

Epicutaneous aeroallergen exposure induces systemic T_H2 immunity that predisposes to allergic nasal responses

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Background: Atopic individuals are predisposed to mounting vigorous T_H2-type immune responses to environmental allergens. The skin is often the first organ that manifests allergic disease and may provide an early entry point for antigen sensitization.

Objective: We sought to determine whether epicutaneous exposure to the aeroallergen *Aspergillus fumigatus* induces nasal allergic responses. Furthermore, we aimed to examine the mechanism involved.

Methods: Wild-type and signal transducer and activator of transcription 6 (STAT6)-deficient mice were exposed to epicutaneous *A. fumigatus* and control antigen ovalbumin. Nasal inflammation and responsiveness to methacholine were monitored.

Results: Exposure to epicutaneous *A. fumigatus* antigen induced a marked atopic dermatitis-like phenotype in a manner significantly more efficient than epicutaneous ovalbumin.

A single *A. fumigatus* intranasal challenge induced clinical nasal responses and hyperresponsiveness to methacholine in the nose as manifested by nasal symptoms, accompanied by allergic airway and nasal inflammation. Mechanistic analysis using gene-targeted mice revealed that the clinical nasal responses and hyperresponsiveness were STAT6-dependent. Although STAT6 was required for changes in nasal responses, it was not required for epicutaneous pathology except eosinophilia.

Conclusion: Epicutaneous exposure to the aeroallergen *A. fumigatus* potently primes for STAT6-dependent nasal responses. These results draw attention to the cooperative interaction between the nasal tract and skin.

Clinical implications: The skin is a potent site for antigen sensitization in the development of experimental allergic rhinitis. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergy, atopic, dermatitis, eosinophilia, epicutaneous, lung, nose, sensitization, STAT6

Despite intense ongoing research, there is currently an epidemic of atopic disease (atopic dermatitis [AD], allergic rhinitis, asthma, and food allergy) in the Western world.^{1,2} Elegant studies have convincingly demonstrated that childhood atopic disease often starts in the skin as AD and later progresses to the respiratory tract.^{3,4} Indeed, epidemiologic studies have found that the development of AD in childhood is a strong predictive factor for persistence of allergic disease into adulthood, but the exact reason for this association is unclear. Whether this association represents a genetic predisposition for both diseases or an etiologic connection has not been established. Notably, a recent clinical study has associated the development of peanut allergy with antigen exposure through abraded skin, further implicating antigen sensitization through atopic skin in early life.⁵ Well designed studies performed by Spergel et al and other groups⁶⁻⁹ have shown that epicutaneous exposure to the antigen ovalbumin under conditions that promote AD-like skin inflammation can prime for subsequent allergic responses in the lung. These results strongly support an etiologic link between the development of AD and subsequent atopic responses; however, whether this response is applicable to other antigens (eg, aeroallergens) and other end-organs (such as the nose) has not been well established.^{3,4,10} Understanding whether this is applicable to aeroallergens and nasal responses is important because the majority of patients with allergic rhinitis have pre-existing atopic disease, often involving the skin or lungs.^{3,4,11,12}

Experimentation in the allergy field has largely focused on analysis of the cellular and molecular events induced by allergen exposure in sensitized animals (primarily mice) and human beings. Although no animal model adequately mimics human disease, experimentation in animals has provided an experimental framework to dissect the key cells and molecules involved in disease pathogenesis. In these studies, mice are typically sensitized with antigen (eg, ovalbumin in the presence of the adjuvant

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Abbreviations used

AD: Atopic dermatitis
 AHR: Airway hyperresponsiveness
 Penh: Enhanced pause
 STAT6: Signal transducer and activator of transcription 6

alum) by intraperitoneal injection. Subsequently, mice are challenged by exposure to local allergen (eg, via intratracheal, intranasal, or nebulized routes for asthma models), and pathological responses are monitored.^{6,13-16} In other murine models, unsensitized mice are repeatedly exposed to mucosal allergens (eg, extracts of *Aspergillus fumigatus*) and eosinophilic inflammation of the lung develops with features that mimic human asthma.¹⁷ Recently, it has been demonstrated that T_H2-associated responses can be induced by repeated epicutaneous exposure to ovalbumin.^{7,8,18-20} Notably, in the absence of systemic priming with adjuvant, application of ovalbumin to shaved skin (via an epicutaneous patch) is able to promote local AD-like skin inflammation and prime for lung inflammation and airway hyperresponsiveness (AHR) after a single exposure to respiratory ovalbumin.^{7,8} Although ovalbumin is a convenient antigen for immunologic investigation, surprisingly, these results have not been extended to allergens that have greater clinical importance. Furthermore, whether epicutaneous antigen sensitization primes for allergic responses in the upper respiratory tract (nose) has not been addressed. This is particularly important because atopic disease often progresses from the skin to the nose, and finally to the lung, suggesting a mechanism that involves progression between these tissues.^{1,3,4,11,12}

We have previously shown that epicutaneous exposure to the aeroallergen *A fumigatus* induces AD-like inflammation and systemic T_H2 responses and primes for allergic responses in the lung and esophagus.²¹ In this study, we aimed to determine whether epicutaneous exposure to *A fumigatus* would prime for subsequent aeroallergen-induced allergic responses in the nasal tract. In addition, we aimed to dissect the mechanism involved, focusing on the role of the transcription factor signal transducer and activator of transcription 6 (STAT6) and associated T_H2 cytokines. Our results demonstrate that after a single respiratory antigen challenge, epicutaneous *A fumigatus* primes for allergic nasal responses. Although clinical nasal responses and eosinophilia were largely STAT6-dependent, cutaneous responses (eg, epithelial thickening) occurred independent of STAT6. These results are the first to demonstrate that cutaneous aeroallergen sensitization can prime for nasal allergic responses, and the first to link cutaneous sensitization to nasal allergy.

METHODS

Mice

BALB/c mice 4 to 8 weeks old and age-matched STAT6-deficient BALB/c mice (Jackson Laboratory, Bar Harbor, Me) were kept in a

specific pathogen-free environment. All procedures were performed in accordance with the ethical guidelines in the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Care and Use Committee approved by the Veterinary Services Department of the Cincinnati Children's Hospital Medical Center Research Foundation.

Epicutaneous antigen sensitization

Mice were sensitized as previously described with some modification.^{8,18} Briefly, mice were anesthetized with Isoflurane (IsoFlo; Abbott Laboratories, North Chicago, Ill), their backs shaved with an electric razor, and allergen applied via a 1 cm × 1 cm patch of sterile gauze that was previously immersed in a diluted allergen solution. The allergen solutions were made as follows: 100 µg ovalbumin (grade V; Sigma, St Louis, Mo) was diluted in 100 µL normal saline, and 100 µg *A fumigatus* antigen (Allergenic Extract; Hollister-Stier, Spokane, Wash) was diluted to 100 µL with normal saline. The allergen diluents consisted of 100 µL normal saline as a control for ovalbumin, or 100 µL 5% glycerin diluted with normal saline as a control for *A fumigatus*. The patch was secured by a Transparent Biocclusive Dressing (Johnson and Johnson Medical Inc, Arlington, Tex) and a Band Aid (Johnson and Johnson Medical Inc) for 1 week. After a rest period of 2 weeks (no patch), this procedure was repeated 3 times over the course of 7 weeks for ovalbumin sensitization, or repeated 2 times over the course of 4 weeks for *A fumigatus* sensitization. The experiments were performed with 4 groups: (1) ovalbumin group, (2) saline group (as the control for the ovalbumin group), (3) *A fumigatus* group, and (4) control group (as the control for the *A fumigatus* group).

Skin symptoms

During the course of sensitization, clinical skin symptoms were monitored and quantified 2 days after patch removal. One point was given for erythema, 1 point for 1 or 2 excoriations, 2 points for 3 or more excoriations, and 1 point for lichenification. On the basis of these criteria, a mouse with none of these abnormalities would receive a score of 0, whereas a mouse with all of these abnormalities, including at least 3 excoriations, would receive a score of 4.

Serum IgE and antigen specific IgG₁

Total serum IgE levels were measured by using BD OptEIA ELISA set (BD Biosciences, San Diego, Calif). The OD was immediately read at 450 nm. The IgE concentration of each sample was calculated by using a standard curve. Ovalbumin and *A fumigatus*-specific IgG₁ were measured as described previously by coating sample wells with 100 µL ovalbumin (100 µg/mL) and *A fumigatus* (50 µg/mL), respectively.²² The OD was read at 450 nm. Data are presented as the serum dilution required to obtain OD = 0.6.

In vivo cytokine production

Blood was taken from the lateral tail vein at day 24 in *A fumigatus* and control groups or at day 44 in ovalbumin and saline groups to measure serum cytokines. The *in vivo* cytokine capture assay was used to monitor *in vivo* production of IL-4 and IFN-γ as previously described.²³ Briefly, to capture secreted IL-4, mice were injected intravenously with 10 µg biotinylated antimouse IL-4 mAb (BVD4-1D11). Mice were bled 24 hours later, and serum levels of IL-4/biotin—anti-IL-4 complexes were determined by ELISA using microtiter plates (Immuron; Dynatech, Chantilly, Va) coated with a nonneutralizing anti-IL-4 mAb (BVD6-24G2.3), which recognizes an IL-4 epitope that is distinct from that recognized by BVD4-1D11. An *in vivo* cytokine capture assay for IFN-γ was

performed similarly by injecting mice with 10 μ g biotinylated anti-mouse IFN- γ mAb (R46-A2) and coating microtiter plates with nonneutralizing anti-mouse IFN- γ mAb, AN18. Streptavidin–horseradish peroxidase (Pierce, Rockford, Ill) followed by incubation with the chromotope SuperSignal ELISA femto-substrate (Pierce) was used to detect the bound complex, and then the plates were read for luminescence with a Fluoroskan Ascent FL luminescence microtiter plate reader (MTX Lab Systems, Vienna, Va). As standards, complexes of recombinant cytokines and biotin-labeled anti-cytokine mAb were used to calculate each cytokine concentration in the serum.

Airway challenge

Two days after the end of epicutaneous sensitization, mice were deeply anesthetized with isoflurane inhalation and given a single intranasal challenge with 25 μ L allergen (1 mg/mL ovalbumin or 1 mg/mL *A fumigatus*) or vehicle alone.

Measurements of AHR

Airway hyperresponsiveness was measured as the percent increase of airway responsiveness to methacholine from baseline readings, which was determined 24 hours after airway antigen challenge, by measuring enhanced pause (Penh), a dimensionless value, using whole-body plethysmography (BUXCO, Troy, NY) as previously described.²⁴ Baseline readings were averaged for 5 minutes after 3 minutes of inhalation of PBS (at the time points of preantigen challenge [0 hours] and 24 hours after antigen challenge). Aerosolized methacholine in increasing concentrations (3.125–25 mg/mL) was inhaled for 3 minutes. Then, Penh readings at each methacholine concentration were averaged for the subsequent 5 minutes. For evaluation of nasal hyperresponsiveness, the number of sneezes was measured during the 5-minute period after each dose of methacholine. The respiratory wave that is specific for sneeze was evaluated for each mouse; sneezing action was confirmed by visual observation.

Nasal symptoms

Twenty-four hours after airway challenge, mice were given another intranasal antigen challenge in a head-down posture without anesthesia, as previously described.²⁵ The number of sneezing and itching episodes (as nasal rubs) during the subsequent 5 minutes was counted.

Tissue processing and staining

Nasal and skin tissues were fixed in 10% formalin immediately after mice were euthanized. Paraffin-embedded tissues were cut into 5- μ m sections, and eosinophils and mast cells were identified by Diff-Quick stain (Fischer, Kalamazoo, Mich) used in a modified fashion after deparaffinization. Briefly, slides were fixed in acetone-methanol at room temperature for 5 min, stained in Diff-Quick solutions (Eosin Y and Blue), followed by a single wash in tap water, and a 1-minute exposure to methanol and xylene. Slides were embedded in the mounting medium (Cytoseal XYL; Richard-Allan Scientific, Kalamazoo, Mich), and differential cell count was performed under light microscopy.

Real-time PCR analysis

The RNA samples (500 ng) were subjected to reverse transcription analysis using Iscript reverse transcriptase (Bio-Rad Laboratories, Hercules, Calif) according to the manufacturer's instructions. IL-18, IL-13, IL-13 receptor (R) α 1, IL-13R α 2, and IFN- γ were quantified by real-time PCR using the LightCycler instrument and LightCycler FastStart DNA master SYBR green I as a ready-to-use reaction mix (Roche Applied Science, Indianapolis, Ind). cDNA

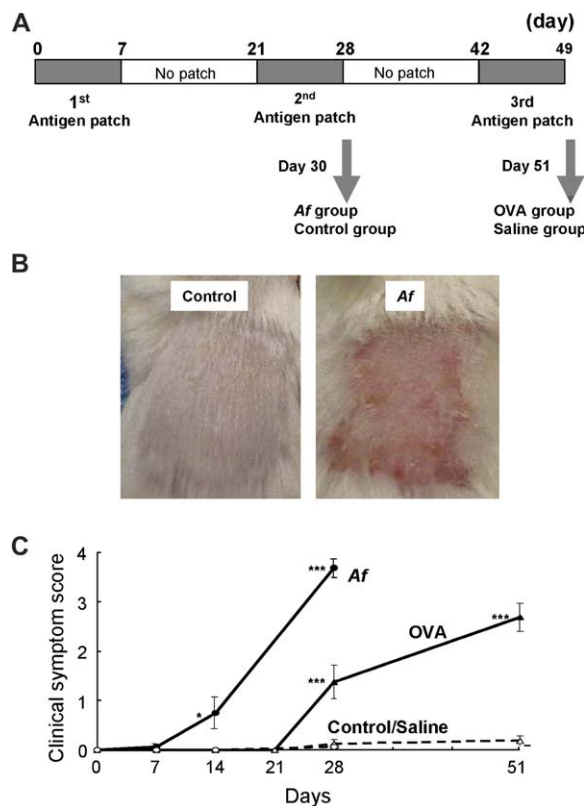


FIG 1. Sensitization protocol and subsequent clinical skin change in *A fumigatus* (Af)-sensitized mice. Mice were sensitized with allergen (Af or ovalbumin [OVA]) or their control solutions by application of an antigen gauze patch. Mice had a 1-week exposure to the antigen, then a 2-week rest interval before the next patch. The Af and control groups had 2 sets of patches with analysis on day 30, whereas the OVA and saline groups had 3 sets of patches with analysis at day 51 (A). Significant inflammatory skin changes were observed in Af group but not in control group (B). During the sensitization protocol, the clinical abnormality score was based on eczema, lichenification, and excoriations (C). The Af group developed skin abnormalities more rapidly than the OVA group. The saline group (saline) or control group (control) did not develop significant skin changes. A representative experiment is shown ($n = 8$ per group). * $P < .05$, *** $P < .001$, compared with control group.

were amplified by using the following primers: mL-13R α 1: catctacaggaccagagga and aagctgagcattctcccttc; mL-18: actgtacaaccgcagataacgg and agtgaacattacagattatccc; mL-13: taactactcaaacgaccgcataat and cttcactccaatcactccac; mL-13R α 2: ggactcatcagactataaagatt and gtgtctcctcatttcaattca; GAPDH: tggaaatcccatccatct and gtctctgggtggcagtgat. For each gene, a standard curve corresponding to a dilution of the respective cDNA was used. Results were normalized to GAPDH amplified from the same cDNA mix and expressed as fold induction compared with the wild-type control group.

Statistical analysis

For all cell counts, stained slides were read randomly and in a blind fashion. The nonparametric Mann-Whitney U test was used for comparison of data between groups, whereas ANOVA was used to evaluate the differences in clinical symptoms, nasal differential cell counts, and airway hyperresponsiveness measurements.

TABLE I. Cutaneous allergic inflammation in wild-type and STAT6-deficient mice (mean $\pm$ SEM, cell count in subepithelial region/mm²)

	Eosinophils	Mast cells	Neutrophils	Mononuclear cells
Wild-type control	6.05 $\pm$ 2.06	69.8 $\pm$ 10.2	24.7 $\pm$ 7.20	34.5 $\pm$ 7.50
Wild-type Af	91.8 $\pm$ 16.0**	191 $\pm$ 25.6*	104 $\pm$ 25.5*	210 $\pm$ 15.9***
STAT6KO Af	14.6 $\pm$ 3.94†	143 $\pm$ 32.5*	149 $\pm$ 24.1**	166 $\pm$ 24.1***

Af, *A fumigatus*.* $P < .05$, ** $P < .01$, *** $P < .001$, compared with wild-type control.† $P < .01$, compared with wild-type Af group.

RESULTS

Clinical symptoms and inflammatory changes after epicutaneous aeroallergen exposure

We were interested in determining whether epicutaneous administration of an aeroallergen was sufficient for induction of atopy. Previous studies have shown that epicutaneous exposure to the food antigen ovalbumin induces AD-like pathological changes in the local skin site.^{6-9,19} To determine whether the ability of ovalbumin to induce T_H2 responses was unique to this antigen, we examined the ability of *A fumigatus* to induce atopic sensitization and disease. For comparison, we also analyzed ovalbumin-treated and saline-treated control mice (Fig 1, A). The mice in both the *A fumigatus* and ovalbumin groups developed skin erythema, excoriation, and lichenification (Fig 1, B, and data not shown). As shown in Fig 1, C, skin symptoms after *A fumigatus* exposure developed more rapidly and severely than in mice exposed to ovalbumin; therefore, the *A fumigatus* protocol was continued for only 4 weeks, whereas the ovalbumin protocol was continued for 7 weeks as previously reported.^{8,19} Histologic analyses revealed eosinophil and mast cell accumulation in the *A fumigatus* group at the end of the 4-week protocol (Table I) consistent with our previous publication.²¹ Also, epithelial thickness increased from 33.5 μ m $\pm$ 4.5 μ m in the *A fumigatus* control group to 90.3 μ m $\pm$ 4.2 μ m in the *A fumigatus* group (means $\pm$ SEMs; $n = 8$; $P < .01$) and from 32.1 μ m $\pm$ 3.1 μ m in the ovalbumin control group to 66.0 μ m $\pm$ 15.1 μ m in the ovalbumin group (means $\pm$ SEMs; $n = 8$; $P < .01$). These results indicate that the aeroallergen *A fumigatus* more rapidly induces AD-like clinical inflammatory skin changes than ovalbumin.

Systemic responses after epicutaneous aeroallergen exposure

We next characterized the systemic responses associated with epicutaneous exposure to *A fumigatus*. Total serum IgE and *A fumigatus*-specific IgG₁ were significantly increased after *A fumigatus* exposure (Fig 2, A and B) consistent with our previous publication.²¹ To examine further the T_H2 response induced by *A fumigatus*, we measured *in vivo* IL-4 and IFN- γ production by an *in vivo* cytokine capture assay.²³ Notably, *A fumigatus* induced significant increases in IL-4, but not IFN- γ production, as measured by captured IL-4 and IFN- γ concentrations in the serum (Fig 2, C and D).

Taken together, these data demonstrate that epicutaneous exposure to *A fumigatus* induces a systemic T_H2 response as measured by antigen-specific serum IgG₁ and total IgE levels.

Epicutaneous aeroallergen exposure primes for allergic nasal responses

Previous studies have shown that epicutaneous exposure to ovalbumin primes for lung hyperresponsiveness, but the specific effect on hyperresponsiveness in the nose was not addressed.^{7,8} We were thus interested in determining whether epicutaneous *A fumigatus* followed by intranasal *A fumigatus* had an effect on the nose. After epicutaneous exposure to *A fumigatus* and respective intranasal allergen challenge, as shown in Fig 3, A, significant nasal symptoms were observed in the *A fumigatus* group compared with the control group. The number of sneezes/5 min was 10.6 $\pm$ 2.2 in the control group and 42.4 $\pm$ 7.5 in the *A fumigatus* group (means $\pm$ SEMs; $n = 8$; $P < .001$); the level of itching/5min was 3.8 $\pm$ 0.6 in the control group and 10.8 $\pm$ 1.6 in the *A fumigatus* group (means $\pm$ SEMs; $n = 8$; $P < .01$). For the evaluation of nasal responsiveness, sneezes were measured after a full dose range of methacholine. Notably, allergen sensitization promoted hyperresponsiveness to methacholine as assessed by nasal symptoms (Fig 3, B). Associated with these clinical changes, significant nasal inflammation was detected by histologic analysis, which was accompanied by eosinophilia, compared with the control group (the number of eosinophils/mm² in the nasal mucosa was 69 $\pm$ 48 in the control group and 3270 $\pm$ 750 in the *A fumigatus* group; means $\pm$ SEMs; $n = 8$; $P < .001$). We next aimed to analyze the development of AHR. We chose analysis by whole-body plethysmography because this would detect changes in the upper respiratory tract (nose) compared with methods that involve intubation of mice. However, it is important to note that the physiological significance of whole-body plethysmography (Penh) in mice is uncertain. Notable AHR was observed in *A fumigatus*-sensitized mice after intranasal *A fumigatus* exposure (Fig 3, C). As a control, AHR was not induced in *A fumigatus*-sensitized mice after the control challenge or in unsensitized control mice. *A fumigatus* was significantly more effective at inducing AHR than ovalbumin at all doses of methacholine between 3.125 and 25 mg/mL ($P < .05$; data not shown). Clinical symptoms (number of sneezes) after a single nasal antigen *A fumigatus* challenge are shown in Fig 3, D.

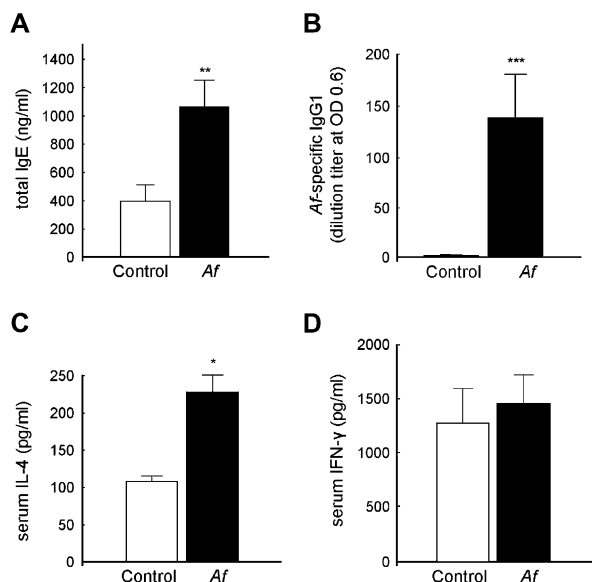


FIG 2. Systemic T_H2 responses after epicutaneous *A fumigatus* (Af) sensitization. The total serum IgE (A), antigen-specific IgG₁ (B), IL-4 (C), and IFN- γ (D) on day 30 of the Af protocol are shown. Values for Af or control sensitized mice are shown. * $P < .05$, ** $P < .01$, *** $P < .001$, compared with control group. A representative experiment is shown (n = 8 per group).

Role of STAT6 after epicutaneous allergen exposure

After epicutaneous *A fumigatus* exposure, mice deficient in STAT6 developed comparable clinical skin abnormalities compared with wild-type mice (Fig 3, A). However, they were completely protected from methacholine induced AHR (Fig 3, B), nasal hyperresponsiveness (Fig 3, C), and clinical nasal symptoms (Fig 3, D). STAT6 was nearly completely required for eosinophil accumulation but not accumulation of neutrophils, mast cells, or mononuclear cells (Fig 3, E and F; and data not shown). Furthermore, serum levels of antigen-specific IgG₁ and total IgE were diminished in STAT6-deficient mice (both lower than the detection limit).

Role of T_H2 -associated cytokines and signaling molecules

IL-18 has been shown to induce T_H2 cytokines in the absence of STAT6.²⁶ We thus examined its expression using real-time PCR in the skin (Fig 4, A) of WT and STAT6 deficient mice (KO) in the control and *A fumigatus* groups. A significant difference was observed between the *A fumigatus* and control groups in both wild-type mice and the STAT6-deficient mice. To assess further the signaling molecules involved, we examined the expression of IL-13 and its receptor components IL-13R α 1 and IL-13R α 2. IL-13 was induced by allergen, and this was primarily STAT6-dependent (Fig 4, B). In contrast, IL-13R α 2 was allergen-induced, and this involved both STAT6-independent and STAT6-dependent pathways (Fig 4, C). IL-13R α 1 and IFN- γ were primarily only induced in the absence of STAT6 (Fig 4, D and E).

DISCUSSION

Allergic responses in the skin (AD) often precede allergic responses in other tissues (such as the respiratory tract), yet the reason for this sequence of events is not clear. In this study, we have demonstrated that repeated exposure to a natural aeroallergen *A fumigatus* can induce AD-like skin changes and a subsequent allergic response in the upper respiratory tract. After a single intranasal allergen challenge, epicutaneous aeroallergen sensitization primes for the development of nasal and airway inflammation (mainly eosinophilia and neutrophilia) and the development of AHR to methacholine. We present evidence that AHR involves nasal hyperresponsiveness, at least in part. Because whole-body plethysmography measurements do not distinguish upper from lower airway responses, we cannot identify the specific region of the respiratory tract responsible for the AHR. Nevertheless, epicutaneous sensitization potently primes for nasal eosinophilia (>40-fold). Our report is the first to identify that epicutaneous sensitization can prime for clinical nasal hyperresponsiveness to methacholine. Our data draws attention to the potential role of epicutaneous sensitization in the development of simultaneous nasal and pulmonary inflammation, a process that occurs in most asthmatics.¹²

To discern the efficacy of *A fumigatus* as a sensitizing agent, we also examined a comparative antigen (ovalbumin), which has been previously investigated in studies of epicutaneous allergen sensitization.^{6-8,18} The allergic phenotype found with *A fumigatus* was also found with ovalbumin, although *A fumigatus* had a greater ability to promote allergic responses. This was especially significant for the clinical skin changes and the subsequent

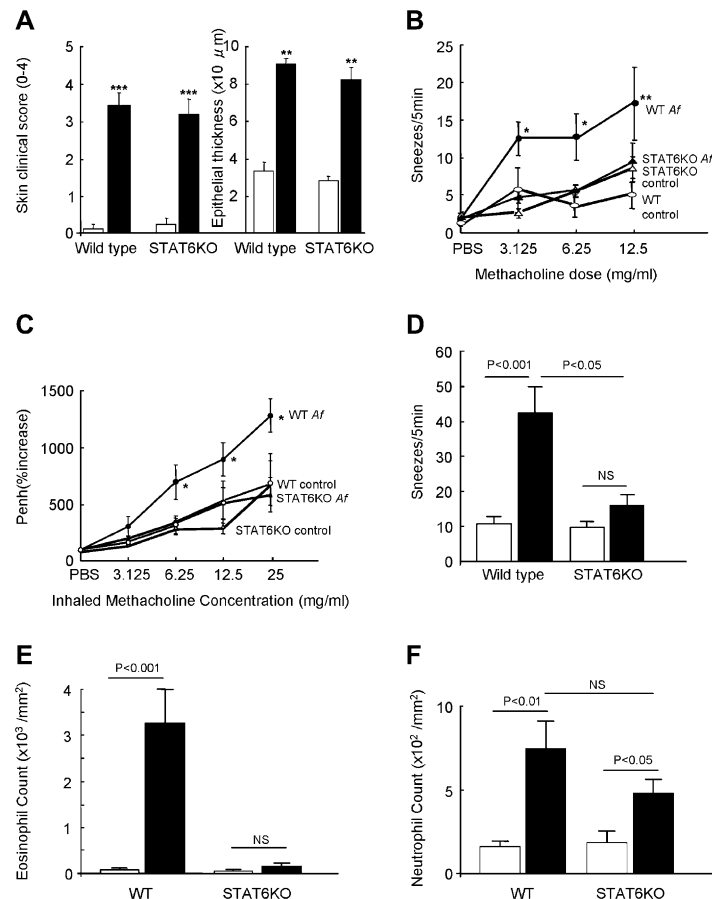


FIG 3. Skin changes and subsequent lower and upper allergic airway responses in epicutaneous sensitized mice in wild-type mice and in STAT6 deficient mice. After epicutaneous *A fumigatus* (Af) exposure, Af group in wild-type mice developed significant clinical skin changes and epithelial thickness (A), methacholine-induced nasal hyperresponsiveness (B), airway hyperresponsiveness (C), clinical nasal symptoms (D), and nasal mucosal eosinophil (E) and neutrophil (F) counts 24 hours after intranasal Af challenge compared with control group of vehicle exposure. Mice deficient in STAT6 developed comparable clinical skin changes compared with wild-type mice and also had significant epithelial thickening (A) and increased nasal neutrophil count (F). However, they were completely protected from nasal hyperresponsiveness (B), airway hyperresponsiveness (C), clinical nasal symptoms (D), and nasal eosinophilia (E). Open bars represent the control group and solid bars the Af group (A and D-F); in B and C, open circles and solid circles represent the control group and the Af group, respectively, of wild-type mice, and open triangles and solid triangles represent the control group and the Af group, respectively, of STAT6-deficient mice. * $P < .05$, ** $P < .01$, *** $P < .001$ compared with each control group. A representative experiment is shown ($n = 8$ per group).

airway responses. Although the mechanism that differentiates their effectiveness in developing systemic allergic responses is unclear, it may be related to the unique characteristics of *A fumigatus*, which has an affinity to organs of the skin, sinuses, and airway.²⁷ It has also been reported that *A fumigatus* contains a unique p2 protein, which is recognized by IgE antibodies from *A fumigatus*-sensitized individuals with allergy.²⁸

We were interested in determining the role of STAT6 signaling in the induction of epicutaneous sensitization and subsequent allergic responses in the skin and nasal tract. This was a relevant objective for several reasons. First, select aspects of *A fumigatus*-induced responses have been shown to be STAT6-independent, but this has not been addressed in the context of a cutaneous

sensitization model. Second, recent studies have shown that immune responses in the lung are distinct from other tissues because of a unique innate dendritic cell population²⁹; thus, it would be important to compare responses in multiple end-organs (skin, nose, and lung). Finally, it was important to determine which cell types correlated with clinical symptoms. Notably, the eosinophilia in the skin and nasal mucosa was suppressed in mice deficient in STAT6, and only the AHR (and not epithelial thickening) was STAT6-dependent. Thus, although the mechanisms that regulate cutaneous and nasal eosinophilia after epicutaneous antigen sensitization are conserved between ovalbumin and *A fumigatus*, skin pathology occurs primarily independent of eosinophils after epicutaneous ovalbumin or *A fumigatus*. Recent studies suggest that

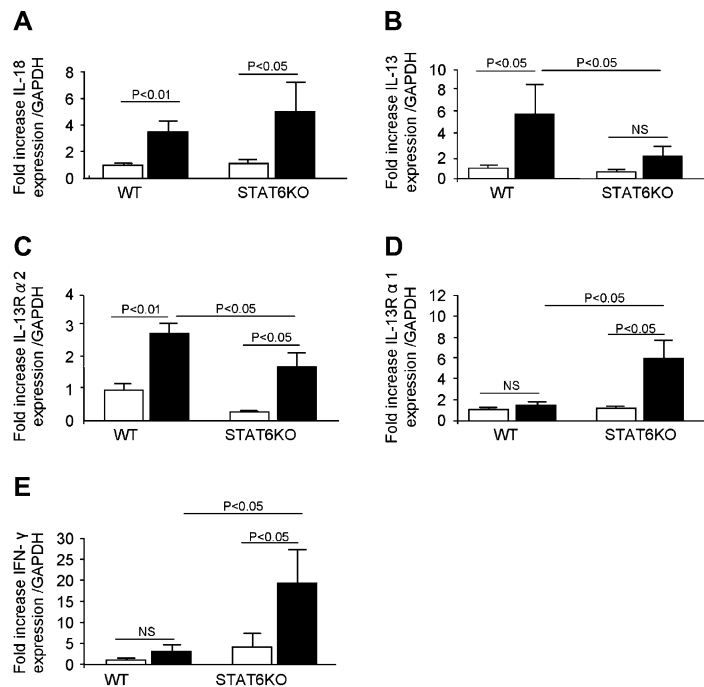


FIG 4. Subsequent nasal inflammation in T_H2 -deficient mice. Each mRNA of IL-18 (**A**), IL-13 (**B**), IL-13R α 2 (**C**), IL-13R α 1 (**D**), and IFN- γ (**E**) was analyzed using real-time PCR in the skin of WT and STAT6-deficient mice (KO) of the control group (open bar) or the *A fumigatus* group (solid bar). Results are expressed as fold increased mRNA expression normalized to GAPDH. A representative experiment is shown (n = 4-7 per group).

the STAT6 pathway is not required to elicit accumulation of neutrophils or lymphocytes in the lung or for peribronchial fibrosis.³⁰⁻³² Indeed, in the absence of T_H2 signaling, T_H1 cells might be vigorously activated and responsible for inducing the T_H2 -independent asthmatic responses.³³ Indeed, we were able to demonstrate overproduction of IFN- γ in the absence of STAT6 (Fig 4, E). As such, several studies have shown coinvolvement of T_H1 and T_H2 cells in the pathogenesis of allergic responses.³⁴⁻³⁸ It remains to be determined whether neutrophils are responsible for the induction of the cutaneous allergic responses.

Recent studies have implicated the involvement of IL-18 in the development of IgE-independent AD-like disease.²⁶ Indeed, we were able to demonstrate overproduction of IL-18 in wild-type and STAT6-deficient mice (Fig 4, A). The involvement of IL-18 in our experimental model of AD deserves further investigation, especially in view of the apparent STAT6-independent pathway for the development of AD.

The role of IL-13 signaling in human atopic dermatitis has recently drawn attention.³⁹ In addition, skin keratinocytes have been shown to express IL-13 receptors and produce T_H2 -associated chemokines such as eotaxin-3 in response to IL-13 signaling.⁴⁰⁻⁴² Although it has been appreciated that IL-13R α 1 is a positive signaling receptor, recent attention has been focused on IL-13R α 2 having an immune regulatory role.⁴³ In particular, several cytokines (including IL-13 and IFN- γ) have been shown to induce expression of a membrane bound and soluble form of IL-13R α 2.^{43,44} IL-13R α 2 has been generally

considered to be a decoy receptor,⁴³ but a recent compelling study has provided evidence that this receptor may trigger the signaling involved in IL-13-induced fibrosis.⁴⁵ It is interesting to speculate that the skin thickening observed in the absence of STAT6 may be mediated by IL-13R α 2, at least in part. In our studies, regulation of IL-13R α 2, but not IL-13R α 1, was at least in part STAT6-independent, supporting a differential role for IL-13R α 2. The role of IL-13 and its receptors as well other receptor signaling molecules such as phosphatidylinositol 3-kinase^{46,47} (implicated in T_H2 -associated responses) in disease induction will be addressed in future studies.

In conclusion, first, we establish that the aeroallergen *A fumigatus* can elicit a strong systemic T_H2 response (notably more efficient than ovalbumin) when delivered by epicutaneous exposure. Second, we demonstrate that cutaneous sensitization with *A fumigatus* potently primes for allergic nasal responses. We establish that eosinophilia (but not mastocytosis or neutrophilia) is regulated by STAT6 after epicutaneous sensitization. We demonstrate that *A fumigatus*-induced skin pathology occurs largely independent of eosinophils, T_H2 -associated cytokines, and immunoglobulins. Taken together, these results draw attention to the potential role of epicutaneous aeroallergen sensitization in the development of systemic allergic disease. These results support a connection between the skin and nasal tract and suggest that the skin is an efficient site for antigen sensitization. As such, these results provide a potential mechanistic basis for the co-occurrence of nasal and cutaneous allergic disease in atopic states.

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