

ALLERGEN INDUCED TFF2 IS EXPRESSED BY MUCUS-PRODUCING AIRWAY EPITHELIAL CELLS BUT IS NOT A MAJOR REGULATOR OF INFLAMMATORY RESPONSES IN THE MURINE LUNG

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□ *Asthma is a complex pulmonary disorder characterized by reversible airflow obstruction, airway hyperresponsiveness, mucus cell metaplasia, and inflammation. Employing animal models of pulmonary inflammation induced by different allergens and Th2 cytokines, the authors have previously described the up-regulation of trefoil factor 2 (TFF2) in the lung. Given the known biological role of trefoil factors in epithelial restitution, it has been postulated that allergen-induced TFF2 might have an important role in asthmatic responses. Here the authors show that TFF2 is induced early and maintained for 2 weeks following allergen challenge in the mouse lung. In situ mRNA hybridization demonstrated expression of TFF2 primarily in a subset of bronchial epithelial cells and TFF2 immunohistochemistry identified expression in alcian blue-positive bronchial epithelial cells. TFF2 gene-deleted mice inoculated with allergen displayed a 10-fold increase in total cellularity compared with saline controls. Although this response was modestly attenuated compared to wild type controls, the loss of TFF2 did not affect gross levels of tissue inflammation. Furthermore, the loss of TFF2 did not affect induction or resolution of mucus cell metaplasia as measured by periodic acid-Schiff (PAS) or alcian blue staining. Thus, TFF2 is an allergen-induced gene,*

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which is expressed in mucus-positive airways, but is not a major contributor to allergen-induced goblet cell metaplasia, mucus production, or inflammatory responses in the lung.

Keywords allergy, asthma, cytokines, mucus, trefoil factor

There is currently an epidemic of asthma and other allergic diseases in the western world and the incidence is on the rise [1]. Experimentation in the asthma field has largely focused on analysis of the cellular and molecular pathways induced by allergen exposure in sensitized animals and humans. These studies have identified elevated production of immunoglobulin E (IgE), mucus hypersecretion, airway obstruction, inflammation, and enhanced bronchial reactivity to spasmogens in the asthmatic response [2–4]. Although these studies have also provided the rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways [5, 6], the development of the asthma phenotype is likely to be related to the complex interplay of a large number of additional genes, and their polymorphic variants [7, 8]. Accordingly, we have identified a panel of genes involved in the pathogenesis of experimental asthma using an empiric approach employing DNA microarray analysis of whole-lung RNA [9].

This article focuses on one such gene that encodes for trefoil factor family peptide (TFF)2. Trefoil peptides are small (7- to 12-kDa) protease-resistant proteins composed of a characteristic 3-loop structure formed by 3 conserved cysteine disulfide bonds and secreted by the gastrointestinal mucosa in a lineage-specific manner [10–13]. TFF2, also known as spasmolytic polypeptide, is expressed in the stomach (and to a lesser extent in the proximal duodenum and biliary tract), whereas TFF1 and TFF3 are expressed and secreted predominantly by gastric pit cells and intestinal goblet cells, respectively. Trefoil factors are critically involved in responses to intestinal injury, primarily by their ability to promote the rapid spreading and migration of existing epithelial cells following injury (restitution) [11]. Although expressed and secreted preferentially by gastric mucus neck cells, TFF2 is up-regulated in diverse pathologic conditions of the gastrointestinal tract including ulceration, inflammatory bowel disease, *Helicobacter pylori* infection, and by injury promoted by nonsteroidal anti-inflammatory drugs [10, 11]. In these conditions, TFF2 is thought to regulate acid production, stabilize the mucin gel layer (by directly interacting with mucin proteins), promote healing, and alter immune response, as supported by recent studies in TFF2-deficient mice [14, 15].

We have previously shown induction of TFF2 in the lungs of mice during allergic airway inflammation triggered by allergens or T helper 2 lymphocyte (Th2) cytokine overexpression [16]. TFF2 has also been identified by microarray analysis as one of only 5 genes up-regulated at least

2-fold in response to ovalbumin (OVA), *Aspergillus fumigatus* (ASP), and trimellitic anhydride (TMA), a small-molecular-weight organic acid that is a known cause of occupational asthma [17]. Furthermore, TFF2 has recently been identified to be up-regulated in the lungs of patients with asthma [18]. Given the known role of TFF2 in diverse responses that are hallmarks of allergic inflammation, it has been speculated that TFF2 is likely to be involved in these responses [13, 19–22], yet this has not been tested. In this study, we examine the consequences of TFF2 deficiency on allergic lung responses.

METHODS

Allergen-Induced Model of Allergic Lung Inflammation

TFF2 (–/–) mice were generated and backcrossed into a BALB/c background. The F4 (–/–) mice were then bred continuously to establish the gene deleted line and verified by PCR. The F4 wild-type mice were bred to generate the wild-type controls in the same genetic background. All mice were maintained under specific pathogen-free conditions. The OVA model of allergic lung disease was established by sensitizing mice with intraperitoneal injection of 50 µg OVA and 1.0 mg alum in 200 µL sterile physiologic saline on 2 occasions separated by 2 weeks. Two weeks after the last sensitization, 2 intranasal doses of 50 µg OVA 3 days apart were administered (Figure 1A). Briefly, mice were lightly anesthetized with isoflourane inhalation, held in a supine position, and 50 µL of normal saline containing 50 µg OVA was applied to the nares using a micropipette. After the liquid was aspirated the mice were held upright until alert. Mice were sacrificed between 18 and 20 hours after the second intranasal challenge. The *Aspergillus fumigatus* antigen (ASP) model of allergic lung disease was established by repeated intranasal doses of ASP 3 times a week for 3 weeks as described previously [23] (Figure 1A).

BALF Collection and Analysis

The mice were euthanized by intraperitoneal injections of 0.2 mg/kg Ketaject (ketamine hydrochloride; Phoenix Pharmaceuticals, St Joseph, MO). Immediately thereafter a midline neck incision was made and the trachea was cannulated. The lungs were lavaged twice with 0.7 mL phosphate-buffered saline (PBS). The recovered bronchoalveolar lavage fluid (BALF) was centrifuged at $500 \times g$ for 5 minutes at 4°C, and resuspended in 200 µL PBS. Lysis of red blood cells was carried out utilizing red blood cell (RBC) lysis buffer (Sigma, St. Louis, MO) according to the manufacturer's recommendations. Total cell numbers were counted with a hemacytometer. Cytospin preparations of 5×10^4 cells were stained with

Giemsa-Diff-Quick (Dade Diagnostics of P.R., Aguada, PR) and differential cell counts were determined.

RNA Isolation and Northern Blot Analysis

For Northern blot analysis, RNA extracted from the right lobe of the lung from each mouse was homogenized using Trizol (Invitrogen, Carlsbad, CA) reagent as per the manufacturer's instructions. RNA (10 µg) was electrophoresed through 1.5% agarose, and transferred to a GeneScreen hybridization membrane (Perkin Elmer, Boston MA). Hybridization was performed with ³²P-labeled cDNA encoding the sequence-confirmed murine TFF2 I.M.A.G.E. clone 438574 (obtained from American Type Tissue Culture Collection, Rockville, MD), and washed under high stringency conditions.

Mucus Production Analysis

Lung tissue samples were inflation fixed with Accustain neutral buffered 10% formalin (Sigma-Aldrich, St. Louis, MO), embedded in paraffin, cut into 5-µm sections, and fixed to polysine slides (Eire Scientific, Portsmouth, NH). Alcian blue staining (Poly Scientific R&D, Bay Shore, NY) was performed on the tissue sections according to the manufacturer's recommendations. The lung sections were taken from the same lobe in each mouse and at least 3 to 4 random sections per mouse were analyzed in a blinded manner. Airway mucus levels were determined by counting mucus-positive airway cells as a percentage of total cells in a blinded manner. Periodic acid-Schiff (PAS) staining (Poly Scientific R&D) was performed on the tissue sections according to the manufacturer's recommendations.

TFF2 Staining in Tissue

Lung tissue samples were inflation fixed with Accustain neutral buffered 10% formalin (Sigma-Aldrich) at a pressure of 25 cm H₂O and immersed in the same fixative overnight, embedded in paraffin, cut into 5-µm sections, and fixed to polysine slides (Eire Scientific). TFF2 protein in the tissue was detected using a rabbit antiserum against human TFF2, which has previously been shown to cross-react with mouse and rat TFF2 [15, 24]. In brief, tissue endogenous peroxidase was quenched with 0.3% hydrogen peroxide in methanol followed by nonspecific protein blocking with normal goat serum. Antigen recovery of tissue sections was performed by microwaving slides in carbonate buffer for 5 minutes (3×). Tissue sections were then incubated with rabbit anti-TFF2 antibody (1:1000) overnight at 4°C, followed by 1:500 dilution of biotinylated goat anti-rabbit IgG secondary antibody and avidin-peroxidase complex (Vector Laboratories) for 60 minutes.

These slides were further treated with nickel diaminobenzidine (DAB)–cobalt chloride solution to form a black precipitate. This was followed by alcian blue staining (Poly Scientific R&D) of the tissue sections according to the manufacturer's recommendations and counterstaining with nuclear fast red.

Clinical Scoring of Lung Inflammation

Lung tissue samples were inflation fixed with Accustain neutral buffered 10% formalin (Sigma-Aldrich) at a pressure of 25 cm H₂O and immersed in the same fixative overnight, embedded in paraffin, cut into 5- μ m sections, and fixed to polysine slides (Eire Scientific). H&E staining (Poly Scientific R&D) was performed on the tissue sections according to the manufacturer's recommendations. The lung sections were taken from the same lobe in each mouse and at least 3 to 4 random sections per mouse were analyzed. A clinical score of 0 to 4 was assigned to each section in a blinded manner (0 = no inflammation; 1 = mild; 2 = moderate; 3 = severe but not in all airways; 4 = severe in all airways).

Analysis of Epithelial Integrity

Lung tissue samples were inflation fixed with Accustain neutral buffered 10% formalin (Sigma-Aldrich) at a pressure of 25 cm H₂O, immersed in the same fixative overnight, embedded in paraffin, cut into 5- μ m sections, and fixed to polysine slides (Eire Scientific). H&E staining (Poly Scientific R&D) was performed on the tissue sections according to the manufacturer's recommendations. The lung sections of allergen or saline challenged lungs were taken from the same lobe in each mouse 18 hours following the last intranasal challenge. At least 3 to 4 random sections per mouse were analyzed under high power magnification (400 $\times$) in a blinded manner. An examination of both proximal and distal airways for erosions and/or gaps in gross epithelial continuity was performed.

Analysis of Epithelial Cell Proliferation

In order to determine the degree of epithelial cell proliferation, 5'-bromodeoxyuridine (5'-BrdU) (Zymed Laboratories, South San Francisco, CA) incorporation analysis was performed. Saline or OVA-treated mice were injected intraperitoneally with 0.25 mL of 5'-BrdU 2 hours before sacrifice. The lungs were fixed with 10% neutral-buffered formalin (Sigma, St. Louis, MO) for 24 hours. After fixation, the tissue was embedded in paraffin and 5- μ m sections were processed using standard histological approaches. Tissues were digested with trypsin (0.125%) for 3 minutes at

37°C followed by incubation for 30 minutes at room temperature. Sections were washed with PBS 3 times for 2 minutes and further incubated with monoclonal biotinylated anti-BrdU antibody (Zymed Laboratories) for 60 minutes at room temperature. Negative controls included replacing the primary antibody with PBS, and positive controls were provided by the manufacturer. BrdU nuclear incorporated cells were detected with streptavidin-peroxidase and diaminobenzidine (DAB) substrate (Zymed Laboratories) followed by counterstaining with hematoxylin.

In Situ Hybridization of Mouse Lung

In situ hybridization was performed as described [25–27]. In brief, the murine TFF2 cDNA from I.M.A.G.E. clone 438574 was obtained (American Type Tissue Culture Collection) and the sequence verified. The pT7T3D-PAC plasmid was linearized by restriction enzyme digest with Not I or EcoRI, and sense and antisense probes were generated by T7 and T3 RNA polymerase, respectively (Riboprobe Gemini Core System II transcription kit; Promega, Madison, WI). The radio-labeled [α S35-UTP] probes were hybridized and washed under high-stringency conditions.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation. Statistical significance comparing different sets of mice was determined by Student's *t* test.

RESULTS

Northern Blot Analysis Demonstrates TFF2 Induction by Allergen

In order to determine the time course of TFF2 expression following allergen challenge in the lung, we subjected OVA/alum sensitized mice to 2 OVA intranasal challenges and monitored TFF2 expression in lung RNA. Northern blot analysis revealed that TFF2 expression was markedly increased by 2 hours following the second OVA challenge compared with mice challenged with saline (Figure 1B). Subsequent kinetic analysis revealed that TFF2 expression remained induced through 2 weeks and eventually decreased to background levels at four weeks following OVA administration (data not shown).

TFF2 In Situ Hybridization Reveals Expression of TFF2 in Airway Epithelial Cells

To address the distribution of TFF2 mRNA-positive cells in the allergic lung, we performed in situ hybridization for TFF2 mRNA.

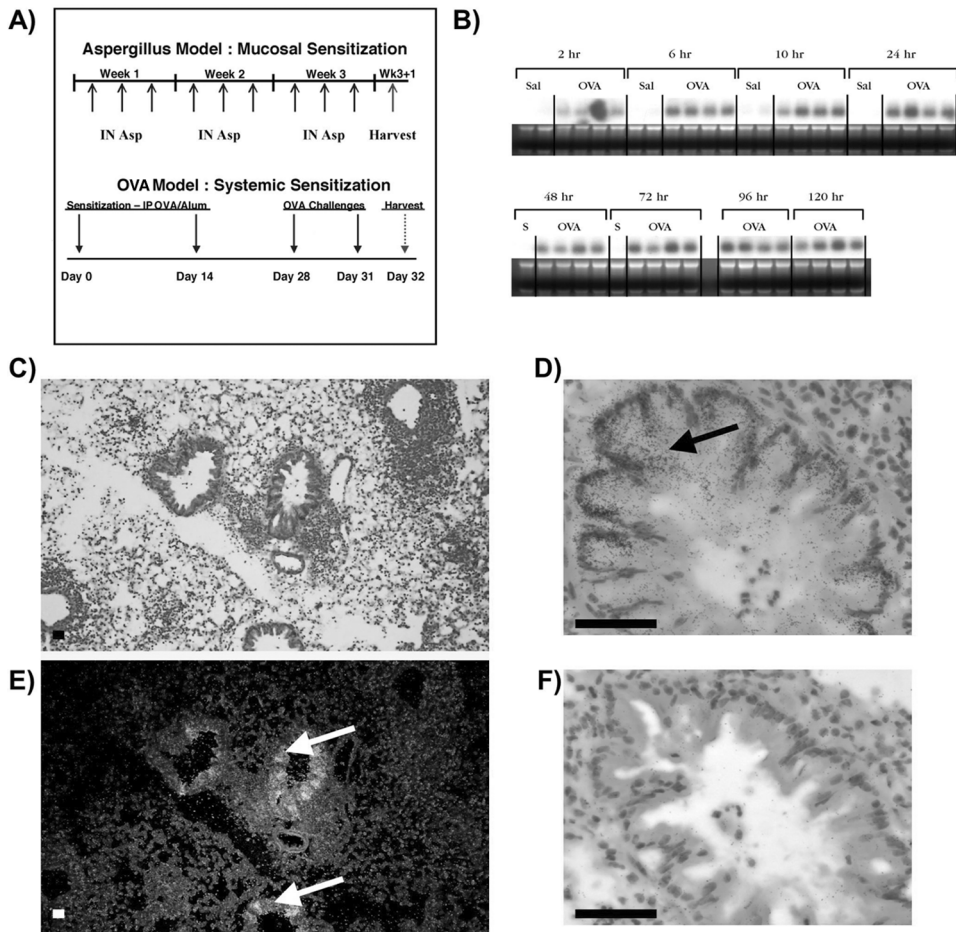


FIGURE 1 Kinetic expression and localization of allergen-induced TFF2. (A) The protocol of allergen-induced inflammation using *Aspergillus fumigatus* antigen or ovalbumin (OVA) to induce allergic inflammation. (B) Northern blot analysis of TFF2 expression following OVA challenge. Time points include 2, 6, 10, 24, 48, 72, 96, and 120 hours after 2 allergen or saline (Sal, S) challenges, each lane represents an individual mouse. The autoradiograph exposure time was 72 hours. The ethidium bromide stained gels are also shown. (C and D) Light field in situ hybridization analysis shows expression of antisense signal in the airway epithelium (black arrow) in OVA-challenged lung 18 hours following the 2nd intranasal challenge. (E) Dark field in situ hybridization analysis shows high expression of antisense TFF2 probe in airway epithelium (white arrows) in OVA-challenged lung 18 hours following the 2nd intranasal challenge. (F) Light field in situ hybridization analysis shows expression of sense signal in the airway epithelium in OVA-challenged lung 18 hours following the 2nd intranasal challenge. (C–F) Scale bars are equivalent to 50 μ m. Original magnification (C) 40 $\times$; (D) 400 $\times$; (E) 40 $\times$; (F) 400 $\times$.

OVA/alum-sensitized mice were challenged with intranasal OVA or saline, and in situ hybridization was performed on lung tissue obtained 18 hours after the second allergen or saline challenge. Antisense staining of OVA-challenged lungs revealed specific TFF2 mRNA expression largely in airway

epithelial cells (Figure 1C–E, arrows). TFF2 expression was associated with airways exhibiting peribronchial inflammation. There was no detectable signal in the smooth muscle cells of the bronchial airways or arterioles, alveolar lining cells, and endothelial cells. As a control, no specific staining was observed when the OVA-challenged lungs were stained with the sense probe (Figure 1F). In addition, anti-sense and sense probing of the saline-challenged lung revealed no detectable staining (data not shown).

TFF2 Protein Colocalizes to Mucin-Positive Airways

To determine if the distribution of TFF2 protein in allergen-challenged lung was associated with mucus-positive cells, TFF2 immunohistochemistry with alcian blue (IHC-AB) costaining was performed. IHC-AB staining of OVA-challenged lungs revealed TFF2 expression in a subset of mucus positive airway epithelial cells (Figure 2C–F). Notably, TFF2-positive cells

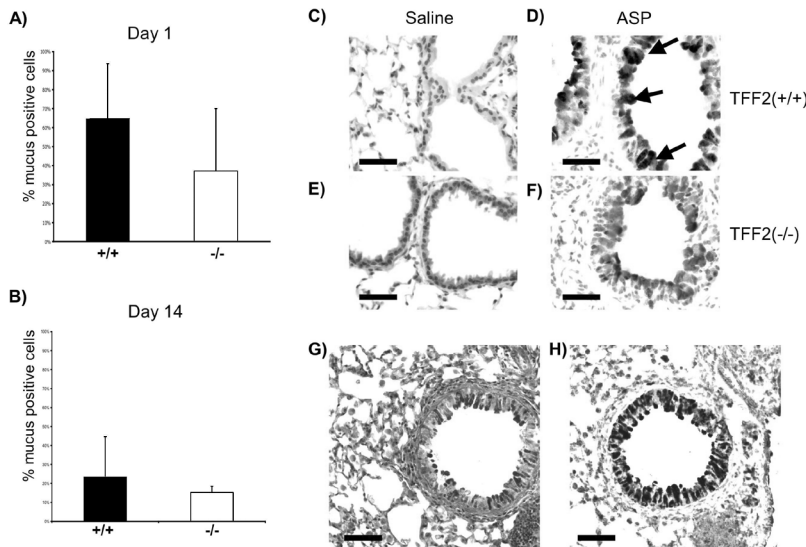


FIGURE 2 TFF2 protein localization to the mucus positive airway epithelium. (A) Morphometric analysis of alcian blue stained mucus cells (positive cells as percentage of total cells) in airway epithelial cells one day following challenge with *Aspergillus fumigatus* antigen. (B) Morphometric analysis of alcian blue stained mucus cells (positive cells as percentage of total cells) in airway epithelial cells 14 days following challenge with *Aspergillus fumigatus* antigen. (A and B) (+/+), wild-type mice; (-/-), TFF2 (-/-). (C) Saline-challenged wild-type mice stained with anti-TFF2 and alcian blue. (D) *Aspergillus fumigatus* antigen-challenged wild-type mice stained with anti-TFF2 and alcian blue. (E) Saline-challenged TFF2 KO mice stained with anti-TFF2 and alcian blue. (F) *Aspergillus fumigatus* antigen-challenged TFF2 KO mice stained with anti-TFF2 and alcian blue. (C–F) Anti-TFF2 IHC is detected by DAB precipitation. (G and H) Serial sections of OVA-challenged wild-type lungs stained with PAS and anti-TFF2, respectively, and IHC detected by DAB precipitation. (C–H) Scale bars are equivalent to 50 μ m. Original magnification (C) 400 $\times$; (D) 400 $\times$; (E) 400 $\times$; (F) 400 $\times$; (G) and (H) 100 $\times$.

were only found in airways which stained positive for mucus. Furthermore, serial sections of lung were stained with periodic acid-Schiff (PAS) followed by TFF2 immunohistochemistry revealed that TFF2 localizes to mucus positive airways in allergen challenged lungs (Figure 2G and H).

TFF2 Is Not Required for Pulmonary Mucus Overproduction

Given the known role of TFF2 as a component of mucus and that TFF2 expression was detected in mucus-positive airway epithelial cells, we were next interested in determining whether loss of TFF2 would effect mucus production in response to allergen challenge. To address this possibility, we examined *Aspergillus fumigatus* antigen-challenged TFF2 gene-deleted mice to determine if the loss of TFF2 would result in an alteration in mucus cell metaplasia. Morphometric analysis revealed that 1 day following ASP antigen challenge, there was no difference in mucus-positive cells in the airway epithelium of lungs, between wild-type and TFF2 (–/–) mice ($65\% \pm 29\%$ versus $37\% \pm 33\%$ mucus-positive cells) ($n = 3$ or 4 per group, $P > .05$) (Figure 2A). Given that we had shown a correlation of TFF2 levels with mucus-positive cells, we were next interested in examining if the loss of TFF2 might accelerate the turnover of mucus-positive cells, and thus effect the regulation of mucus cell homeostasis in allergen-challenged airways. To address this possibility we performed a kinetic analysis of allergen-challenged lungs harvested 1 to 14 days following allergen challenge. The *Aspergillus fumigatus* model was chosen because the kinetic analysis of mucus cell metaplasia offers a robust local inflammatory response. Initial studies of small distal airways (<250 cells) indicated that there was no difference on days 1 and 7 between wild-type and TFF2 (–/–) mice, mucin levels were decreased in TFF2 (–/–) mucus-positive cells at day 14, and there was complete resolution by day 21 (data not shown). Morphometric analysis revealed that there was no statistically significant difference in large airway mucin levels at 14 days following ASP antigen challenge between wild-type and TFF2 (–/–) mice ($23\% \pm 21\%$ versus $15\% \pm 3\%$ mucus-positive cells) ($n = 3$ or 4 per group, $P > .05$) (Figure 2B). These data suggest that TFF2 does not have a role in mucus cell development following allergen challenge.

Cellular Inflammation in TFF2-Deficient Mice

We next determined if the loss of TFF2 would effect the cellular inflammation induced by allergen challenge given that TFF2 has previously been shown to modulate the immune response in gastrointestinal models of inflammation [14, 28–30]. H&E staining in OVA challenged lung shows

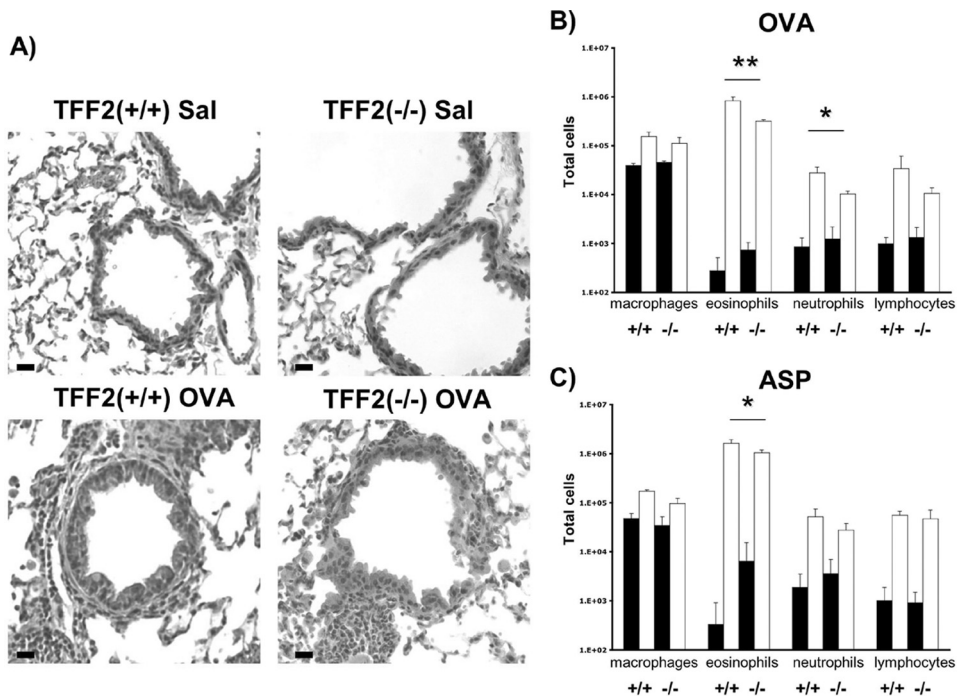


FIGURE 3 Tissue inflammation and inflammatory cell differential counts in the bronchoalveolar lavage fluid of allergen challenged mice. (A) H&E-stained section of OVA or saline-challenged lungs in wild-type (+/+) or TFF2 (-/-) mice. Scale bars are equivalent to 50 μ m. Original magnification, 100 $\times$. (B) BALF inflammation induced by OVA antigen (open bar) or saline (filled bar) in wild-type (+/+) or TFF2 (-/-) mice (n = 3 or n = 4 mice per experimental group; * $P < .05$, ** $P < .01$). (C) BALF inflammation induced by *Aspergillus fumigatus* antigen (open bar) or saline (filled bar) in wild-type (+/+) or TFF2 (-/-) mice (n = 3 or n = 4 mice per experimental group; * $P < .05$).

increased inflammatory cells in TFF2 (-/-) and wild-type mice compared to saline controls ($P < .01$) (Figure 3A). OVA antigen-challenge of TFF2 (-/-) mice resulted in a marked increase in the cellular infiltrate into the BALF following OVA challenge compared to saline controls (4.49×10^5 versus 4.91×10^4 total cells). The infiltration of both total and individual cell populations into the BALF following allergen administration resulted in a modest ~ 2 -fold reduction in total cellularity in TFF2 (-/-) mice compared with wild-type mice. Notably, there was a reduction in eosinophils in allergen challenged wild-type mice compared to TFF2 (-/-) mice (8.25×10^5 versus 3.16×10^5 total cells) (n = 3, $P < .01$) and neutrophils (2.78×10^4 versus 1.03×10^4 total cells) (n = 3, $P < .05$) (Figure 3B). Similar results were seen in mice treated with ASP antigen. *Aspergillus fumigatus* antigen-challenge of TFF2 (-/-) mice resulted in a marked increase in the cellular infiltrate into the BALF following OVA challenge compared to saline controls (1.21×10^6 versus 4.56×10^4 total cells). The

infiltration of both total and individual cell populations into the BALF following allergen administration resulted in a modest reduction in total cellularity in TFF2 (–/–) mice compared with wild-type mice. Notably, there was a reduction in eosinophils in allergen-challenged wild-type mice compared to TFF2 (–/–) mice (1.63×10^6 versus 1.04×10^6 total cells) ($n = 3$, $P < .05$) (Figure 3C).

We were next interested in determining if the modest reduction in inflammatory cells coincided with a reduction in inflammation in the lung tissue. Using a quantitative scale of inflammation (0 to 4), we examined multiple small airways per tissue section in a blinded manner. This analysis indicated that there is no significant difference in overall inflammation in the lungs of allergen challenged mice between wild-type and TFF2 (–/–) mice following either 2 or 9 intranasal OVA challenges (Figure 4A and 4B). Subsequent analysis of ASP-challenged mice also showed no detectable decrease in inflammation in TFF2 (–/–) mice compared with wild-type mice (Figure 4C). Taken together these results indicate that TFF2 does not have a major role in regulating tissue recruitment of inflammatory cells in response to allergen challenge.

Loss of TFF2 Does Not Result in Increased Epithelial Damage or Proliferation in Allergen-Challenged Lungs

To assess the possibility that TFF2 (–/–) mice might be susceptible to increased epithelial cell damage, an analysis of epithelial cell integrity was done to detect gross changes in epithelial morphology (erosions of the epithelial cell layer). This analysis showed no detectable damage in the allergen-treated mice of either genotype when compared to saline-treated control mice (data not shown). An analysis of BrdU incorporation was performed in order to test whether TFF2 might increase cellular proliferation as epithelial damage results in proliferation and differentiation. Notably, there was no detectable BrdU staining in either the large or small airway epithelium of TFF2 (–/–) or wild-type mice, although staining in regions associated with inflammatory cells was observed (data not shown). This suggests that loss of TFF2 does not grossly impair epithelial integrity or epithelial proliferation in response to allergen challenge.

DISCUSSION

To further understand the role of TFF2 in the effector phase of allergic airway inflammation, we examined the loss of TFF2 in models of allergen-induced pulmonary inflammation with particular emphasis

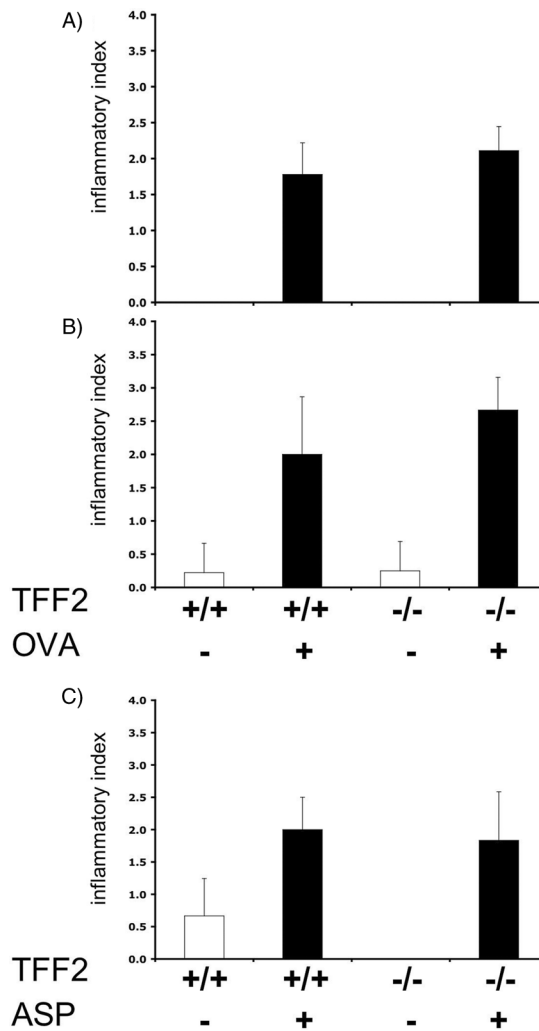


FIGURE 4 Inflammatory cell index in lung tissue of allergen challenged mice. (A) Inflammatory index in lung tissue of wild-type ($+/+$) or TFF2 ($-/-$) mice 18 hours following challenge with OVA (filled bars) or saline ($2\times$) (open bars) ($n = 3$ mice per experimental group). (B) Inflammatory index in lung tissue of wild-type or TFF2 ($-/-$) mice 18 hours following challenge with OVA (filled bars) or saline ($9\times$) (open bars) ($n = 3$ or $n = 4$ mice per experimental group). (C) Inflammatory index in lung tissue of wild-type ($+/+$) or TFF2 ($-/-$) mice 18 hours following challenge with *Aspergillus fumigatus* antigen (filled bars) or saline ($9\times$) (open bars) ($n = 2$ or $n = 3$ mice per experimental group).

on mucus production. Our results have uncovered several principles concerning TFF2 and experimental asthma. First, OVA induced a sustained overexpression of TFF2, lasting at least 2 weeks following OVA challenge; this correlates with the observed expression patterns of mucus-positive goblet cells in the airway epithelium of allergen-challenged mice.

Second, TFF2 is expressed in a subset of airway cells that display a mucus cell phenotype. Third, our study indicates that there is not a major alteration in the inflammatory response in the lungs of TFF2 gene deleted mice. It has previously been speculated that injury-associated epithelial hyperplasia and epithelial differentiation (e.g., mucus cell metaplasia), processes known to be regulated by TFF2 in the gastrointestinal tract, may also be mediated by TFF2 in the lung [11, 16, 31]. Our results indicate that TFF2 is not an essential component of the initiation or resolution of mucus cell metaplasia in allergen-challenged lungs. Lastly, although TFF2 has been implicated in epithelial restitution, its absence does not appear to predispose to epithelial damage in the allergic lung. Perhaps a stronger lung injury model might reveal a role for TFF2 in lung epithelial cell restitution; however, preliminary studies with a naphthalene-induced epithelial injury model involving extensive epithelial cell death revealed comparable epithelial denudation in wild type and TFF2 gene targeted mice (data not shown).

Under healthy conditions, TFF2 is predominantly expressed in the stomach with lower levels in the proximal duodenum and biliary tract [10, 11]. In the stomach, TFF2 is expressed by gastric mucus neck cells, and is secreted onto the mucosal surface associated with mucin proteins. TFF2 is up-regulated in diverse injury-associated pathological conditions in the gastrointestinal tract including ulceration associated with *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drug use, and Crohn's disease of the gastrointestinal tract [32–34]. Although in vitro studies have shown that TFF2 can modulate cell-cell contacts and stimulate motogenic signaling in bronchial epithelial cells, processes essential in epithelial restitution, we did not observe a role for TFF2 in promoting epithelial protection in the lung, perhaps because the experimental conditions were insufficient to induce sufficient lung injury [20, 22, 35–37].

In conclusion, we report that (1) TFF2 is expressed in a subset of mucus-positive airway epithelial cells; (2) TFF2 gene deleted mice show no alteration in the initiation or resolution of mucus cell metaplasia following allergen challenge; (3) TFF2 gene deleted mice show no major alteration in the degree of inflammation in the lung; and (4) TFF2 gene deleted mice show no alteration in the susceptibility to allergen-induced damage to or increased proliferation of the airway epithelium. Collectively, these results indicate that TFF2 is not a major contributory factor to the classic features of experimental allergic lung disease in mice. Despite the lack of a profound phenotype in TFF2-deficient mice, in allergen-challenged lungs it is interesting to note consistent decreases in inflammatory cell numbers. This finding warrants further study as it suggests that TFF2 might alter antigen processing or uptake, potentially by modulating the mucosal microenvironment.

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