosmo B, Tarallo A, Stripp B, Whitsett J, and Flavell RA. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci USA* 93: 7821–7825, 1996.
32. Robinson DS, Durham SR, and Kay AB. Cytokines. 3. Cytokines in asthma. *Thorax* 48: 845–853, 1993.
33. Rosenwasser LJ. Promoter polymorphism in the candidate genes, IL-4, IL-9, TGF- β 1, for atopy and asthma. *Int Arch Allergy Immunol* 118: 268–270, 1999.
34. Rothenberg ME, Luster AD, and Leder P. Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc Natl Acad Sci USA* 92: 8960–8964, 1995.
35. Sadat MA, Kumatori A, Suzuki S, Yamaguchi Y, Tsuji Y, and Nakamura M. GATA-3 represses gp91phox gene expression in eosinophil-committed HL-60-C15 cells. *FEBS Lett* 436: 390–394, 1998.
36. Siegel MD, Zhang DH, Ray P, and Ray A. Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLEO elements. *J Biol Chem* 270: 24548–24555, 1995.
37. Snapper CM and Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236: 944–947, 1987.
38. Stellato C, Matsukura S, Fal A, White J, Beck LA, Proud D, and Schleimer RP. Differential regulation of epithelial-derived C-C chemokine expression by IL-4 and the glucocorticoid budesonide. *J Immunol* 163: 5624–5632, 1999.
39. Stelts D, Egan RW, Falcone A, Garlisi CG, Gleich GJ, Kreutner W, Kung TT, Nahrebne DK, Chapman RW, and Minnicozzi M. Eosinophils retain their granule major basic protein in a murine model of allergic pulmonary inflammation. *Am J Respir Cell Mol Biol* 18: 463–470, 1998.
40. Swain SL, Weinberg AD, English M, and Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol* 145: 3796–3806, 1990.

41. **Wills-Karp M.** Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 17: 255–281, 1999.
42. **Yamaguchi Y, Ackerman SJ, Minegishi N, Takiguchi M, Yamamoto M, and Suda.** Mechanisms of transcription in eosinophils: GATA-1, but not GATA-2, transactivates the promoter of the eosinophil granule major basic protein gene. *Blood* 91: 3447–3458, 1998.
43. **Yamaguchi Y, Hayashi Y, Sugama Y, Miura Y, Kasahara T, Kitamura S, Torisu M, Mita S, Tominaga A, and Takatsu K.** Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J Exp Med* 167: 1737–1742, 1988.
44. **Zhang DH, Cohn L, Ray P, Bottomly K, and Ray A.** Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* 272: 21597–21603, 1997.
45. **Zhang DH, Yang L, Cohn L, Parkyn L, Homer R, Ray P, and Ray A.** Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* 11: 473–482, 1999.
46. **Zhang DH, Yang L, and Ray A.** Differential responsiveness of the IL-5 and IL-4 genes to transcription factor GATA-3. *J Immunol* 161: 3817–3821, 1998.
47. **Zheng W and Flavell RA.** The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89: 587–596, 1997.
48. **Zon LI, Yamaguchi Y, Yee K, Albee EA, Kimura A, Bennett JC, Orkin SH, and Ackerman SJ.** Expression of mRNA for the GATA-binding proteins in human eosinophils and basophils: potential role in gene transcription. *Blood* 81: 3234–3241, 1993.

