

Expression and Regulation of Small Proline-Rich Protein 2 in Allergic Inflammation

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Asthma is a complex inflammatory pulmonary disorder that is on the rise despite intense ongoing research. We aimed to elucidate novel pathways involved in the pathogenesis of asthma. Employing asthma models induced by different allergens (ovalbumin and *Aspergillus fumigatus*), we uncovered the involvement of two members of the small proline-rich protein (SPRR) family, SPRR2a and SPRR2b, known to be involved in epithelial differentiation but not allergic disease. *In situ* hybridization revealed induction of SPRR2 signal in a subset of bronchial epithelial cells and mononuclear cells associated with inflammation after allergen challenge. Allergen-induced SPRR2 mRNA accumulation in the lung occurred in a time-dependent manner, with peak expression 10–96 h after a second ovalbumin challenge. Transgenic overexpression of interleukin (IL)-13 in the lungs resulted in a marked increase of SPRR2 expression, and allergen-induced SPRR2 expression was significantly decreased in IL-13-deficient mice. Studies in gene-targeted mice revealed that allergen-induced SPRR2 was dependent upon STAT6. Finally, we aimed to determine if the induction of SPRR2 by allergen was tissue specific. Notably, SPRR2 was markedly increased in the small intestine after induction of allergic gastrointestinal inflammation. Thus, SPRR2 is an allergen- and IL-13-induced gene in experimental allergic responses that may be involved in disease pathophysiology.

Keywords: asthma; allergy; small proline-rich protein

There is currently an epidemic of allergic diseases in the western world (1). Experimentation in the asthma field has largely focused on analysis of the cellular and molecular events induced by allergen exposure in sensitized animals (primarily mice) and humans. These studies have identified elevated production of IgE, airway obstruction, eosinophilic inflammation, enhanced bronchial reactivity to spasmogens, epithelial metaplasia (including goblet cell formation), and epithelial injury in the asthmatic response (2). Clinical and experimental investigations have demonstrated a strong correlation between the presence of CD4⁺ T helper 2 lymphocytes (Th2 cells) and disease severity, suggesting an integral role for these cells in the pathophysiology of asthma (3, 4). Th2 cells are thought to induce asthma through the secre-

tion of an array of cytokines (interleukin [IL]-4, -5, -9, -13) that activate inflammatory and residential effector pathways both directly and indirectly (5, 6). In particular, IL-13 is produced at elevated levels in the asthmatic lung and is thought to be a central regulator of many of the hallmark features of disease (7). IL-13 induces IgE production, mucus hypersecretion, eosinophil recruitment and survival, airway hyperreactivity, the expression of CD23, adhesion systems, and chemokines (7–9). IL-13 signaling uses the IL-4 receptor α chain (10, 11) and induces janus kinase 1 and signal-transducer-and-activator-of-transcription (STAT)6 (12–14). Indeed, STAT6-deficient mice have attenuated development of several features of experimental asthma. Although recent studies provide a rationale for the development of several therapeutic agents that interfere with specific inflammatory pathways (15, 16), 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.

Aiming to elucidate novel pathways involved in the pathogenesis of allergic asthma, we took an empiric approach using DNA microarray analysis of whole lung RNA (17, 18). To identify allergen-induced gene expression in the lung that was not specific to a particular experimental regime, we induced experimental asthma using two distinct experimental protocols. In one model, mice were intraperitoneally sensitized with the allergen ovalbumin (OVA) and subsequently challenged with intranasal OVA or control saline on two occasions separated by 3 d. In another model, experimental asthma was induced by the repeated intranasal applications of *Aspergillus fumigatus* antigen. It is noteworthy that this model involves a unique mucosal sensitization route (19) and that *Aspergillus fumigatus* is a ubiquitous common aeroallergen. Importantly, both asthma models exhibit similar phenotypes, including Th2-associated eosinophilic inflammation, mucus overproduction, and airway hyperresponsiveness. Lung RNA from saline- and allergen-challenged mice (18 h after the last challenge) was subjected to microarray analysis using the Affymetrix chip U74Av2 that contains oligonucleotide probe sets representing 12,422 genetic elements, one of the largest collections of characterized mouse genes commercially available. This led to the identification of 242 genes that were commonly involved in disease pathogenesis, rather than unique to a particular allergen or mode of disease induction (17). These “asthma signature” genes provide a valuable opportunity to define new pathways involved in the pathogenesis of allergic airway inflammation. This article focuses on genes encoding SPRR2a and b.

Small proline-rich proteins (SPRR) are encoded by a family of at least 17 members located in a closely linked locus on chromosomes 3 and 1 in mice and humans, respectively (20). Each SPRR gene contains two exons, with the entire open reading frame contained in the second exon. Although there are three SPRR subfamilies, most studies of gene expression and function have been performed with a member of the first family, SPRR1a. This gene,

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along with SPRR2a and SPRR2b, is expressed predominantly in squamous epithelium, where it has been thought to contribute to the formation of the insoluble cornified crosslinked envelope that provides structural integrity and limits permeability. Differential expression of SPRR proteins in different organs in response to various stimuli (such as cigarette smoke or ultraviolet irradiation) correlates with modulation of biomechanical properties of the epithelium, such as elasticity and flexibility (21). For instance, SPRR1-positive squamous epithelium is found in tissues that require significant flexibility (e.g., lips, tongue, and esophagus). In addition to their function as structural proteins, nuclear expression of SPRR-encoded proteins, particularly at the time of cell cycle entry into G0, and interactions between SPRR-encoded proteins and nuclear proteins have suggested a role for SPRR family members in gene regulation, possibly in limiting proliferation and promoting differentiation (22). In the lungs, increased SPRR1 gene expression has been associated with squamous metaplasia and especially with the appearance of squamous cell cancer (23). However, increased SPRR expression has not been studied in asthma. Finally, the gene for human SPRR2 is located on chromosome 1q21, within an area that demonstrated linkage to atopic dermatitis (24). Due to the potential involvement of SPRR2 proteins in epithelial changes observed in asthma, and the paucity of information on the involvement of SPRR in asthma, we tested the hypothesis that SPRR2 is an allergen- and IL-13-induced gene product that is regulated by STAT6-dependent signaling pathways in experimental allergic lung disease. Furthermore, we tested the hypothesis that SPRR2 induction in allergic inflammation is not restricted to the lung but also occurs in Th2-associated intestinal inflammation.

MATERIALS AND METHODS

Mice

Balb/c mice were obtained from the National Cancer Institute (Frederick, MD) and housed under specific pathogen-free conditions. Mice deficient in STAT6 in the Balb/c background were obtained from Jackson Laboratories (Bar Harbor, ME). IL-13-deficient mice were kindly provided by Dr. Andrew MacKenzie (25). Mice carrying the tetracycline inducible IL-13 transgene under the regulation of the Clara cell 10 lung promoter have been previously described (26).

Experimental Allergy Induction

Mice were sensitized by intraperitoneal injection with 100 µg of OVA and 1 mg aluminum hydroxide (alum) in saline on Days 0 and 14. On Days 24 and 27, mice were lightly anesthetized with inhaled isoflurane and challenged intranasally with 50 µg OVA or saline. Alternatively, 2 wk after the last intraperitoneal sensitization, mice were held in the supine position, three times a week (every other day), and intragastrically administered 250 µl of sterile saline that contained 50 mg of OVA, as previously reported (27). In other experiments, mice were challenged with nine doses of intranasal *Aspergillus fumigatus* antigen over the course of 3 wk (28). The allergen challenge was performed by applying 50 µl to the nares using a micropipette with the mouse held in a supine position. Mice were killed 3 or 18 h after allergen challenge.

One lobe of the lungs was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin using standard histologic techniques. The remaining lungs and 2–3 cm of jejunal segments were placed in Trizol (Gibco BRL) and homogenized for RNA isolation.

Microarray Hybridization and Analysis

Microarray hybridization was performed by the Affymetrix Gene Chip Core facility at Cincinnati Children's Hospital Medical Center, as previously described (17, 18). Briefly, RNA was converted to cDNA with Superscript choice for cDNA synthesis (Invitrogen, Carlsbad, CA) and subsequently converted to biotinylated cRNA with an Enzo High Yield RNA Transcript labeling kit (Enzo diagnostics, Farmingdale, NY).

After hybridization to the murine U74Av2 GeneChip (Affymetrix, Santa Clara, CA), the gene chips were automatically washed and stained with streptavidin-phycoerythrin by using a fluidics system. The chips were scanned with a Hewlett Packard GeneArray Scanner (Loveland, CO). This analysis was performed with one mouse per chip ($n > 3$ for each allergen challenge condition and $n > 2$ for each saline challenge condition). From data image files, gene transcript levels were determined using algorithms in the Microarray Analysis Suite Version 5 software (Affymetrix). Differences between saline- and OVA-treated mice were also determined using the GeneSpring software (Silicon Genetics, Redwood City, CA). Data for each allergen challenge time point was normalized to the average of the saline-treated mice. Details of the microarray analysis have been published previously (17, 18).

Northern Blot Analysis

RNA (10–20 µg) was electrophoresed in an agarose-formaldehyde gel, transferred to Gene Screen transfer membranes (NEN, Boston, MA) in 10× SSC, and cross-linked by ultraviolet radiation. Probes were obtained from clones from the IMAGE consortium, and labeled with ³²P using the Klenow reaction with random priming. Blots were hybridized under standard conditions. Following washes, membranes were exposed to film for 1–5 d.

In Situ Hybridization of Mouse Lung

In situ hybridization was performed as described (18). In brief, murine SPRR2a cDNA in plasmid pT7T3 (Incyte Genomics, St. Louis, MO) was linearized by Eco RI or Not I digestion, and antisense and sense RNA probes, respectively, were generated by T3 and T7 RNA polymerase (Riboprobe Gemini Core System II transcription kit; Promega, Madison, WI), respectively. The radio-labeled [α^{35} S-UTP] probes were hybridized and washed under high-stringency conditions.

RT-PCR Analysis

Total RNA from saline and OVA-challenged lungs was isolated as above, and RNA from esophagus was used as a positive control. cDNA was prepared by reverse transcription and PCR performed using gene-specific primers for members of the SPRR2 family, as described previously. (29) SPRR2c and 2j were not used because they contain a nonsense and frameshift mutation, respectively, and 2c is likely a pseudogene (29).

RESULTS

DNA Microarray Analysis Identifies SPRR2a and SPRR2b as Allergen-Induced Genes in Experimental Asthma

Global quantitative microarray analysis revealed that SPRR2a and SPRR2b mRNA expression was significantly increased in both the *Aspergillus*- and OVA-induced asthma models (Figures 1A and 1B). Interestingly, microarray analysis revealed very specific dysregulation of SPRR2a and SPRR2b compared with other members of the SPRR2 family of proteins that were represented on a chip. In particular, SPRR2c-k were also represented on the microarray chip; however, only 2e and 2i had a detectable hybridization signal and neither was increased during induction of experimental asthma (data not shown).

It is important to note that the coding sequences of SPRR2 family members are highly homologous. Although the Affymetrix chip uses gene specific sequences, it remained possible that other members of the family were cross-reacting with the SPRR2a or b probes. Members of the SPRR2 family mainly differ in the number of repeats (of the sequence PKCPEPCPP) in the central domain. Thus, the design of unique probe sets that recognize only one of the SPRR2 family members was important. However, because probe sets for family members other than 2a and 2b were either called “absent” or did not change with allergen challenge, it is unlikely that these family members contribute to hybridization to probes for 2a and 2b. Additionally, it remained possible that only one of SPRR2a or 2b was increased, but it cross-hybridized to the probe for the

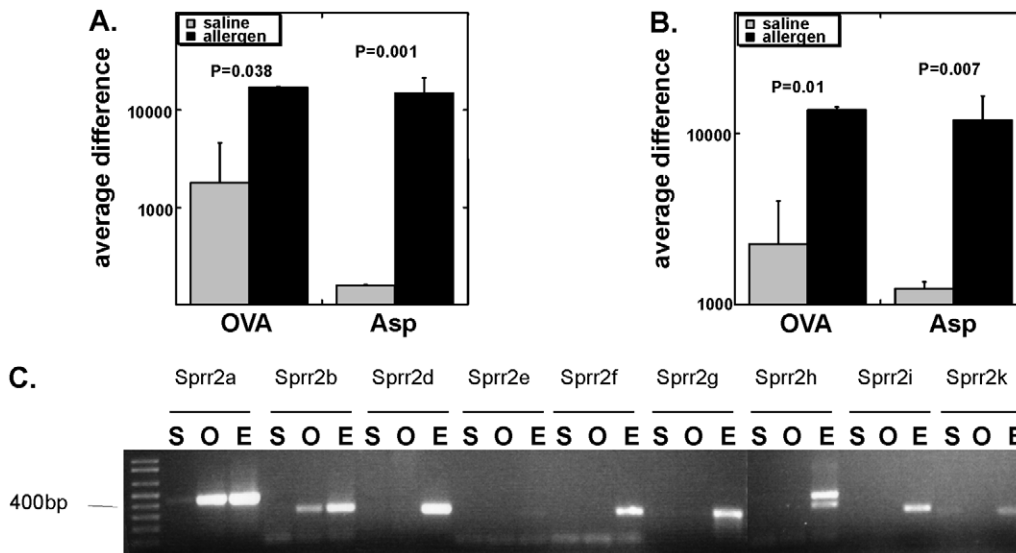


Figure 1. SPRR2 expression is induced in experimental asthma. The expression of SPRR2a (A) and SPRR2b (B) in lungs of OVA- or Asp-challenged mice as measured by microarray analysis is shown. The average difference for the hybridization signal after saline (white bar) and allergen (black bar) challenge is depicted. Error bars represent the standard deviation. In C, RT-PCR for SPRR2 family members is shown. Lanes are: S-saline-challenged lung; O-OVA-challenged lung and E-esophagus. Low level of SPRR2k expression was observed in only a minority of control lung samples.

other, resulting in a positive signal. Thus, we aligned individual probe sequences from the probe set for SPRR2a with sequence for SPRR2b and vice versa. This analysis revealed that at least half of the probes were polymorphic, with at least 1 to 5 polymorphisms per probe (data not shown). Considering the stringency of the software analysis that we employed, it is unlikely that transcripts are cross-reacting with probes designed to test other family members. However, to verify this by an independent method, we performed RT-PCR analysis using primers unique for each SPRR2 transcript (29). This analysis demonstrated that indeed SPRR2a and SPRR2b are specifically increased with allergen challenge. RNA from the esophagus was used as a positive control for other SPRR2 family members (Figure 1C).

Northern Blot Analysis Demonstrates SPRR2 Induction by Allergen

We subsequently determined by Northern blot analysis that SPRR2 was indeed induced by allergen challenge. The cDNA probe used for Northern blot analysis comprises the open reading frame of SPRR2a; due to high homology between SPRR2 family members, this probe does not distinguish between different SPRR2 gene transcripts. OVA-induced SPRR2 expression was time- and dose-dependent during the progression of experimental asthma (Figure 2). After the first OVA challenge, SPRR2

mRNA peaked at 24 h and declined to baseline by 2 wk. After two allergen challenges, SPRR2 mRNA accumulated at a much higher level, peaking at 10 h and remaining elevated even after 96 h compared with saline-challenged lungs. These data suggest that the adaptive immune response enhances SPRR2 induction. We were next interested in correlating SPRR2 mRNA accumulation with differential leukocyte recruitment into the BALF (see Figure 2 in Ref. 30). While peak SPRR2 mRNA expression correlated with neutrophil levels, the sustained SPRR2 expression more strongly correlated with eosinophils and mononuclear cells. Similar to the results with the OVA model, we observed increased SPRR2 expression when allergic inflammation was induced by the *Aspergillus fumigatus* antigen (see below). It remained possible that SPRR2 induction was a nonspecific response to any stimulus. Thus, we tested the expression of SPRR2 in *Haemophilus influenzae* type B-infected mice, because this experimental system induces a strong inflammation in the lung. Notably, SPRR2 expression was not induced by this infection model (data not shown).

SPRR2 *In Situ* Hybridization

To begin to address the distribution of SPRR2 mRNA-positive cells in the allergic lung, we performed *in situ* hybridization for SPRR2 mRNA. OVA/alum-sensitized mice were challenged with intranasal OVA or saline and *in situ* hybridization was

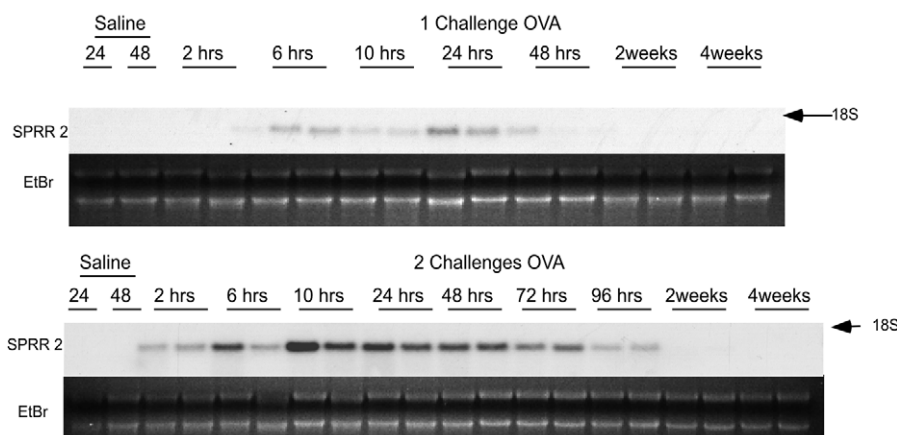


Figure 2. Northern blot analysis of SPRR2 mRNA expression. Northern blot analysis of SPRR2 expression after one (top) and two (bottom) OVA challenges are shown. The ethidium bromide (EtBr)-stained gel is also shown. Each lane represents one mouse.

performed on lung tissue obtained 18 h after the second allergen or saline challenge. Antisense staining of OVA challenged lungs revealed strong SPRR2 mRNA expression in individual or small clusters of bronchial epithelial cells (Figures 3A and 3B). Epithelial cell staining did not reveal a pattern consistent with a specific cell type (i.e., goblet cells), nor was there a correlation with the size of bronchi (data not shown). Squamous metaplasia was not observed in the bronchial mucosa. Interestingly, a small subset of mononuclear cells associated with the inflammatory lesions was positive for SPRR2 (Figures 3C and 3D). Identifiable eosinophils appeared negative (*arrowheads* in Figure 3D *inset* and data not shown). There was no signal in the smooth muscle cells of the bronchial airways, or arterioles, and endothelial cells. As controls, antisense and sense staining of the saline-challenged lung revealed no detectable staining (Figures 3E and 3F, and data not shown). Additionally, no specific staining was observed when the OVA-challenged lungs were stained with the sense probe (data not shown).

IL-13 Induces Lung SPRR2

Because asthma is a Th2-associated process, we were interested in testing the hypothesis that chronic overexpression of the Th2 cytokine IL-13, which has been shown to promote epithelial differentiation (e.g., goblet cell formation) particularly in the

lungs, was sufficient for induction of SPRR2. Mice that overexpress the IL-13 transgene in pulmonary epithelium (under the control of the Clara cell 10 promoter) have several features of asthma including eosinophil-rich inflammatory cell infiltrates, mucus production, and changes in baseline airway tone (31). We hypothesized that SPRR2 mRNA would be induced by the IL-13 transgene. Indeed, mice with the IL-13 transgene under the control of the doxycycline-regulated promoter had low levels of SPRR2 mRNA already after 1 d of doxycycline administration, as shown in Figure 4A. The expression of SPRR2 mRNA continued to increase during 30 d of doxycycline treatment (Figure 4A). Similar to results with OVA-challenged mice, *in situ* hybridization revealed two distinct populations of SPRR2-positive cells: bronchial epithelial cells (either in clusters or as individual cells) and scattered mononuclear cells (Figure 4B). No specific staining was observed when the IL-13 transgenic lungs were stained with the sense probe (data not shown).

SPRR2 Expression Requires IL-13

Next, we were interested in testing the hypothesis that IL-13 is required for allergen-induced SPRR2 expression. To test this hypothesis, wild-type and IL-13-deficient mice were challenged with OVA and *Aspergillus* antigens as above. Interestingly, the expression of SPRR2 was significantly attenuated in IL-13-deficient

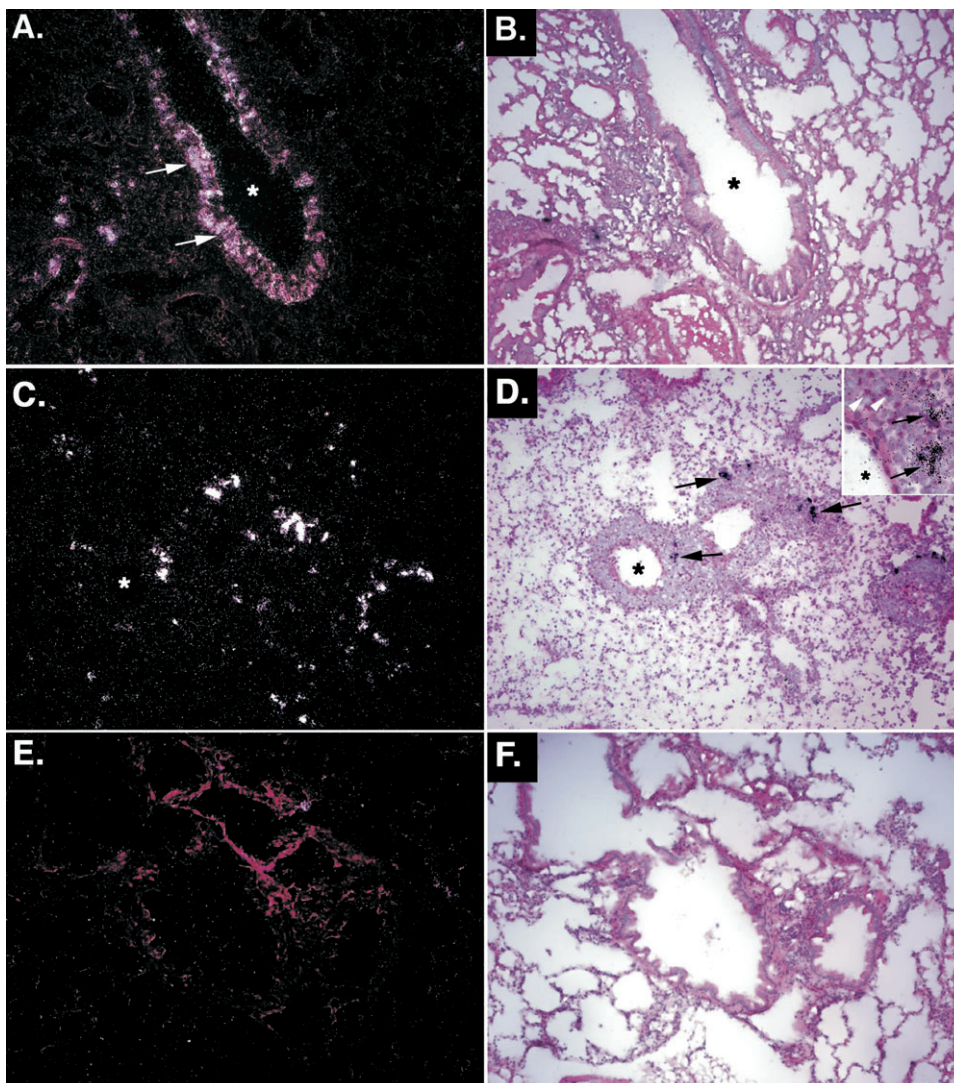


Figure 3. SPRR2 mRNA *in situ* hybridization. Lung sections from allergen- (A–D) and saline- (E and F) challenged mice (18 h after two OVA challenges) hybridized with the antisense riboprobe for SPRR2 at $\times 100$ original magnification are shown. The darkfield (A, C, and E) and brightfield (B, D, and F) images are shown. In A, there is strong signal (*bright white grains*) in the bronchial epithelium. In C and D, positive signal is shown in cells in the inflammatory infiltrate (*arrows*). The *inset* in D shows a magnified area demonstrating positive signal in a small subpopulation of the infiltrating cells. *White arrowheads* demonstrate eosinophils that are not positive for SPRR2. *Asterisk* denotes airway lumen.

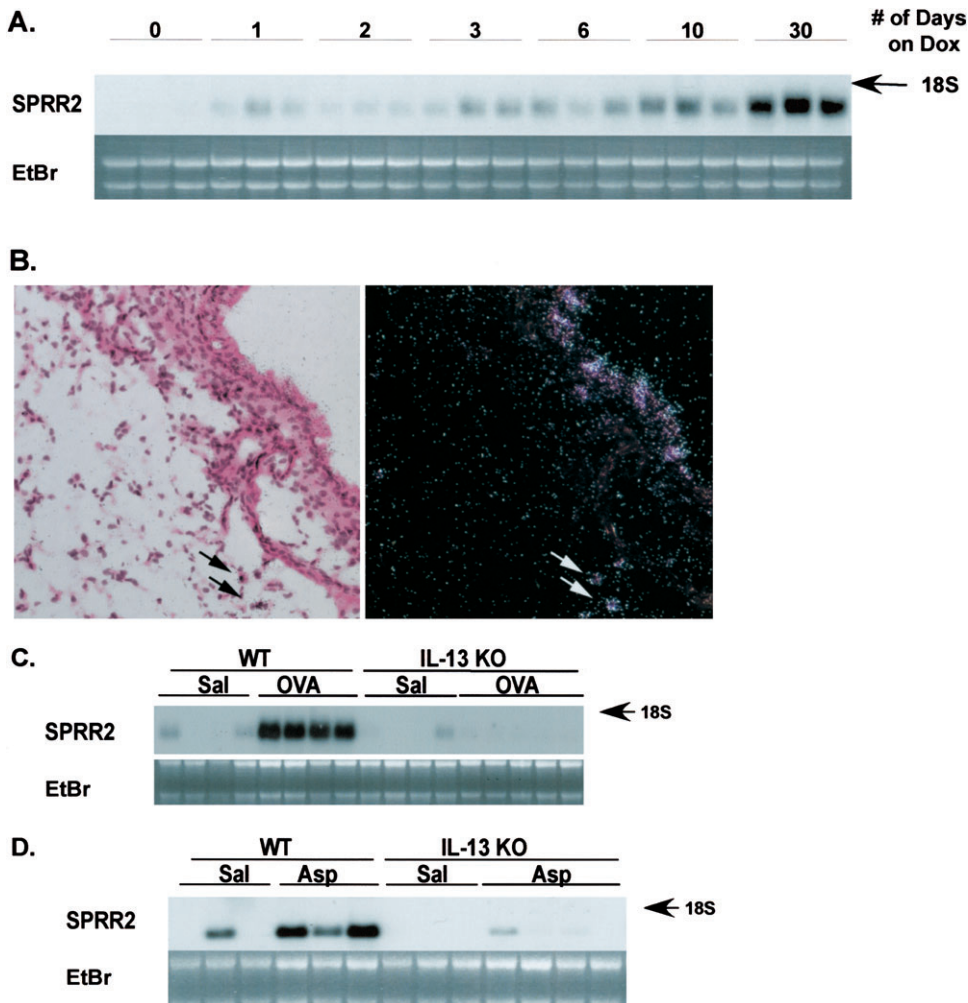


Figure 4. IL-13 is sufficient and required for SPRR2 expression in the lung. In *A*, IL-13 lung transgenic mice were examined for SPRR2 mRNA expression by Northern blot analysis. The IL-13 inducible transgenic mice were fed a control or doxycycline-supplemented diet for indicated periods of time. In *B*, brightfield (left panel) and darkfield (right panel) images of *in situ* hybridization of IL-13 transgenic mice are shown. Wild-type (WT), and IL-13-deficient mice were challenged with saline, OVA (*C*), or *Aspergillus fumigatus* (*D*) allergen as indicated. The ethidium bromide (EtBr)-stained gels are also shown. Each lane represents one mouse.

mice challenged with OVA (Figure 4C) and *Aspergillus* (Figure 4D). These data suggest that IL-13 is both sufficient and required for SPRR2 expression in the lungs. It is interesting to note that one of the saline-challenged mice had detectable expression of SPRR2. While expression in saline-challenged mice was sporadically observed especially with long autoradiograph exposures, there was a clear statistically significant increase after allergen challenge ($P < 0.002$). There are several explanations for this finding, including possible contamination by trace amounts of esophageal tissue in the lung preparation. In addition, it remains possible that factors unassociated with the experimental allergen challenge could also induce SPRR2 mRNA. We elected to include this representative Northern blot to bring this issue to the reader's attention.

SPRR2 Expression Is Primarily STAT6-Dependent

IL-13 signaling involves both STAT6-dependent and -independent events. To test the role of STAT6 in the induction of SPRR2, we induced experimental asthma in mice genetically deficient in STAT6, and examined the impact on SPRR2 induction. Mice deficient in STAT6 had markedly reduced expression of SPRR2 after OVA challenge compared with OVA-challenged wild-type mice (Figure 5A). Similarly, *A. fumigatus*-induced SPRR2 was largely STAT6-dependent (Figure 5B). Finally, intratracheal delivery of IL-13 to STAT6-deficient mice demonstrated that SPRR2 induction was largely STAT6-dependent

(data not shown). These data indicate that allergen-induced SPRR2 expression is STAT6-dependent.

SPRR2 mRNA Is Induced by Allergic Gastrointestinal Inflammation

We were interested in determining if the induction of SPRR2 by allergen challenge was specific to the respiratory tract. To test this, we examined the induction of SPRR2 during allergic gastrointestinal inflammation. In particular, when OVA/alum-sensitized Balb/c mice are exposed to repeated doses of oral OVA, they develop an impressive Th2-associated intestinal inflammation accompanied by allergic diarrhea (27). Notably, after oral OVA challenge, there was strong induction of SPRR2 mRNA compared with saline challenge (Figure 6). SPRR2 expression was already increased at two challenges, and increased further with three and four challenges. Notably, mice do not exhibit symptoms (e.g., diarrhea) after only two challenges, suggesting that SPRR2 expression precedes occurrence of symptoms.

DISCUSSION

To understand the complex mechanisms involved in the pathogenesis of asthma, we employed transcript expression profile analysis to define a set of "asthma signature" transcripts. We focused on the discovery of SPRR2 because this molecule had properties potentially important in allergic responses and because SPRR2 was not previously implicated in the pathogenesis

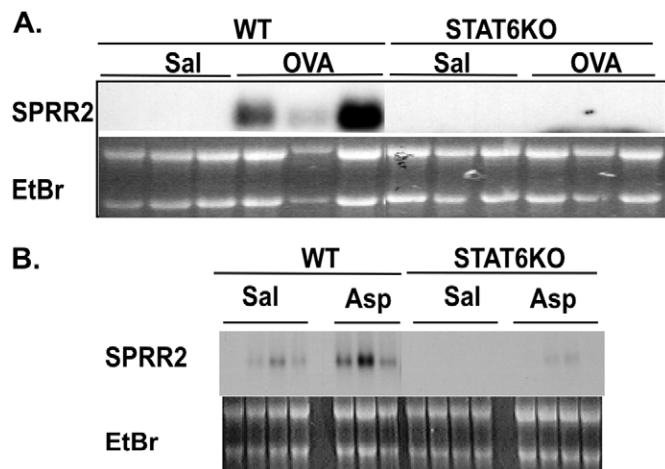


Figure 5. Regulation of allergen-induced SPRR2 expression by STAT6. Northern blot analyses of SPRR2 lung mRNA expression in saline or OVA- (A) and Aspergillus- (B) challenged mice. Wild-type (WT) and STAT6-deficient (KO) mice were challenged with saline, OVA, or *Aspergillus fumigatus* allergen as indicated in the figures. The ethidium bromide (EtBr)-stained gels are also shown. Each lane represents one mouse.

of allergy. Our results have uncovered several principles concerning SPRRs and allergic responses. First, allergic lung inflammation, triggered by diverse allergens and modes of disease induction, is shown to be associated with marked and specific ectopic expression of SPRR2a and SPRR2b in the lung. The expression of these two specific SPRR2 family members was confirmed by PCR analysis. Using RT-PCR, which is a more sensitive technique than microarray analysis, we were also able to detect upregulated expression of SPRR1a in allergen-challenged lungs (data not shown), but the significance of this is under investigation. Before these findings, SPRR expression was thought to be primarily restricted to squamous epithelium (under nonmalignant conditions), such as skin and parts of the gastrointestinal tract. Second, our study demonstrates that the Th2 cytokine IL-13 is a potent inducer of SPRR2 in the lung and that allergen induction of pulmonary SPRR2 expression is largely IL-13 and STAT6-dependent. Because there are two consensus STAT-binding sites in the 5' flanking region of SPRR2a, this may be a direct effect of STAT6 on SPRR2a transcription. As such, this broadens the potential function of the SPRR family to a completely new set of conditions. However, a portion of SPRR2 expression was not dependent on STAT6. These results are consistent with recent studies demonstrating that certain aspects of asthma, specifically in the chronic phase of disease, are STAT6-independent (17, 32–34). Although the mechanism of this finding warrants future investigations, it is interesting to speculate on several possibilities. Because SPRR2 expression was found in at least two distinct cell types, it remains possible that each cell type may have distinct STAT6 dependence. Alter-

natively, SPRR2 may be regulated by transcription factors that are Th2-associated, but not Th2-restricted (e.g., NF- κ B); thus there may be alternative signaling pathways induced in the absence of STAT6. Prior studies have determined that Th2 responses can occur independent of STAT6 in the absence of the Bcl6 or CTLA4 molecules (35, 36). Thus, the observed STAT6-independent SPRR2 expression may arise from suppression of Bcl6-associated transcription and/or by strong T cell co-stimulation. Occasionally, a saline-challenged mouse demonstrated SPRR2 expression. Due to high expression of SPRR2 proteins in the esophagus and anatomical proximity to the lungs, it remains possible that this is due to contamination with esophageal RNA. Third, *in situ* hybridization localized SPRR2 expression to clusters of epithelial cells. Because there was no pattern consistent with expression in a specific cell type (e.g., goblet cells) or bronchus size, these data suggest that SPRR2 is likely to be transiently expressed in a broad spectrum of lung epithelial cells. In addition, we identify that a small subset of mononuclear cells associated with the inflammatory lesions is positive for SPRR2. To our knowledge, this is the first demonstration of SPRR2 expression in cells associated with an inflammatory infiltrate. It remains possible that expression may be cell cycle-specific, similar to the expression of SPRR1 in Chinese Hamster Ovary cells (22). Finally, we have demonstrated that SPRR2 expression was also induced in the gastrointestinal tract after oral allergen challenge. The induction of expression of molecules involved in core features of the allergic phenotype is expected to occur regardless of the organ or tissue involved. Our data demonstrate that SPRR2 is one such gene and suggest that SPRR2 may be involved in the pathophysiology of allergic responses in diverse mucosal tissues.

Although we have not yet elucidated the function of SPRR proteins in experimental asthma, it is interesting to speculate about their potential role. In response to allergen challenge or other harmful stimuli, epithelial injury is accompanied by rapid migration of neighboring cells (a process termed restitution). Notably, we have recently described the induction of trefoil factor 2, a key molecule involved in epithelial restitution, in the murine asthmatic lung (34), suggesting that a series of gene products involved in this process are acutely induced after allergen challenge. Epithelial restitution is accompanied by the proliferation of mature epithelium, as well as “dedifferentiation” of basal and secretory epithelium into highly proliferative progenitor-like stem cells, which can then give rise to epithelium with multiple phenotypes. It is tempting to speculate that SPRR2 is expressed in multipotent progenitor-like stem cells in the asthmatic lung and is involved in regulating cellular differentiation. This is an attractive hypothesis consistent with early SPRR induction (even 1 d on doxycycline in IL-13 transgenic mice and 6 h after initial allergen challenge) and disappearance of SPRR expression once the stimulus is removed. SPRR may also be expressed in cells that are destined to become squamous cells. Indeed, squamous cell metaplasia has been observed in patients with asthma (37, 38) and SPRR expression has been detected in respiratory epithelia undergoing squamous metaplasia after exposure to cigarette smoke (21). Squamous metaplasia is a common reaction to noxious

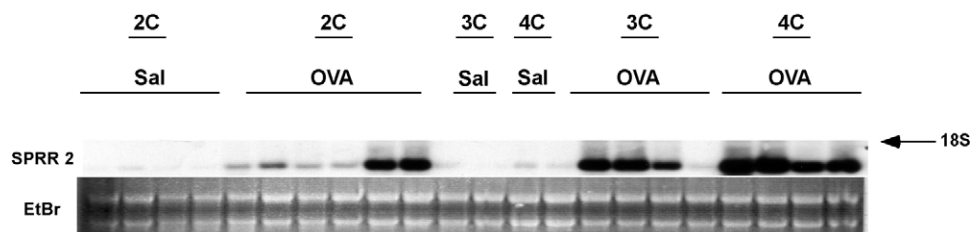


Figure 6. Intestinal SPRR2 expression is induced by experimental gastrointestinal inflammation. Northern blot analysis of SPRR2 expression after two (2C), three (3C), or four (4C) intragastric OVA or saline challenges is shown. The ethidium bromide (EtBr)-stained gel is also shown. Each lane represents one mouse.

stimuli (such as acid in Barrett's esophagus) due to superior biomechanical strength of the squamous epithelium. Although squamous metaplasia is not morphologically evident in our experimental asthma model, it remains possible that SPRR2 expression precedes morphologic changes or that other stimuli are required for squamous metaplasia to develop. However, in more chronic models, squamous metaplasia, with persistent SPRR expression, may occur.

Our results suggest that the pathogenesis of IL-13-associated allergic lung responses (which involves epithelial injury and repair) may be mediated by SPRR2, at least in part. It is tempting to speculate that epithelial hyperplasia and differentiation (e.g., mucus cell metaplasia) observed in asthma may be mediated by SPRR2 in the lung. Notably, IL-13-deficient, allergen-challenged mice have significantly reduced epithelial cell mucus production, while airway inflammation and hyperreactivity remain comparable to wild-type mice (39). Moreover, a recent study demonstrated that IL-13 acting directly on epithelial cells causes mucus overproduction (40). This correlates with our findings that allergen-challenged IL-13-deficient mice have decreased SPRR2 expression and that SPRR2 is primarily induced in epithelial cells. Future studies will need to address the exact role of SPRR2 in the pathogenesis of allergic responses.

In summary, we report that (1) SPRR2 is an allergen-induced transcript in the asthmatic lung; (2) SPRR2 expression is induced in a subset of airway epithelial and mononuclear cells associated with the inflammation in the asthmatic lung; (3) the Th2 cytokine IL-13 induces SPRR2; (4) SPRR2 induction by allergen is dependent on IL-13 and STAT6; and (5) that SPRR2 is induced in allergic inflammation in the gastrointestinal tract. Collectively, these results raise the possibility that SPRR2 may have an important role in the pathogenesis of allergic responses, especially with processes regulated by SPRR2 under other conditions including epithelial differentiation. In addition, these results broaden the involvement of SPRR into primary inflammatory conditions.

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