

Treatment of Allergic Airway Inflammation and Hyperresponsiveness by Antisense-induced Local Blockade of GATA-3 Expression

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Abstract

Recent studies in transgenic mice have revealed that expression of a dominant negative form of the transcription factor GATA-3 in T cells can prevent T helper cell type 2 (Th2)-mediated allergic airway inflammation in mice. However, it remains unclear whether GATA-3 plays a role in the effector phase of allergic airway inflammation and whether antagonizing the expression and/or function of GATA-3 can be used for the therapy of allergic airway inflammation and hyperresponsiveness. Here, we analyzed the effects of locally antagonizing GATA-3 function in a murine model of asthma. We could suppress GATA-3 expression in interleukin (IL)-4-producing T cells in vitro and in vivo by an antisense phosphorothioate oligonucleotide overlapping the translation start site of GATA-3, whereas nonsense control oligonucleotides were virtually inactive. In a murine model of asthma associated with allergic pulmonary inflammation and hyperresponsiveness in ovalbumin (OVA)-sensitized mice, local intranasal administration of fluorescein isothiocyanate-labeled GATA-3 antisense oligonucleotides led to DNA uptake in lung cells associated with a reduction of intracellular GATA-3 expression. Such intrapulmonary blockade of GATA-3 expression caused an abrogation of signs of lung inflammation including infiltration of eosinophils and Th2 cytokine production. Furthermore, treatment with antisense but not nonsense oligonucleotides induced a significant reduction of airway hyperresponsiveness in OVA-sensitized mice to levels comparable to saline-treated control mice, as assessed by both enhanced pause (PenH) responses and pulmonary resistance determined by body plethysmography. These data indicate a critical role for GATA-3 in the effector phase of a murine asthma model and suggest that local delivery of GATA-3 antisense oligonucleotides may be a novel approach for the treatment of airway hyperresponsiveness such as in asthma. This approach has the potential advantage of suppressing the expression of various proinflammatory Th2 cytokines simultaneously rather than suppressing the activity of a single cytokine.

Key words: GATA-3 • antisense DNA • asthma • T cells • Th2 cytokines

Introduction

Allergic asthma is a chronic pulmonary disease associated with bronchoconstriction and chronic inflammation of the airways (1–3). In allergic asthma, there is a polarization of T

lymphocyte responses and enhanced secretion of cytokines involved in regulation of IgE, mast cells, basophils, and eosinophils, ultimately leading to inflammation and disease (4). The proinflammatory cytokines produced by T cells contribute to the initiation and perpetuation of allergic asthma (1–3). In particular, it has been shown that allergic airway inflammation in patients with asthma and animal models of allergic airway inflammation is associated with increased

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Th2 (IL-4, IL-5, IL-13) cytokine production (5–8). In this regard, IL-4 has been postulated to be critical for the development of Th2 T cells in asthmatic airways and the reduced expression of the IL-12 receptor β 2 chain on lung Th1 cells (6, 9, 10). Furthermore, it is believed to induce IgE isotype switching in B cells in asthma and promotes goblet cell metaplasia, mucus hypersecretion, and the recruitment of eosinophils by upregulating vascular cell adhesion molecule 1 expression in pulmonary endothelial cells (10). The importance of IL-4 is also underlined by the observation that inhalation of IL-4 causes the development of sputum eosinophilia and increased responsiveness of the airways and the finding that neutralizing antibodies to IL-4 are therapeutically effective for asthma (1, 11, 12).

GATA-3 is a pleiotropic transcription factor of the C4 zinc finger family expressed in T cells, mast cells, eosinophils, basophils, and embryonic brain and kidney that binds to a 5'-WGATAR-3' consensus DNA sequence (13–16). GATA-3 has been shown to be essential for the development of the earliest T cell progenitor cells (17). Furthermore, GATA-3 was found to be selectively expressed in Th2 but not in Th1 cells and to play an important role in cytokine gene expression in T cells (18–21). In particular, GATA-3 is important for the expression of IL-5 in T cells by transactivation of the IL-5 promoter together with Ets-1 and Ets-2 proteins (20, 21). Furthermore, GATA-3 weakly transactivates the IL-4 promoter in T cells (22). These functions of GATA-3 on Th2 cytokine gene promoters can be suppressed by repressor of GATA (ROG), a recently cloned lymphoid-specific repressor of GATA-3–induced transactivation (23). Finally, ectopic expression of GATA-3 in developing Th1 cells leads to upregulation of IL-4 and IL-5 and downregulation of IFN- γ . The latter effect appears to be partly due to downregulation of the IL-12 receptor β 2 chain (18, 19, 24).

Activation studies in retroviral infected T cells have shown that the activation of GATA-3 in T cells occurs upon activation of the IL-4/signal transducer and activator of transcription (STAT)¹–6 signaling pathway (25), suggesting that the exposure of naive T cells to IL-4 may be a very early event that induces GATA-3 activation and Th2 cell differentiation. However, GATA-3 can fully reconstitute Th2 development in STAT-6–deficient T cells, suggesting that it is a master switch both in STAT-6–dependent and –independent Th2 development (26). Finally, GATA-3 has been shown to exert STAT-6–independent autoactivation, creating a feedback pathway stabilizing Th2 commitment (26).

Based on the above data, it was of particular interest to analyze the expression and functional role of GATA-3 in patients with atopic asthma. Indeed, in a recent study Nakamura and co-workers (27) showed an increased expres-

sion of GATA-3 mRNA in asthmatic airways, suggesting that GATA-3 may be involved in the regulation of Th2 cytokine responses in asthma. Based on this observation, we analyzed in the present study the role of GATA-3 in a mouse model of asthma (28) using an antisense DNA strategy. We observed increased local production of GATA-3 in this model and demonstrate that local treatment with GATA-3 antisense oligonucleotides could be successfully used to downregulate Th2-induced pulmonary inflammation and airway hyperresponsiveness.

Materials and Methods

Reagents. The following reagents were used: water-soluble dexamethasone (DEX) and aluminum potassium sulfate (alum; Sigma-Aldrich); crystalline OVA (Pierce Chemical Co.); biotinylated monoclonal anti–mouse IL-4 (BD Pharmingen), monoclonal and polyclonal goat antibody anti–GATA-3, (Santa Cruz Biotechnology, Inc.) and monoclonal antiactin (Santa Cruz Biotechnology, Inc.); horseradish peroxidase–conjugated anti–rabbit Ig and anti–mouse Ig (Amersham Pharmacia Biotech), the ECL Western blotting system (Amersham Pharmacia Biotech); biotinylated anti–goat IgG (Vector Laboratories); Cy3/AMCA Streptavidin conjugated (Dianova); and phosphorothioate oligonucleotides (Roth GmbH and GIBCO BRL).

Phosphorothioate Oligonucleotides. Oligonucleotides were synthesized with a phosphorothioate backbone to improve resistance to endonucleases. The antisense oligonucleotides consisted of 18-mer analogues to the 5' end of the murine GATA-3 sequence which spans the translation initiation site. This sequence had no C plus G dinucleotide or a quadruple G sequence element that are known to cause unspecific effects. In addition, several control (mismatched and nonsense) oligonucleotides were prepared. The nonsense oligonucleotides contained the same nucleotide composition as the antisense oligonucleotides.

The sequences were as follows (mismatches are underlined): GATA-3 antisense DNA (with or without FITC), 5'-AGT CAC CTC CAT GTC CTC-3'; GATA-3 nonsense DNA, 5'-CTA TGT CAT CCG CTC CAC-3'; GATA-3 mismatched DNA, 5'-AGC CAC CTA CAT TTC CTA-3'; and GATA-3 mismatched DNA 2, 5'-AGC CAC CTA GGC ATC CTC-3'.

Coincubation of DNA with Murine Spleen CD4⁺ Th2 Cells. Naive splenic CD4⁺ T cells (purity > 97%) were obtained using monoclonal antibodies to CD4 coupled to magnetic beads (Miltenyi Biotec) according to the protocol provided by the manufacturer. Cells were cultured on 10 μ g/ml anti-CD3–coated wells in the presence of 2 μ g/ml anti-CD28, 100 U/ml rmlL-2, and 1,000 U/ml rmlL-4 for 3 d. Cells were then cultured for an additional 2 d in the presence of rmlL-2 and IL-4 only. During the last 12 h cells were challenged with 50 ng/ml PMA and 1 μ g/ml ionomycin. For coincubation experiments phosphorothioate oligonucleotides (12–15 μ M) were preincubated for 45 min with Lipofectamine (Life Technologies/GIBCO BRL). At days 2 and 4 the oligonucleotides were added to the cell cultures. Cells treated with FITC–conjugated antisense DNA were washed, fixed in 4% paraformaldehyde, and analyzed by FACS[®] analysis for uptake efficiency. Cell viability was assessed by trypan blue staining and supernatants were analyzed for IL-4 and IL-9 production by ELISA.

Enrichment of Antisense-transfected Cells by Immunomagnetic Cell Sorting. To enrich for antisense-transfected T cells, T cells were cotransfected on day 4 with the pMACS K^k.II vector (Miltenyi

¹Abbreviations used in this paper: BAL, bronchioalveolar lavage; BALF, BAL fluid; DEX, dexamethasone; EMSA, electrophoretic mobility shift assay; HPF, high power field; MACS, magnetic cell sorting; MCh, methacholine; PAS, periodic acid-Schiff; PenH, enhanced pause; RL, pulmonary resistance; STAT, signal transducer and activator of transcription.

Biotech) expressing a truncated H2-K^k molecule (that is not expressed in BALB/c mice) and FITC-labeled or unlabeled phosphorothioate oligonucleotides. Cells were harvested 48 h after cotransfection, resuspended in PBE (PBS with 5 mM EDTA) buffer, and incubated with MACSelect K^k microbeads (Miltenyi Biotech) followed by magnetic separation. FITC-positive cells were visualized by fluorescence microscopy or confocal laser microscopy using a Bio-Rad Laboratories MRC-1024/MP microscope. To assess enrichment of antisense-transfected cells, T cell nuclei were counterstained with DAPI (Vector Laboratories) and the percentage of FITC-positive cells was determined by counting 12 randomly selected high power fields (HPFs) in three samples per condition by fluorescence microscopy. Cytokine production in magnetic cell sorting (MACS)-selected T cells was assessed after 18 h stimulation with 50 ng/ml PMA plus 1 µg/ml ionomycin by ELISA.

Allergen Sensitization/Challenge Protocol. Female BALB/c mice (6–8 wk of age) received an intraperitoneal injection of 100 µg/ml OVA (0.2 ml of 500 µg/ml in normal saline) complexed with alum on days 0 and 14. On days 25, 26, and 27, the mice underwent anesthesia with 0.2 ml of avertine intraperitoneally (1 mg/ml of triplometan in amylalcohol; 2.5% in PBS) before receiving 50 µg OVA in saline intranasally. Control animals received saline intraperitoneally with alum on days 0 and 14 and 50 µl of saline intravenously on days 25, 26, and 27.

Antisense GATA-3 or DEX Treatment in OVA-sensitized Mice. To assess the effect of antisense GATA-3 treatment in airway inflammation, antisense or control oligonucleotides were given by intranasal administration (200 µg/treatment) at days 24, 25, 26, and 27 (30 min before intravenous injection of OVA) to OVA-sensitized mice. To localize antisense oligonucleotides *in vivo*, we performed the last intranasal administration with FITC-labeled antisense oligonucleotides.

To compare the efficiency of antisense GATA-3 treatment to the classical treatment with glucocorticoids, a group of OVA-treated mice was given 1 mg (50 µl vol) of water soluble DEX intravenously in saline on days 24, 25, 26, and 27.

Collection and Analysis of Bronchioalveolar Lavage Fluid, Cytospins, and ELISA for IFN-γ and IL-4. 24 h after the last intranasal challenge with either OVA or saline at day 27, bronchioalveolar lavage (BAL) of the right lung was performed after tying off the left lung at the mainstem bronchus. Total BAL fluid (BALF; 1 ml of saline three times) was collected and cells were counted using a 100-µl aliquot. Samples were centrifuged at 1,200 rpm for 5 min at 4°C, and cell pellets were resuspended in PBS. Cytospins were made by centrifugation at 450 rpm for 5 min at room temperature. Eosinophils were detected by staining according to May-Grünwald Giemsa and subsequently analyzed with a ZEISS microscope at 400×.

To determine cytokine concentrations in BALF, samples were centrifuged at 1,200 rpm for 5 min at 4°C. Supernatants were analyzed by specific ELISA for content of IFN-γ and IL-4 using commercially available kit systems (BD PharMingen).

Lung Histology. 24 h after the last intranasal challenge with either OVA or saline, lungs were analyzed by histology (day 28). The right lung was frozen immediately and stored in liquid nitrogen until use. The left lung was fixed in 10% formaline, dehydrated, mounted in paraffin, sectioned, and stained with hematoxylin/eosin. Eosinophils in the lungs were quantified blindly by the same pathologist (H.A. Lehr) using a Leitz D33 microscope, a JVC1 chip camera, the program Photoshop®, and an Image Processing Tool Kit (v2.1). Three HPFs in the area of peripheral

bronchi were randomly selected for quantification. Counts are given as eosinophils per square millimeter.

Airway mucus was identified by the alcian blue/periodic acid-Schiff (PAS) reaction by using a standard protocol in use in our Department of Pathology. PAS-positive mucosubstances in the small and large airways were also assessed by semiquantitative analysis in four to five mice per group using the following grading scale: –, no mucosubstances; +, mucosubstances detectable; ++, high amount of mucosubstances.

Immunohistochemistry. Immunohistochemistry was performed as described previously (29–31). In brief, frozen sections were fixed in 4% paraformaldehyde and immunostained for GATA-3 and IL-4. In brief, sections were preincubated with 2% normal rabbit serum in PBS/1% BSA/0.4% saponin for 45 min followed by an incubation with anti-GATA-3 goat polyclonal antibodies (5 µg/ml) in PBS/1% BSA/0.4% saponin/2% normal rabbit serum overnight at 4°C. The next day sections were incubated with biotinylated rabbit anti-goat IgG (1:100 dilution) followed by Streptavidin-Cy3 (1:1,000 dilution).

For IL-4 staining studies, sections were then incubated overnight with an anti-IL-4 biotinylated antibody (10 µg/ml; BD PharMingen) in PBS/1% BSA/0.4% saponin followed by the Streptavidin-conjugated Cy3 complex (1:1,000 in PBS). Sections were analyzed with an Olympus fluorescence microscope.

Morphometric Analysis. GATA-3- and IL-4-positive cells in the lungs were quantified blindly by the same pathologist using an Olympus microscope and an Image Processing Tool Kit (v2.1). 10 HPFs in the area of peripheral bronchi were randomly selected in 4–5 mice per group for quantification. Counts are given as positive cells per HPF.

Protein Analysis. Lung tissues were homogenized by using an electrical homogenizer, and proteins were extracted in PBS in the presence of protease inhibitors (6.75% aprotinin, 312 µg/ml trypsin inhibitor) and 0.62% NP-40. Protein concentrations were determined by a protein assay according to the manufacturer's instructions (Bio-Rad Laboratories). The standard curve was performed by using 0, 4, 8, and 16 µg of BSA per milliliter. Under our experimental conditions ~4 mg of proteins per lung could be recovered.

Western Blot Analysis. 50 µg of total proteins isolated from the whole lung was separated by 15% SDS-PAGE and blotted onto a nitrocellulose membrane overnight (31). Equal loading was assessed with Ponceau's solution (Sigma-Aldrich). The membrane was then incubated in blocking solution (5% dry milk in PBS/0.05% Tween 20) for 1 h at room temperature, and subsequently exposed to 0.8 µg/ml of monoclonal antibodies anti-GATA-3 or anti-β-actin overnight at 4°C. The day after, the membrane was incubated with peroxidase-conjugated anti-mouse Igs (1:2,500) for 1 h at room temperature. Finally, enzyme activity was detected with the ECL-Plus Western blotting detection system according to the manufacturer's instructions (Amersham Pharmacia Biotech).

Electrophoretic Mobility Shift Assay. Electrophoretic mobility shift assay (EMSA) was carried out by the addition of 25,000 cpm radiolabeled GATA-3, OCT-1, and SP1 consensus DNA probes (Santa Cruz Biotechnology, Inc.) to the binding reaction that also contained 1 µg synthetic DNA duplex of poly(dIdC), 30 µg of total lung proteins, and binding buffer (32). Complex formation was allowed to proceed for 30 min. Finally, the complexes were separated from unbound DNA by native PAGE on 5% gels. The GATA-3 bands on the EMSA gels were analyzed by densitometry as specified in Results. For supershift analysis, 1 µg GATA-

3-specific antibodies and control antibodies (obtained from Santa Cruz Biotechnology, Inc.) were used as specified in Results.

Assessment of Airway Reactivity by Body Plethysmography. Airway reactivity was assessed in 12 mice per group by head-out ple-

thysmography as described previously (33). In brief, mice were placed in four body plethysmographs attached to an exposure chamber (Crown Glass). Airflow was measured with a PTM 378/1.2 pneumotachograph (Hugo Sachs Electronics) and an 8-T2

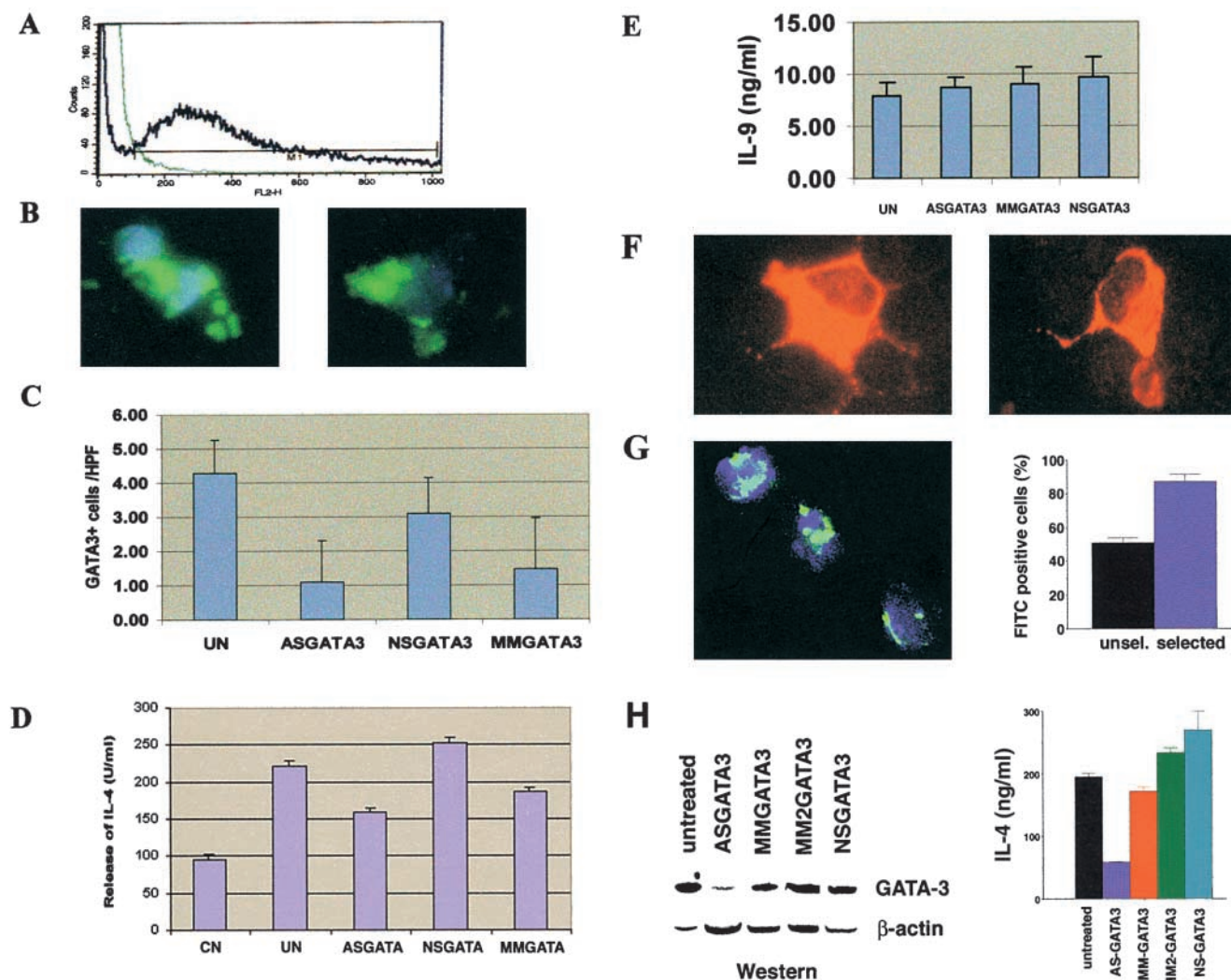


Figure 1. Suppression of IL-4 production and GATA-3 expression in Th2 T cells by a specific phosphorothioate oligonucleotide to the translation start site of GATA-3. To induce Th2 T cell development naive splenic CD4⁺ T cells (purity > 97%) were cultured on anti-CD3-coated wells in the presence of soluble anti-CD28, rmlL-2, and rmlL-4 for 3 d. Cells were then cultured for an additional 2 d in the presence of rmlL-2 and IL-4 only. During the last 12 h, cells were challenged with PMA (50 ng/ml)/ionomycin (1 μg/ml). At days 2 and 4, oligonucleotides were preincubated with Lipofectamine and added to the cell cultures as indicated. In vitro-differentiated Th2 cells showed high viability under all experimental conditions (UN, 85–95%; ASGATA3, 90–98%; NSGATA3, 75–95%) in three independent experiments as assessed by trypan blue exclusion (data not shown). Coincubation with FITC-labeled antisense oligonucleotides to GATA-3 led to a high degree of DNA uptake (50–70% of cells) as assessed by FACS[®] analysis (A) and quantitative fluorescence microscopy using nuclear counterstaining with DAPI (B). Antisense oligonucleotides to GATA-3 led to a significant ($P < 0.05$) reduction of the number of GATA-3-expressing Th2 cells, whereas control nonsense oligonucleotides had no such effect (C). In addition, mismatched oligonucleotides led to a reduction in the number of GATA-3-expressing cells, although this effect was not statistically significant. Cytokine production from untreated and GATA-3 antisense or control-treated cells was assessed by ELISA. The treatment with antisense DNA to GATA-3 significantly ($P < 0.05$) reduced IL-4 production (D) compared with untreated cells but, in contrast, IL-9 release remained unaffected after antisense GATA-3 treatment (E). In addition, control oligonucleotides had no significant effect on both IL-4 and IL-9 production, although mismatched DNA caused a reduction of the average IL-4 production. (F) High GATA-3 expression in in vitro-differentiated Th2 cells. (G) To enrich for antisense-transfected Th2 cells, we used a cotransfection system with a plasmid expressing a truncated H2-K^k molecule followed by magnetic selection of transfected cells (see Materials and Methods). As shown by confocal laser microscopy (G, left), there was a high DNA uptake in MACS-selected cells, and quantification of FITC-positive cells showed an enrichment of cells transfected with FITC-labeled antisense DNA compared with unselected cells (G, right). There was a downregulation of GATA-3 expression in MACS-selected, antisense-transfected cells compared with untreated MACS-selected T cells as shown by Western blot analysis (H, left). Such downregulation was not observed after treatment with GATA-3 nonsense DNA and two mismatched oligonucleotides and was associated with a marked downregulation of IL-4 production as assessed by ELISA (H, right). AS, antisense; ASGATA3, GATA-3 antisense DNA; NSGATA3, GATA-3 nonsense DNA; NS, nonsense DNA; MM, mismatched oligonucleotides; UN, untreated cells; CN, unstimulated control.

differential pressure transducer (Gaeltec). In addition, airflow was analyzed in response to various concentrations of methacholine (MCh) (25, 50, 75, 100, 150 mg/ml for 1 min) delivered by a jet nebulizer (Pari-Boy; Pariwerke). Finally, the concentration of MCh that caused a 50% reduction in expiratory airflow (MCh₅₀) was determined.

In an additional series of experiments, airway responsiveness was assessed by analyzing enhanced pause (PenH) responses of saline- or OVA-treated mice in a body plethysmograph (model PLY 3211; Buxco Electronics, Inc.) (34). Measurements of MCh responsiveness were obtained by exposing mice for 5 min to 200 mg/ml of aerosolized MCh and monitoring PenH. Results were

expressed as the peak of fold-increase of PenH within 5 min after MCh treatment.

In addition, pulmonary resistance (RL) was measured in the same cohort of untreated and treated OVA-sensitized mice as described previously (35, 36). In brief, dose-response curves to MCh were obtained in anesthetized mice after administering increasing doses of intravenous MCh (33–1,000 μg/kg). Data were expressed as mean values of RL ± SEM.

Statistical Analysis. Differences were evaluated for significance ($P < 0.05$) by the Student's two-tailed *t* test for independent events (Statworks). Data are given as mean values ± SEM.

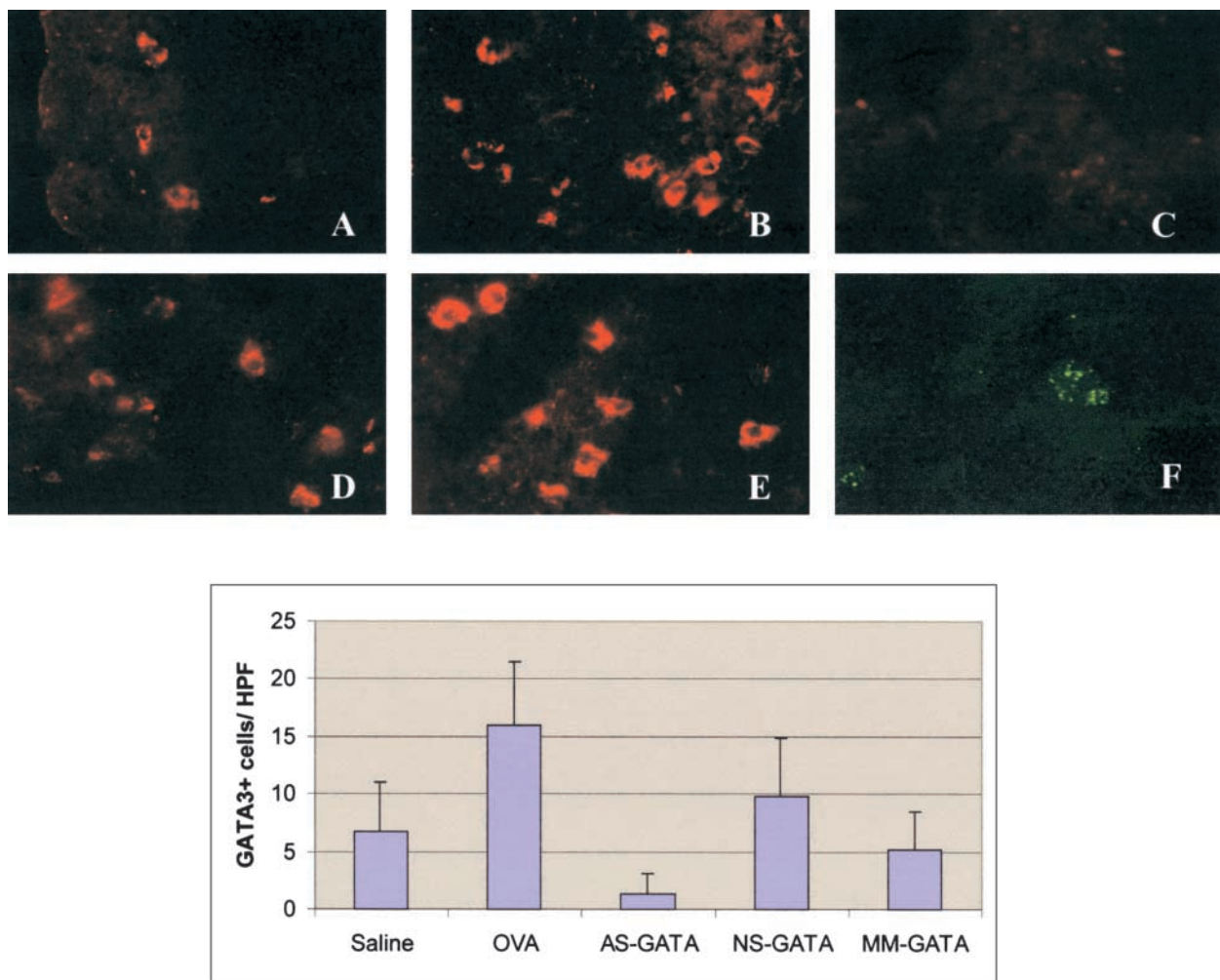


Figure 2. Increased number of GATA-3-producing lung cells in OVA-sensitized mice and inhibition of GATA-3 protein expression after local administration of antisense oligonucleotides. Sections of OVA-immunized (B) and saline-treated control mice (A) were stained with an antibody against GATA-3 (Cy3). OVA immunization caused an upregulation of GATA-3 (B) expression. Intranasal administration of GATA-3 antisense oligonucleotides suppressed GATA-3 (C) expression in the lung of OVA-immunized mice (original magnification: ×400). The same field analyzed for FITC-labeled antisense DNA uptake is shown in F (FITC; emission wavelength, 520 nm). Double staining showed the absence of GATA-3 protein expression in cells with intracellular FITC-labeled antisense DNA to GATA-3 (compare C and F). Local treatment with control oligonucleotides did not change significantly the number of GATA-3-positive cells in the lung compared with OVA-sensitized airways (nonsense and mismatched oligonucleotide treatment is shown in E and D, respectively), although mismatched DNA led to a reduction in the average number of GATA-3-expressing cells per HPF (bottom panel). In these quantitative studies, GATA-3-positive cells in OVA-immunized mice, GATA-3 antisense, mismatched, and nonsense-treated OVA-immunized mice, and saline-treated control mice were assessed by immunohistochemistry. GATA-3-expressing cells in the lungs were quantified blindly by the same observer (see Materials and Methods). 10 HPFs in the area of peripheral bronchi were randomly selected in nine lung sections from each mouse (five mice per group) for quantification. Counts are given as cells per HPF. AS-GATA, GATA-3 antisense; MM-GATA, mismatched GATA-3; NS-GATA, nonsense GATA-3; OVA, OVA-immunized mice.

For the analysis of RL data the multivariate analysis MANOVA (Wilks Lambda) was used.

Results

Suppression of IL-4 but Not IL-9 Release in Th2 CD4⁺ T Cells by a Specific Antisense Phosphorothioate Oligonucleotide to the Translation Start Site of GATA-3. In an attempt to specifically downregulate expression of GATA-3 in T lymphocytes in vitro and in vivo, we used an antisense DNA strategy. Accordingly, we designed specific CpG-lacking antisense phosphorothioate oligonucleotides targeting the translation start site of murine GATA-3 (see Materials and Methods). Then we tested the ability of such oligonucleotides to specifically downregulate GATA-3 expression in CD4⁺ cells isolated from the spleen during Th2 cell differentiation in vitro. We observed in coincubation studies an efficient uptake of FITC-labeled antisense oligonucleotides

in cultured and already differentiated Th2 cells (day 6: 50–70% of cells) and Jurkat cell lines (>65% as assessed by FACS[®] analysis and immunofluorescence; Fig. 1, A and B) 24 h after the treatment with antisense oligonucleotides to GATA-3. This finding was associated with a downregulation of GATA-3 expression and a significant reduction of IL-4 release 12 h after incubation with PMA/ionomycin (Fig. 1, C and D, respectively). However, IL-9 production by spleen T cells remained unchanged after antisense GATA-3 treatment (Fig. 1 E). It should be noted that the observed 50% reduction of PMA plus ionomycin-inducible IL-4 production upon antisense treatment is remarkable, as only 50–70% of the spleen cells showed DNA uptake. Indeed, cotransfection-based enrichment of antisense-transfected T cells led to a more pronounced downregulation of IL-4 production by Th2 cells (Fig. 1).

Treatment with control nonsense oligonucleotides did not affect GATA-3 expression or IL-4 and IL-9 release un-

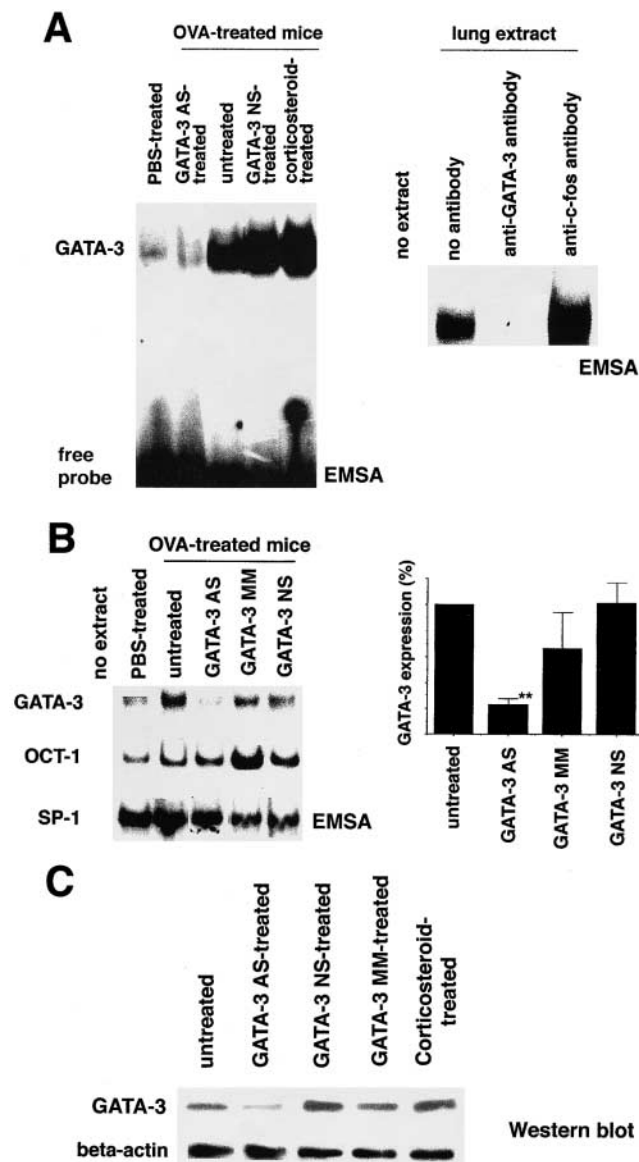


Figure 3. Detection of GATA-3 in the lungs of OVA-immunized mice by gel retardation assay (EMSA) and Western blot analysis. (A) Expression of GATA-3 in cellular extracts from lung cells was assessed by EMSA using a GATA-3-specific reference binding site. Unbound radiolabeled probe was separated from DNA-protein complexes by gel electrophoresis under non-denaturing conditions. OVA immunization led to a strong increase of GATA-3 expression in the lung compared with lungs from saline-treated control mice. Whereas treatment with GATA-3 antisense oligonucleotides caused a suppression of GATA-3 expression, treatment with GATA-3 nonsense DNA or corticosteroids had no effect on GATA-3 expression. Specificity of the GATA-3 signal was shown by competition assays using unlabeled GATA-3 oligonucleotides (data not shown) and supershift assays with specific antibodies to GATA-3. Whereas a control antibody had no effect on the GATA-3 complex in EMSA, a complete abrogation of the retarded complex was observed upon addition of the GATA-3-specific antibody (right). (B) Specificity of the effects of GATA-3 antisense oligonucleotides on GATA-3 expression. The expression of GATA-3, OCT-1, and SP-1 transcription factors in cellular extracts from lung cells was assessed by EMSA using specific reference binding sites. Although antisense oligonucleotides to GATA-3 caused a marked reduction of GATA-3 expression, they had no such effect on the expression of OCT-1 and SP-1. The expression of GATA-3 in lung extracts was finally assessed in three independent experiments using EMSA analysis. Quantification of the EMSA bands in these three experiments is depicted on the right (signal in untreated conditions was defined as 100%). Whereas GATA-3 antisense DNA led to a significant ($P < 0.01$) reduction of GATA-3 expression, nonsense DNA had no such effect. Mismatched DNA had no significant effect on GATA-3 expression, although a reduction in the average GATA-3 signal was noted. $^{**}P < 0.01$. (C) Western blot analysis for GATA-3 and β -actin expression in the lungs of OVA-immunized mice. Extracts from lung tissue were analyzed using a GATA-3-specific antibody. GATA-3 antisense DNA treatment led to a suppression of GATA-3 expression, whereas control nonsense GATA-3 oligonucleotides and corticosteroids had no such effect. One representative out of three lungs per group is shown. 50 μ g of proteins per lane was loaded. Equal loading was also confirmed after 5 min staining of the nitrocellulose membrane with Ponceau's solution (data not shown). AS, antisense; NS, nonsense; MM, mismatched.

der the same experimental conditions. Treatment with mismatched DNA also led to a reduction of the average number of GATA-3-expressing cells and IL-4 release, possibly due to a remaining weak hybridization of the mismatched DNA to the GATA-3 target mRNA (Fig. 1). However, this effect was less pronounced compared with the antisense DNA effect, and the mismatched DNA-induced changes were not statistically significant. Furthermore, this effect was not seen using a second mismatched control oligonucleotide (Fig. 1 H), suggesting that antisense oligonucleotides to GATA-3 cause a specific suppression of GATA-3 expression and IL-4 production by cultured T cells.

Intranasal Administration of GATA-3 Antisense Oligonucleotides Decreases Lung GATA-3 Protein Expression in a Murine Model of Asthma. Based on previous studies showing a key role for GATA-3 in Th2 cytokine production and increased Th2 cytokine production in asthma (2, 18), we analyzed in a consecutive series of studies the expression of GATA-3 in a murine model of asthma (37) associated with late phase allergic pulmonary inflammation in OVA-sensitized mice. Accordingly, we immunostained lung sections from OVA-treated and control mice with anti-GATA-3 antibodies. As shown in Fig. 2, OVA immunization caused a striking increase in lung-infiltrating cells expressing GATA-3 compared with saline treatment.

In further studies, we wanted to directly test the effects of GATA-3 antisense phosphorothioate oligonucleotides on the expression of GATA-3 during the late phase allergic asthmatic reaction in vivo. Intranasal delivery of FITC-labeled GATA-3 antisense oligonucleotides led to a DNA uptake by GATA-3-expressing lung cells in vivo (Fig. 2, C and F). This finding was accompanied by a significant decrease of GATA-3 expression in the lung of OVA-sensitized mice (Fig. 2, B and C, and bottom panel), while control nonsense oligonucleotides had no such effect (Fig. 2 E, and bottom panel). Mismatched DNA led to a reduction of the number of GATA-3-positive cells in the lung (Fig. 2 D, and bottom panel), although this effect was not statistically significant.

In additional studies, we performed Western blot analysis of total proteins derived from the lung to assess local GATA-3 expression. As shown in Fig. 3, these studies confirmed the downregulation of GATA-3 protein expression in antisense GATA-3-treated lungs but not in lungs treated with control nonsense oligonucleotides (Fig. 3 C). Similarly, analysis of lung proteins for GATA-3 expression by gel retardation assays (EMSA) using a GATA-3 consensus oligonucleotide confirmed the downregulation of GATA-3 upon antisense treatment (Fig. 3 A). Densitometric analysis of the EMSA bands showed a significant downregulation of GATA-3 upon antisense DNA treatment (Fig. 3 B). In contrast, the transcription factors OCT-1 and SP-1 were not affected by GATA-3 antisense DNA (Fig. 3 B). Furthermore, nonsense control oligonucleotides were virtually inactive with regard to GATA-3 expression. Taken together, these results indicated that local administration of

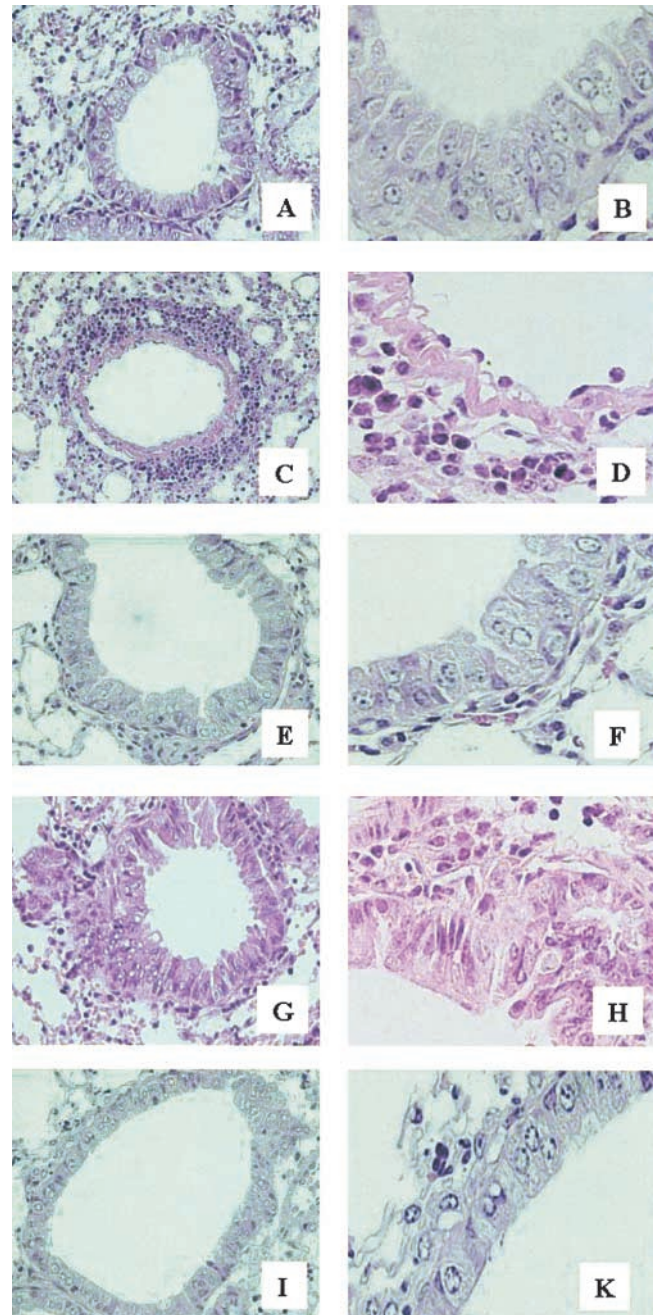


Figure 4. Histologic evidence of airway inflammation in OVA-sensitized and control mice; effect of GATA-3 antisense treatment. Lung tissue was analyzed from untreated (C and D) and antisense-treated (E and F) OVA-sensitized mice. In untreated mice, a massive peribronchial infiltration with eosinophils, thickening of the basement membrane, and de-epithelialization were seen (see higher magnification in D: $\times 400$). In contrast, after treatment with antisense DNA to GATA-3 an intact bronchial epithelial layer and no eosinophil infiltration were seen (E and F) comparable to DEX treatment (I and K). Lung tissues taken from sham (saline)-sensitized mice (A and B) and OVA-immunized mice treated with nonsense DNA (G and H) are shown as control. Lung sections were stained with hematoxylin and eosin and examined by light microscopy. Original magnifications: (A, C, E, G, and H) $\times 200$; (B, D, F, H, and K) $\times 400$.

specific antisense oligonucleotides leads to specific down-regulation of pulmonary GATA-3 expression in an asthma model in vivo.

Inhibition of Allergen-induced Airway Inflammation by GATA-3 Antisense Oligonucleotides. To assess the histologic effects of GATA-3 antisense phosphorothioate oligonucleotides on allergen-induced airway inflammation, we analyzed lung tissues 24 h after the last of three sequential OVA challenges (days 25–27) on day 28. In untreated OVA-sensitized mice, an infiltration of the bronchial interstitium with eosinophils was observed (Fig. 4, C and D). Treatment of OVA-sensitized mice with nonsense GATA-3 oligonucleotides had no detectable effects (Fig. 4, G and H). In contrast, no inflammatory signs were seen in OVA-sensitized mice upon treatment with corticosteroids or GATA-3 antisense oligonucleotides (Fig. 4, I, K, E, and F).

Control mice were treated with saline complexed with alum and rechallenged intranasally with saline; they show an intact airway epithelium and no sign of inflammation (Fig. 4, A and B).

Antisense Oligonucleotides to GATA-3 Inhibit Infiltration of Eosinophils in the Lung. In further studies on the potential mechanisms of antisense-induced suppression of lung inflammation, we assessed allergen-induced infiltration of eosinophils in the lung. By morphometric analysis, the influx of eosinophils into the lung interstitium was significantly reduced after administration of GATA-3 antisense oligonucleotides compared with untreated mice (Fig. 5). Antisense-induced suppression of the number of eosinophils in the BALF was comparable to that induced by intranasal DEX treatment (BAL cell viability $77.5 \pm 9.8\%$). However, no difference was seen in cell viability in the

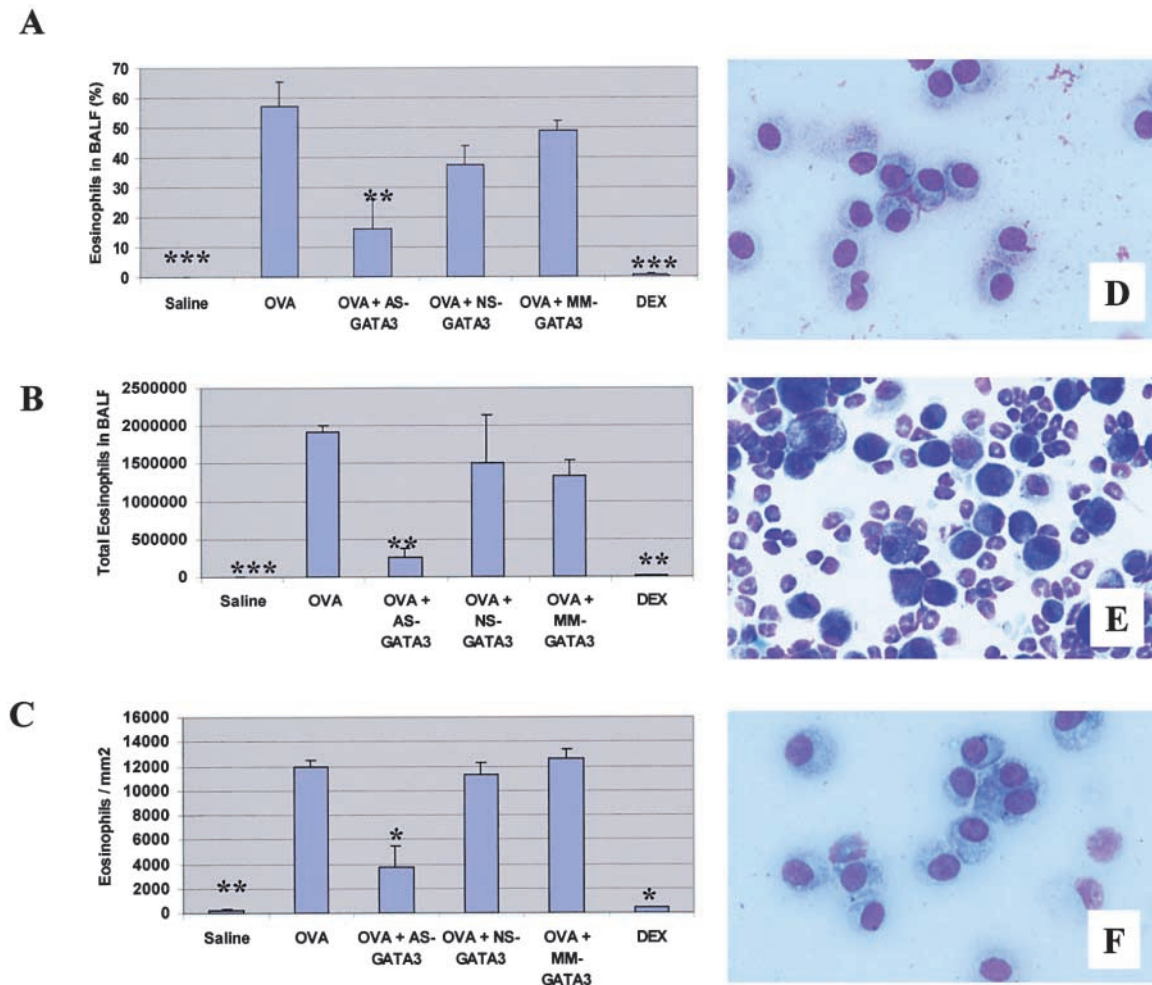


Figure 5. Infiltration of eosinophils into the airways is abrogated by local administration of GATA-3 antisense oligonucleotides (significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). (A) Eosinophils in the BALF were detected on cytopins after staining according to May-Grünwald Giemsa and quantified after counting of 200 cells. Data are reported as the mean percentage of eosinophils in the BAL \pm SEM ($n = 4-5$ animals per group). (B) Absolute eosinophil numbers \pm SEM in the recovered BALF. Results were obtained by multiplying the percentage of eosinophils with the total cell number per milliliter and the recovered volume of the BALF. (C) Three representative fields around peripheral bronchi of similar size were randomly selected from lung sections and eosinophils were counted with a computerized system (see Materials and Methods). Data are reported as the mean number of eosinophils per $\text{mm}^2 \pm$ SEM. Significant differences compared with OVA treatment are indicated. (D–F) A representative field of the BALF from a saline-treated mouse (D) and an OVA-sensitized animal with (F) or without (E) antisense GATA-3 DNA treatment are shown. Cytopins were stained with May-Grünwald Giemsa. AS, antisense; NS, nonsense; MM, mismatched.

BALF after antisense treatment in OVA-sensitized lungs compared with saline treatment ($84.4 \pm 3.5\%$ and $91.15 \pm 6.3\%$ viability, respectively, as assessed by trypan blue exclusion). In contrast, treatment with control mismatched (BAL cell viability $87.27 \pm 3.8\%$) and nonsense oligonucleotides (BAL cell viability $79.15 \pm 5.45\%$) had no significant effect on the OVA-induced influx of eosinophils in the airways (Fig. 5).

Antisense Oligonucleotides to GATA-3 Inhibit Th2 Cytokine Production and Infiltration of Eosinophils in the Lung of OVA-sensitized Mice. Since T cell cytokines are known to regulate airway inflammation in vivo, we next determined Th1 and Th2 cytokine levels in the BALF of OVA-sensitized mice (Fig. 6). Interestingly, neither OVA nor antisense treatment altered IFN- γ levels in the BALF compared with saline-treated mice. However, IL-4 concentration in the BAL was significantly suppressed ($P < 0.05$) by GATA-3 antisense DNA treatment, suggesting that blockade of GATA-3 expression selectively inhibits Th2 cytokine levels in the BAL. Nonsense control DNA did not cause significant changes in IL-4 production, whereas mismatched oligonucleotides led to downregulation of the average IL-4 production. However, this effect was not statistically significant.

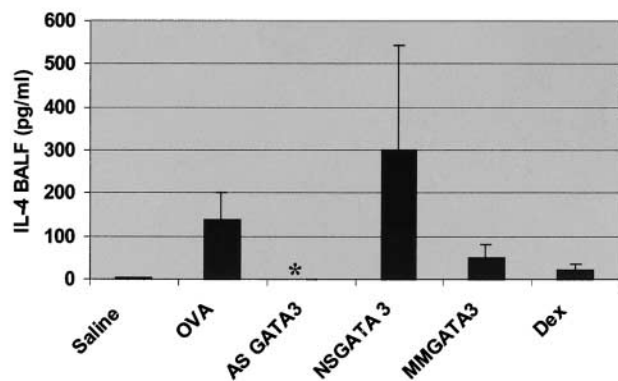
Local Treatment with Antisense Oligonucleotides Decreased Alcian Blue/PAS-positive Cells in the Airways of OVA-sensitized Mice. Many mucus-producing cells (acidic sulfated and neutral mucosubstances) were seen in the airways of

mice sensitized to OVA (Fig. 7) compared with saline-treated mice (saline), as assessed by the alcian blue/PAS reaction. These inflammatory changes were absent in mice that were treated with GATA-3 antisense DNA (AS-GATA-3). DEX treatment reduced PAS-positive cells in the airways. In contrast, no changes were seen after treatment with nonsense control oligonucleotides.

Local Treatment with Antisense Oligonucleotides to GATA-3 Suppresses Airway Hyperreactivity in OVA-sensitized Mice. In a final series of experiments, we analyzed whether antisense oligonucleotides to GATA-3 would affect airway hyperreactivity in OVA-sensitized mice. This point was of particular importance since it was unclear whether modulation of the function of GATA-3 would not only affect airway inflammation but also airway hyperresponsiveness.

In initial experiments, we subjected 12 OVA-sensitized mice or control mice per group to treatment with antisense oligonucleotides, control nonsense oligonucleotides, or saline and assessed airway hyperreactivity by noninvasive head-out body plethysmography after MCh challenge at day 28. As shown in Fig. 8, A and B, OVA-sensitized mice showed an increase in airway hyperreactivity compared with saline-treated control mice after challenge. Furthermore, it was found that antisense oligonucleotides to GATA-3 lead to a significant reduction of airway hyperreactivity to levels comparable to saline-treated control mice. Finally, we observed that control nonsense oligonucleotides had no effect on airway hyperreactivity in OVA-sensitized mice.

A



B

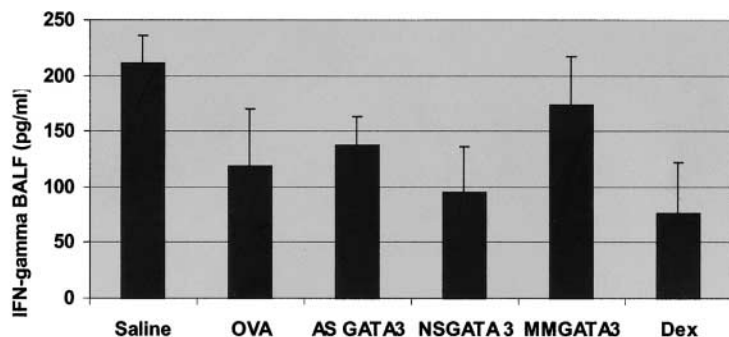
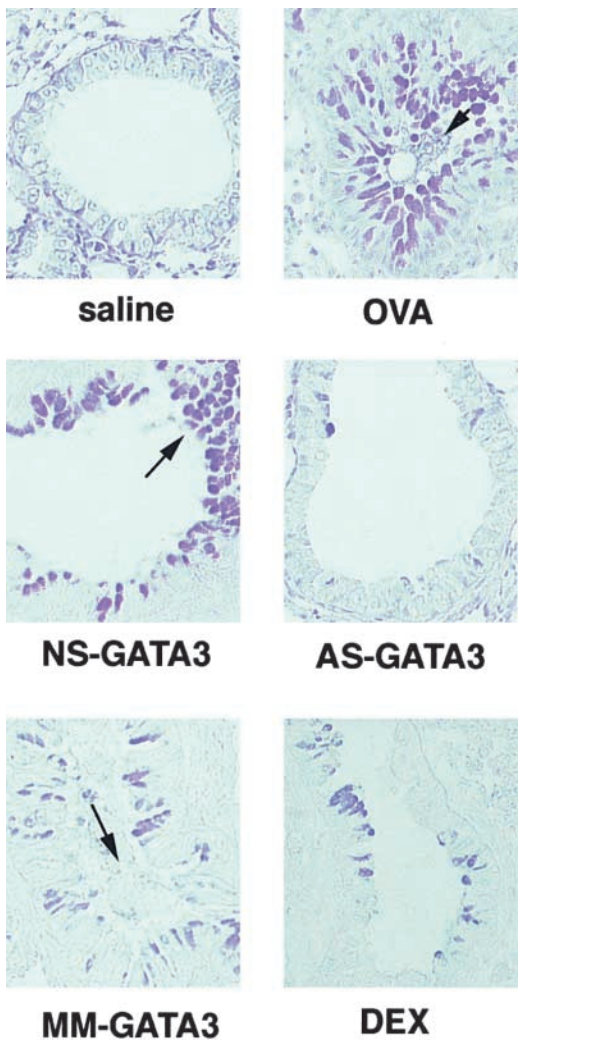


Figure 6. GATA-3 antisense treatment selectively reduces Th2 cytokine concentration in the BALF. Analysis of Th1 and Th2 cytokine concentration in the BAL from lungs of saline-treated mice (PBS; $n = 4$), OVA-sensitized mice ($n = 4$), and OVA-sensitized mice treated with GATA-3 antisense DNA ($n = 5$), GATA-3 nonsense DNA ($n = 4$), GATA-3 mismatched DNA ($n = 4$), or DEX ($n = 4$). Cytokine levels (IFN- γ , IL-4) were determined by specific ELISA and are reported as mean values \pm SEM. IFN- γ and IL-4 levels were between 17 and 234 pg/ml and 35 and 435 pg/ml, respectively. AS GATA3, OVA-sensitized mice treated with GATA-3 antisense DNA; MMGATA3, GATA-3 mismatched DNA; NSGATA3, GATA-3 nonsense DNA; OVA, OVA-sensitized mice.



	saline	OVA	NS-GATA3	AS-GATA3	MM-GATA3	DEX
large airways	-	++	++	-	+	-
small airways	-	++	++	-	+	+/-

Figure 7. Mucus occlusion of lower airways in OVA-treated mice (OVA) compared with saline-treated mice (saline). The acidic and neutral mucosubstances are stained in magenta by the alcian blue/PAS reaction. The treatment with antisense DNA to GATA-3 (AS-GATA3) led to a strong reduction of the PAS reaction in the airways of OVA-sensitized mice. This effect was comparable to DEX treatment. No effect was observed after nonsense DNA treatment (NS-GATA3), whereas mismatched DNA (MM-GATA3) led to a small reduction in mucus production. The differences in mucus production were also assessed by semiquantitative analysis of mucosubstances in the small and large airways of four to five mice per group. The mean values are shown. Antisense DNA to GATA-3 led to an abrogation of mucus production, whereas nonsense DNA had no effects compared with OVA-sensitized untreated mice. Mismatched DNA led to a detectable reduction in mucus production, although this effect was much lower compared with GATA-3 antisense DNA. Bottom table: -, no mucosubstances; +, mucosubstances detectable; ++, high amount of mucosubstances.

To validate our findings on a significant downregulation of airway hyperreactivity upon antisense treatment, we assessed in subsequent studies PenH responses of OVA-sensitized mice in a body plethysmograph (Fig. 8 C). It was found that administration of antisense oligonucleotides to GATA-3 results in a significant downregulation of MCh responsiveness, whereas nonsense control oligonucleotides had no such effect. Finally, to assess the MCh response in the lower airways, we measured RL and dose-response curves to intravenous MCh in anesthetized OVA-treated mice by body plethysmography (Fig. 8 D). These experiments demonstrated a significant reduction of RL in OVA-sensitized mice upon treatment with antisense but not nonsense control oligonucleotides. Thus, using three independent methods to assess airway hyperreactivity, these data showed that airway hyperreactivity was significantly suppressed by administration of antisense oligonucleotides to GATA-3.

Discussion

Recent studies in transgenic mice have revealed that expression of a dominant negative form of GATA-3 can prevent allergic airway inflammation (38), indicating that GATA-3 plays a crucial role in the development of Th2-mediated lung inflammation (39). However, it remains unclear whether GATA-3 plays a role in the effector phase of allergic airway inflammation and whether antagonizing the expression and/or function of GATA-3 can be used for the therapy of allergic airway inflammation and hyperresponsiveness. Furthermore, the potential mechanisms of action of such therapy remain to be elucidated. In this study, we demonstrate a key role of the transcription factor GATA-3 for cytokine production of T cells in the effector phase of a murine asthma model induced by OVA. We found that specific blockade of GATA-3 expression in the lung by antisense oligonucleotides leads to suppression of airway inflammation and Th2 cytokine production both in the BAL and in the interstitium of the lung. Antisense-induced blockade of GATA-3 was at least as effective to suppress lung inflammation as the administration of corticosteroids. Furthermore, using three independent methods to assess airway hyperresponsiveness to MCh, we demonstrate that GATA-3 antisense oligonucleotides significantly reduce airway hyperresponsiveness in a model of allergic asthma, suggesting a novel function of GATA-3 as a key regulator of airway hyperresponsiveness in allergic asthma. Taken together with the recently described increased expression of GATA-3 mRNA in lung CD3⁺ T cells of patients with allergic asthma (27), these data suggest the potential therapeutic utility of GATA-3 antisense oligonucleotides as a novel molecular approach for the treatment of patients with allergic asthma in humans. This approach has the potential advantage of blocking the production of several Th2 cytokines simultaneously rather than suppressing the activity of a single cytokine.

The GATA family of zinc finger transcription factors has pleiotropic roles in cell development and differentiation.

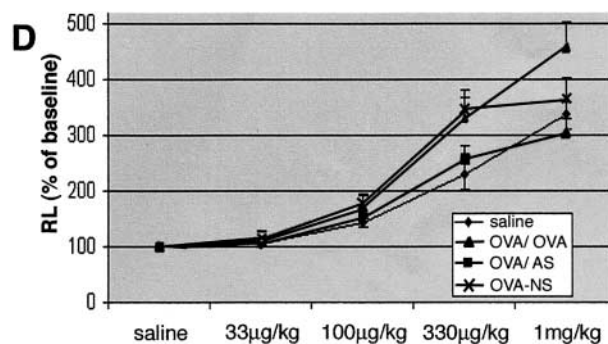
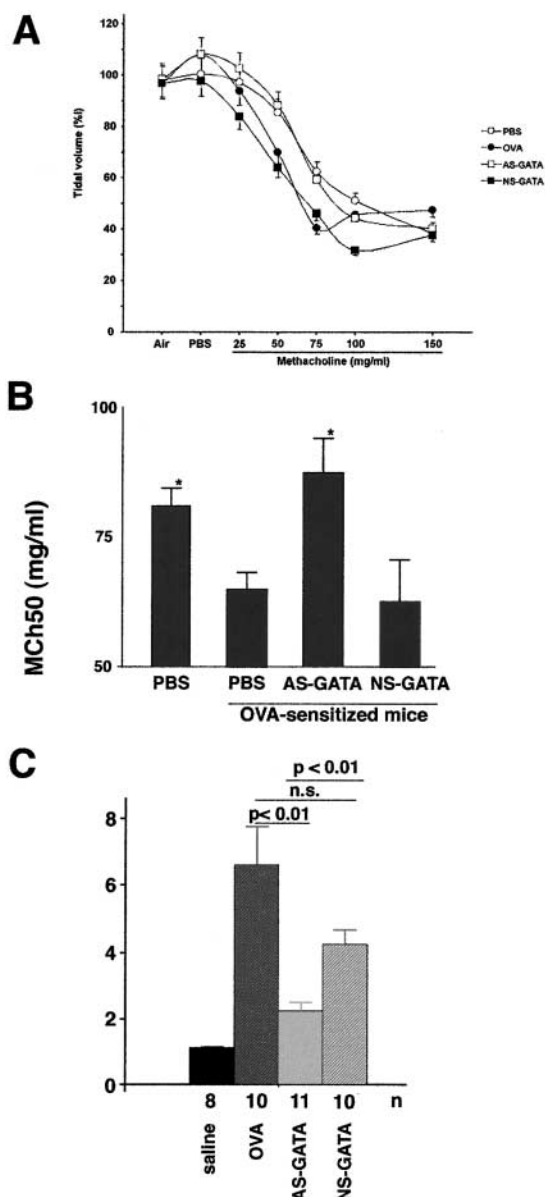


Figure 8. Assessment of airway reactivity in BALB/c mice after GATA-3 antisense treatment using three independent methods to assess airway hyperreactivity in vivo. (A) 12 BALB/c mice per group were sensitized to OVA accompanied by local treatment with OVA alone (PBS, OVA-sensitized mice), OVA followed by either GATA-3 antisense oligonucleotides (AS-GATA), or GATA-3 nonsense oligonucleotides (NS-GATA). Body plethysmography was performed 24 h after the last local treatment in all 12 mice per group as specified in Materials and Methods. Airway reactivity was measured in nonanaesthetized, spontaneously breathing mice simultaneously during MCh aerosol exposure. Basal values were measured (air), followed by measuring the response to aerosolized saline (PBS) and to increasing concentrations of MCh (25–150 mg/ml). Values expressed are mean \pm SEM of tidal volume (%). (B) Analysis of the MCh concentration that caused a 50% reduction in expiratory airflow (MCh50; reference 33) in the plethysmography experiments described in A. Data represent mean values \pm SEM. * $P < 0.05$. (C) Airway responsiveness in OVA- and saline-treated mice was assessed by analyzing PenH responses in a body plethysmograph. Mice were treated with saline ($n = 8$) or sensitized to OVA followed by local treatment with OVA alone (OVA; $n = 10$), GATA-3 antisense oligonucleotides (AS-GATA; $n = 11$) or GATA-3 nonsense oligonucleotides (NS-GATA; $n = 10$), as described above. Measurements of MCh responsiveness were made after exposure for 5 min to 200 mg/ml of aerosolized MCh and monitoring PenH. Results are expressed as the mean peak of PenH within 5 min after MCh treatment \pm SEM. Data were pooled from two independent experiments. Whereas treatment with antisense oligonucleotides to GATA-3 caused a significant reduction of PenH values ($P < 0.01$), treatment with nonsense control DNA did not have a significant effect. Furthermore, treatment of OVA-sensitized mice with antisense DNA led to a significant reduction of PenH values ($P < 0.01$) compared with nonsense, control-treated mice. (D) Analysis of RL in anesthetized, OVA-, or saline-treated mice by body plethysmography. Mice were treated with saline ($n = 8$) or sensitized to OVA followed by local treatment with OVA alone (OVA/OVA; $n = 11$), GATA-3 antisense oligonucleotides (OVA/AS; $n = 11$), or GATA-3-nonsense oligonucleotides (OVA/NS; $n = 12$). Body plethysmography was performed 24 h after the last local treatment in all mice. Dose–response curves to MCh were obtained after administering indicated doses of intravenous MCh. Data were pooled from two independent experiments and are expressed as mean values of RL \pm SEM. Multivariate analysis showed a significant increase of RL after OVA treatment compared with saline treatment ($P < 0.05$). Treatment with antisense

oligonucleotides to GATA-3 led to a significant suppression of RL values compared with untreated, OVA-sensitized mice ($P < 0.01$), whereas nonsense control DNA did not have a significant effect. Furthermore, treatment of OVA-sensitized mice with antisense DNA caused a significant suppression of RL values compared with nonsense-treated, OVA-sensitized mice ($P < 0.05$).

While GATA-1 and GATA-2 are involved in the development of the erythroid and megakaryocytic lineages, GATA-3 is highly expressed in early T lymphocytes and controls T cell receptor gene expression and thymocyte development (17, 14). Furthermore, within mature T cells GATA-3 was found to be selectively expressed in naive T cells and to control Th2 T cell development (18, 21, 40, 41). These data prompted us to determine the expression and functional role of GATA-3 in a murine model of asthma associated with Th2 cytokine production. We observed that OVA sensitization of mice caused a strong up-regulation of pulmonary GATA-3 expression. This finding led us to further investigate the functional role of this factor

using an antisense DNA strategy. We found that exposure of cultured spleen Th2 cells to an antisense GATA oligonucleotide suppressed IL-4 expression. This effect on IL-4 production could be augmented by MACS-based selection of antisense-transfected T cells using a cotransfection protocol with a plasmid expressing a truncated H2-K^k molecule. In the supernatant of antisense-treated T cells, we could not see any decrease in IL-9 production after treatment with antisense to GATA-3, possibly because the IL-9 promoter has no binding sites for GATA-3 (42). Using antisense oligonucleotides we then tested the potential pathogenic role of GATA-3 in the OVA-dependent asthma model in vivo. We observed that intranasal administration

of FITC-labeled GATA-3 antisense phosphorothioate oligonucleotides causes an efficient uptake in interstitial lung cells that coexpress large amounts of GATA-3 and IL-4 upon OVA sensitization. This uptake was followed by downregulation of lung GATA-3 expression (compared with OVA-sensitized mice without treatment) within 24 h after the last antisense administration. The specificity of the antisense effect on GATA-3 expression was confirmed according to several previously described criteria (43, 44): (a) control nonsense oligonucleotides did not have an effect on GATA-3 expression; (b) the antisense oligonucleotide had no effect on a nontargeted protein (SP-1, OCT-1); and (c) direct toxic effects of the GATA-3 antisense oligonucleotide were excluded by cell viability tests. Interestingly, mismatched control DNA led to a reduction of GATA-3 expression and IL-4 production in T cells, probably due to a remaining weak hybridization of the mismatched DNA to the GATA-3 target mRNA. However, these changes were less pronounced compared with the antisense DNA effect and did not reach statistical significance.

The antisense-induced suppression of GATA-3 expression was accompanied by a suppression of Th2 cytokine synthesis *in vitro* and *in vivo* and the accumulation of eosinophils in the airways, whereas treatment with control nonsense oligonucleotides had no such effects. Mucus occlusion of the airway lumen in the OVA-treated mice was present 24 h after the last intravenous OVA administration on day 28. Furthermore, *in vivo* noninvasive pulmonary function tests using body plethysmography showed that the OVA-immunized challenged mice reproducibly developed increased airway responsiveness to MCh that could be completely reversed by treatment with antisense DNA to GATA-3 but not by control nonsense DNA. This is an important novel information that highlights GATA-3 as a key regulator of both T cell effector function and airway hyperresponsiveness in allergic airway inflammation. It should be noted that the above finding on antisense-induced suppression of airway hyperresponsiveness was validated by two additional independent methods using assessment of PenH responses and of RL in anesthetized mice. All three methods demonstrated a significant downregulation of OVA-induced airway hyperresponsiveness upon administration of antisense oligonucleotides to GATA-3, definitively demonstrating for the first time a critical regulatory role of GATA-3 in airway hyperresponsiveness in allergic asthma.

The potential relevance of this GATA-3 antisense-based approach to inhibit Th2 cytokine production is underlined by various functional studies showing a key regulatory role for both IL-4 and IL-5 in asthma. For instance, studies in transgenic and knockout mice showed that the Th2 cytokine IL-4 is important for IgE mRNA production in allergic asthmatic reactions (45, 46). Furthermore, studies in a knockout model for the IL-4-dependent STAT-6 have shown that suppression of IL-4 signal transduction profoundly inhibits influx of eosinophils into the lung (47). Finally, suppression of IL-5 has been shown to prevent allergic inflammation in an animal model (48), possibly due to suppression of IL-5-dependent activation of eosinophils (49).

Recent *in vitro* and *in vivo* studies have shown that various transcription factors such as c-maf, nuclear factor of activated T cells c1-4, NIP 45, STAT-6, and GATA-3 are important for Th2-type cytokine production in T cells (14, 50). Here, we show that local GATA-3 antisense DNA treatment directly affects Th2 cytokine production and lung inflammation in asthma, although the oligonucleotides lack CpG motifs. These data support and significantly extend data from a very recent report (38) showing that expression of a dominant negative mutant of GATA-3 in T cells from transgenic mice leads to profound attenuation of OVA-induced eosinophilia and Th2 cytokine production. However, we demonstrate in this study for the first time that GATA-3 plays a role in the effector phase of allergic airway inflammation using mice with a normal immune system. Furthermore, we show that blockade of GATA-3 expression can be used as a therapeutic approach to suppress lung inflammation and mucus production in a murine model of asthma *in vivo*. Finally, we have found that GATA-3 is a critical factor for the maintenance of airway hyperresponsiveness in allergic airway inflammation.

Taken together, these data suggest that antisense DNA treatment to GATA-3 is a novel selective way to simultaneously suppress the production of several Th2 type cytokines in asthmatic reactions *in vivo*. Furthermore, we have demonstrated here that GATA-3 antisense DNA treatment of asthma is at least as effective as corticosteroid treatment, suggesting that antisense oligonucleotides to GATA-3 may be a novel molecular approach for the treatment of inflammatory disorders such as asthma in humans.

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