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A non-redundant role for mouse Serpinb3a in the induction of mucus production in asthma

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Abstract

Background—Asthma is a major public health burden worldwide. Studies from our group and others have demonstrated that SERPINB3 and B4 are induced in asthmatics; however their mechanistic role in asthma has yet to be determined.

Objective—To evaluate the role of Serpin3a, the murine homolog of human SERPINB3 and B4, in asthma.

Methods—We studied wild type Balb/c and Serpinb3a null mice in house dust mite or IL-13 induced asthma models and evaluated airway hyperresponsiveness, inflammation, and goblet cell hyperplasia.

Results—Airway hyperresponsiveness and goblet cell hyperplasia were markedly attenuated in the Serpinb3a null mice compared to the wild type mice following allergen challenge, with

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minimal effects on inflammation. Expression of SPDEF, a transcription factor that mediates goblet cell hyperplasia, was decreased in the absence of Serpinb3a. IL-13 treated Serpinb3a null mice showed attenuated AHR, inflammation, and mucus production.

Conclusions—Excessive mucus production and mucus plugging are key pathologic features of asthma, yet the mechanisms responsible for mucus production are not well understood. Our data reveal a novel non-redundant role for Serpinb3a in mediating mucus production through regulation of SPDEF expression. This pathway may be used to effectively target mucus hypersecretion.

Keywords

goblet cells; SPDEF; IL-13; hyperplasia

INTRODUCTION

Asthma is a chronic debilitating condition that affects over 22 million Americans, more than 6 million of whom are children. While current therapies have improved overall patient quality of life, a significant number of asthmatics do not respond well to existing therapies¹. Elucidating the key molecular components involved in the asthmatic response is crucial in identifying targets for novel asthma therapies. Recent microarray analysis of nasal epithelial cells from children suffering from an acute asthma attack identified 161 genes that were consistently and differentially regulated compared to samples from non-asthmatic controls². Several serpin (serine protease inhibitor) genes were upregulated, suggesting that this family of proteins may play key roles in mediating the asthmatic response.

Serpins were first identified as serine protease inhibitors, but have also been reported to inhibit cysteine proteases and caspases. Serpins mediate their inhibitory action by covalently binding to the target protease and adopting a highly stable conformation, at the same time destabilizing the protease³. In humans, SERPINB4 and its closely related homolog SERPINB3, were first identified as proteins elevated in squamous cell carcinomas⁴. SERPINB3 and B4 are expressed in the Hassall's corpuscles of the thymus, suprabasal layers of the stratified squamous epithelium of a variety of tissues and in the pseudo-stratified columnar epithelium of the conducting airways⁵. SERPINB4 and B3 share over 92% amino acid identity, with only 54% identity in the protease binding reactive center loop, which provides substrate specificity⁶. The known substrates for SERPINB3 include the cysteine proteases cathepsin S, K, L and papain⁷ whereas SERPINB4 inhibits the serine proteases cathepsin G and mast cell chymase⁸, and the cysteine protease Derp1, found in house dust mite⁹. SERPINB4 is an intracellular serpin that can be detected in the serum¹⁰. It has diverse functions, including regulation of E-cadherin expression and cell migration¹¹, and inhibition of apoptosis by preventing cytochrome c release¹². The human SCCA locus (chromosome 18q21) contains two paralogous genes, *SERPINB3* and *B4*. In mice, the locus (on chromosome 1D) is amplified to include four genes (*Serpinb3a*, *b3b*, *b3c*, and *b3d*) and three pseudogenes. Serpinb3a most closely resembles SERPINB4 and B3¹³, and targets both serine and cysteine like proteases¹⁴ serving the function of SERPINB4 and B3.

SERPINB4 and B3 have been implicated in allergic disorders. Elevated SERPINB4 and B3 levels are detected in the serum of patients with atopic dermatitis¹⁵ and asthma¹⁶. Recent studies suggests the use of SERPINB4 and B3 as potential biomarkers for atopic dermatitis¹⁷ and asthma¹⁸. SERPINB4 and B3 expression is induced in bronchial epithelial cells treated with IL-4 and IL-13¹⁹ in a STAT6-dependent fashion²⁰. Using a murine asthma model, we provide evidence that Serpinb3a contributes to airway hyperresponsiveness (AHR), goblet cell hyperplasia, and mucus production following house dust mite challenge, through a mechanism that results in induction of the transcription factors SPDEF and

FOXA3. Serpinb3a function lies downstream of IL-13 and Serpinb3a mediates pro-inflammatory signaling pathways downstream of IL-13 in addition to mucus production in the lungs. This is the first study that provides mechanistic insight into the role of Serpinb3a in asthma.

METHODS

Generation of Serpinb3a-deficient mice

Serpinb3a-deficient mice were generated by homologous recombination in 129/SV ES cells²¹. Exon 8, which encodes the critical reactive site loop (RSL) was replaced by a neomycin resistance cassette using linearized plasmid pAB20. A thymidine kinase gene (Fig. 1B, TK, green) was included to select against non-homologous recombination events. A successful homologous recombination event (Fig. 1B, bottom) resulted in the replacement of the coding region of exon 8 with the Neo^r gene (Fig. 1B, blue) and the generation of a novel BamHI restriction site. The probe used to discriminate between wild-type and recombinant alleles contained exon 5 and a portion of intron 5. Wild-type (+/+) and Serpinb3a-deficient (-/+, heterozygotes; -/-, homozygotes) mice were genotyped by Southern blotting of BamHI-digested genomic DNA. The genomic DNA probe detected 2.0 kbp recombinant and 4.5 kbp wild-type *Serpinb3a* alleles. The probe also detected the 6.5 kbp wild-type allele of *Serpinb3b* (Fig. 1C, green arrow). The screening over 1000 ES cell clones yielded a single recombinant with the correct genotype. This recombinant clone was injected into C57BL/6 blastocysts and the resulting progeny were examined for germline transmission. Heterozygous mutant animals were crossed and the Serpinb3a mutant and wild-type alleles segregated at the expected Mendelian frequency (~1:2:1). Mutant alleles in the BALB/c background were generated by outcrossing Serpinb3a+/- mice to BALB/c mice and then backcrossing 8 generations using the BALB/c N3 Max Bax speed congenics marker panel (Charles River Labs, Troy, NY). Absence of Serpinb3a expression was assessed by RT-PCR analysis (See Supplemental Methods for details).

Murine asthma model

All animal protocols were approved by the IACUC. House Dust Mite (*Dermatophagoides pteronyssinus*) (HDM) was purchased from Greer Laboratories (Lenoir, NC). Wild type Balb/c mice, purchased from Jackson Labs (Bar Harbor, ME), and Serpinb3a null mice (Balb/c background) were sensitized with 10µg HDM delivered intraperitoneally on days 0 and 7 (or PBS as control). The mice were then challenged with 100µg of HDM (or PBS as control) delivered intratracheally on days 14 and 21. Airway hyperresponsiveness (AHR) was measured using the airway pressure time index (APTI, described in the Supplemental Methods) on Day 23. For IL-13 treatment wild type Balb/c mice and Serpinb3a null mice were treated with 5µg of hIL-13 (Peprotech) delivered intratracheally on days 0, 3 and 6. On day 7 AHR was measured using APTI.

BALF collection and analysis

After the measurement of AHR, bronchoalveolar lavage was performed by cannulation of the trachea. The lungs were lavaged twice with 1.0 ml HBSS. The collected BALF was centrifuged, the cell pellet treated with red blood cell lysis buffer (Sigma Aldrich, St. Louis, MO), and the total cell numbers counted with a hemacytometer. Cells were spun onto slides and stained with the HEMA3 stain set (Fisher Scientific, Kalamazoo, MI). 200 cells were counted and the total number of each cell type calculated.

Flow Cytometry

Cells isolated from lung draining lymph nodes were stained for dead cells (7AAD from Pharmingen/BD Biosciences, San Jose, CA), B-cells (CD45R/B220-FITC from Pharmingen) and T cells (CD3e-APC, CD4-PE/Cy7 and CD8b-PE from BioLegend, San Diego, CA). Lymph node cells (5×10^5 cells) were incubated on ice for 1 hour with conjugated antibody, washed twice and resuspended in the presence of 7AAD. Acquisition was done on a FACS Canto II (Becton Dickinson, Mountain View, CA) and analyzed used FlowJo software (Tree Star, Ashland, OR).

IgG1 and IgE ELISAs

Plasma was diluted 1:100 for IgE and 1:10,000 for IgG1. BALF was used undiluted. ELISAs were performed using the appropriate kit from BD Biosciences. For measurement of HDM-specific IgE and IgG1 levels, wells were coated with 0.01% HDM (Greer Laboratories) overnight and the rest of the protocol was carried out as recommended in the kits.

Histology and Immunohistochemistry

The single left lobe of the lung was fixed in formalin, paraffin embedded and cut into 4 μ m sections. Sections were stained with hematoxylin and eosin (H&E) or PAS according to the manufacturer's recommendations. For Muc5AC staining, tissue sections were treated with proteinase K (Invitrogen, Carlsbad, CA) followed by a biotinylated pan Muc5AC antibody (NeoMarkers, Fremont, CA). CLCA3 immunostaining was performed using a rabbit polyclonal anti-mouse CLCA3 antibody (Abcam, Cambridge, MA; ab46512) as previously described²², but the pronase digestion step was eliminated. SPDEF staining has previously been described²³. FOXA3 staining was performed using the FOXA3 antibody from Santa Cruz Biotechnology (N-19, Santa Cruz, CA) as per the manufacturers' recommendations. The airways were scored for number of positive airways and staining intensity in each airway.

Scoring of Immunohistochemistry—To score the number and intensity of staining in the airways, the total number of distal airways in a section from the left lobe of the lung of each mouse was counted. Each airway was assigned a grade as follows: Grade 0- no positively stained cells, Grade 1 - < 30% of the airway was stained for the antigen, Grade 2 - 30–70% of the airway was stained positive, Grade 3 - > 70% of the airway was stained positive. Airways with a significant attenuation in the amount of staining per cell were also considered Grade 1 or Grade 2 airways. Representative airways from each grade for Muc5AC (Fig S3A), CLCA3 (Fig S3B), SPDEF (Fig S4A), FOXA3 (Fig S4B) and Muc5AC following IL-13 treatment (Fig S4C) are shown. The percent positive airways within each grade were then calculated. Statistical analysis of airway ranking is described in the statistical analysis section below. A minimum of five mice from each group from at least two independent experiments was scored.

Analysis of bronchial epithelial cells

Primary bronchial epithelial cells were isolated from subjects and cultured *in vitro* as previously described²⁴. Cells were treated with 10ng/ml IL-13 for 24 hours, harvested, RNA isolated and cDNA prepared using the Superscript First Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). Immortalized Human Bronchial Epithelial Cells (HBEC)²⁵ were treated for 8 hours with 50ng/ml IL-13 (Peprotech), 10ng/ml IL-4 (Peprotech), 50ng/ml TNF- α (R&D Systems) or 10ng/ml IFN- γ (R&D Systems). Cells were harvested, RNA isolated and cDNA prepared using the T-Primed First Strand Kit (Amersham Biosciences, Piscataway, NJ). Experiments were done in triplicate and repeated three times.

Statistical Analysis

All statistical analysis was done using PRISM software (GraphPad Software Inc., La Jolla, CA). Statistical significance was assessed using one-way ANOVA followed by a Tukey-Kramer post test. If there were significant differences, precise p-values were calculated using a two-tailed t-test comparing the two groups. In figure 7A, statistical significance was established using an unpaired t-test. Statistical analysis of immunohistochemistry was performed using the Chi-squared test on the number of positive airways/grade.

RESULTS

Generation and characterization of the Serpinb3a null mice

Like SERPINB4 and B3, the mouse homolog, Serpinb3a, is expressed in the lung and is a potent inhibitor of proteases¹⁴. To examine the role of SERPINB4 and B3 in asthma we generated a Serpinb3a-null allele and backcrossed it onto the Balb/c background (Fig. 1A, B, and C). Compared to the wild-type gene (Fig. 1B, middle), most of exon 8, including the critical reactive site loop, was replaced by the Neo cassette in pAB20. Serpins missing the RSL are incapable of inhibiting peptidases. Absence of Serpinb3a mRNA (but not Serpinb3b) was verified by RT-PCR analysis (Figure 1D). Serpinb3a null mice showed normal development and weight gain with no systemic abnormalities, with no differences in the levels of T and B lymphocyte subsets in their spleens (Fig. 1E) and lymph nodes (Fig. 1F) compared to wild type Balb/c mice indicating that the absence of Serpinb3a does not affect lymphoid cell development.

HDM induced airway hyperresponsiveness is attenuated in Serpinb3a null mice

In a murine model of asthma, allergen-induced airway hyperresponsiveness (AHR), as assessed by airway pressure time index (APTI), was significantly attenuated in Serpinb3a null mice compared to wild-type (WT) mice ($p=0.002$) (Fig. 2A). This suggests that while the AHR is not exclusively dependent on Serpinb3a, this protein has a non-redundant contribution to AHR in mice.

Serpinb3a does not contribute to allergen sensitization or inflammation induced by HDM

To assess the effect of Serpinb3a deletion on allergen sensitization, we measured the levels of IgG1 and IgE antibodies. Total and HDM-specific IgG1 and IgE in the BALF increased in HDM-treated mice, with no differences between the WT and Serpinb3a null mice (Fig. 2B, C, Supplementary Fig S1), indicating that Serpinb3a does not modulate allergen sensitization to HDM in our asthma model. We next assessed T and B cell populations in the lymph nodes. As expected, the percent of B cells increased following allergen challenge, with a concomitant decrease in the percent of CD3+ T cells (Fig 2D, E). Consistent with the IgG1 and IgE levels, there were no differences in the B- and T-cell numbers between the WT and Serpinb3a null mice. Together these data indicate that Serpinb3a does not affect the ability of the mice to initiate an immune response following allergen challenge.

A hallmark of asthma is increased migration of inflammatory cells into the lung and the BALF. We observed similar increases in the total numbers of cells in the bronchoalveolar lavage fluid (BALF) of HDM (house dust mite) challenged WT and Serpinb3a null mice (Fig. 3A). Differential staining revealed similar significant increases in eosinophil numbers in the HDM challenged WT and Serpinb3a null mice. Histological analysis of H&E stained lung sections revealed similar increases in airway and vascular smooth muscle thickness and increased numbers of inflammatory cells in the airways following HDM exposure, with no differences in the absence of Serpinb3a (Fig. 3B). Collectively, these data indicate that Serpinb3a does not regulate airway inflammation following HDM challenge.

Serpinb3a contributes to mucus production and goblet cell hyperplasia following HDM exposure

Mucin production was assessed using Muc5ac immunohistochemistry (a component of mucus upregulated in allergic airway responses ²⁶). The staining for each airway was assigned a grade from 0–3 based on the intensity and extent of staining. The differences in frequency distribution across the four grades were calculated by a chi-square test. WT mice showed a dramatic increase in Muc5ac positive cells and mucus in the airways following HDM challenge, which was significantly attenuated in the absence of Serpinb3a (p-val =0.005) (Fig. 4A, B), suggesting a non-redundant role for Serpinb3a in the induction of mucin accumulation in this model of asthma.

We performed immunohistochemistry for CLCA3, a specific and early marker of goblet cell differentiation ²⁷. Serpinb3a null mice displayed significantly less staining for CLCA3 after HDM challenge compared to WT mice (Fig. 4C). Quantification of the CLCA3 staining revealed an approximately 30% decrease in strongly positive airways in Serpinb3a-null versus WT mice (p-val =0.003, by chi-square analysis) (Fig. 4D), indicating that the observed decrease in mucus production is due to a reduction in the number of mucin producing goblet cells in the distal airways of Serpinb3a null mice. Notably, we did not observe a complete disappearance of goblet cells in the absence of Serpinb3a.

Expression of SPDEF and FOXA3 is attenuated in the Serpinb3a null mice

The transcription factor SPDEF is expressed at low levels in normal lungs, but its expression is strongly induced following allergen challenge, and increased expression of SPDEF in airway epithelial cells caused goblet cell hyperplasia in an IL-13 and STAT6 dependent manner ^{23, 28}. Thus SPDEF has been implicated in the pathogenesis of goblet cell hyperplasia. FOXA3 is also induced following allergen exposure and is associated with goblet cell hyperplasia ²⁸. Immunostaining of SPDEF and FOXA3 increased in WT mice following HDM treatment, but these increases were attenuated in the Serpinb3a null mice (pval < 0.001, by chi-square analysis) (Fig. 5A, B and Supplementary Fig. S2A, B respectively). Thus, Serpinb3a has a non-redundant role in the induction of these transcription factors following HDM challenge.

IL-13 induced AHR, mucus production, and airway inflammation are ameliorated in Serpinb3a null mice

IL-13 delivery directly to the airways of mice induces allergic airway disease, with increased airway hyperresponsiveness, inflammation, mucus production, and goblet cell hyperplasia, and blocking IL-13 activity inhibits AHR and mucus production following allergen challenge ²⁹. SPDEF expression is also induced in an IL-13-dependent manner ²³. Serpinb3a is induced by IL-13 in bronchial epithelial cells. To determine whether Serpinb3a was acting downstream of IL-13, we tested the ability of IL-13 to induce AHR and mucus production in Serpinb3a null mice. IL-13-dependent AHR was significantly attenuated in the Serpinb3a null mice (p=0.032) (Fig. 6A). Surprisingly, Serpinb3a absence reduced the number of inflammatory cells in the BALF following IL-13 treatment (Fig. 6B) supporting an additional role for Serpinb3a in IL-13-dependent airway inflammation. Consistent with the results following HDM exposure, mucin production was also decreased in Serpinb3a null mice following IL-13 treatment, indicating that effects of Serpinb3a are downstream of IL-13 (Fig. 6C,). Since there were significantly fewer positive airways in the WT IL-13 treated compared to the WT HDM treated lungs, we did not quantify the results of the immunostaining.

SERPINB4 expression is increased in bronchial epithelial cells from asthmatics treated with IL-13

Our previous studies had revealed that SERPINB4 is induced in nasal epithelial cells of children with uncontrolled (exacerbated) asthma compared to cells from control children without asthma or those with controlled (stable) asthma². We characterized SERPINB4 expression in human bronchial epithelial cells (HBE) from asthmatics and non-asthmatics. Primary HBE cells from asthmatics and non-asthmatics had similar low levels of SERPINB4 expression at baseline (data not shown). IL-13 treatment of primary bronchial epithelial cells from normal and asthmatic patients resulted in induction of SERPINB4 expression, but the magnitude of the increase was significantly higher in the asthmatic samples compared to the control group (Fig. 7A). Similarly, SERPINB4 was strongly induced in a human bronchial epithelial cell (HBEC) line treated with IL-13. Expression of SERPINB4 was also increased after treatment with IL-4, but not by TNF α or IFN γ (Fig. 7B). We examined other cell types, including human lung fibroblasts and primary pulmonary artery smooth muscle cells, but could not detect any SERPINB4 transcript, indicating that SERPINB4 expression is restricted to epithelial cells.

DISCUSSION

Our data establishes a novel role for Serpinb3a as a critical mediator of mucus production and increased AHR in murine models of asthma. While Serpinb3a is not required for goblet cell differentiation, it has a non-redundant role in the induction of IL-13- or allergen-induced goblet cell differentiation and mucin production, because Serpinb3a null mice failed to induce the magnitude of goblet cell hyperplasia and mucin production observed in the WT mice. In addition to the effects on mucus production and AHR, IL-13 treated (but not HDM treated) Serpinb3a null mice also showed attenuated migration of inflammatory cells into the BALF when compared to WT mice. This suggests that HDM induces inflammatory cell accumulation via pathways independent of IL-13 and Serpinb3a. Additionally, it places Serpinb3a farther upstream in the IL-13 signaling pathway, regulating both mucus production and inflammation.

There are three paralogs of *Serpinb3a* (*b3b*, *b3c*, and *b3d*) in mice. Serpinb3c and b3d expression were undetectable in the lung in our experiments, consistent with previous reports³⁰. However it remains possible that Serpinb3b could compensate partially for the absence of Serpinb3a in our mouse models such that the observed phenotype in the Serpinb3a null mice would be greater in the absence of Serpinb3a and B3b.

The mechanism by which Serpinb3a contributes to goblet cell hyperplasia involves increased protein levels of the transcription factors SPDEF and FOXA3. This likely involves a post-transcriptional mechanism since our data reveals that SPDEF mRNA was induced following allergen challenge equally in the presence or absence of Serpinb3a (Supplementary Figure S5). It is interesting to speculate that SPDEF is degraded by proteases that are inhibited by Serpinb3a, or perhaps that non-protease inhibitory functions of Serpinb3a regulate the activity and/or stability of this transcription factor.

Alternatively, the impact of Serpinb3a on SPDEF may be mediated indirectly through other Serpinb3a targets. Known targets for SERPINB4 include cathepsin G, mast cell chymase and Derp1^{9, 14}. Mast cell chymase and cathepsin G are pro-inflammatory mediators that have been implicated in mucus production in asthma^{31, 32}. Der p1 is a protease found in HDM and the protease activity is important in mediating the allergic response³³. While it is possible that Der p1 is a critical target for Serpinb3a, it is unlikely to be the sole target, because inflammation and mucin production are also attenuated in the Serpinb3a null mice following direct delivery of IL-13 (independent of allergen). If mast cell chymase and

cathepsin G were the sole targets for Serpinb3a, we would expect that Serpinb3a null mice would show exacerbated responses after allergen challenge. On the contrary, we observed an attenuated response to HDM in the Serpinb3a null mice, suggesting that mast cells chymase and cathepsin G are not major factors in the response to allergen, or that other mechanisms exist to inhibit these proteases in the absence of Serpinb3a. In any case, there are likely other functional targets of Serpinb3a that are protective in asthma. Since studies in tumor cell lines demonstrate increased apoptosis in response to UV damage in the absence of SERPINB3³⁴, we also evaluated apoptosis in the lungs following HDM treatment. We observed no induction of apoptosis by TUNEL assay (data not shown) in the mouse lungs, suggesting that the anti-apoptotic function was not the relevant mechanism in our asthma model.

Comparison of cultured bronchial epithelial cells of normal subjects and asthmatics revealed no differences in basal SERPINB4 expression. However, the magnitude of IL-13-induced SERPINB4 expression was enhanced in the HBE cells derived from asthmatics suggesting that asthmatic airway epithelial cells are more likely to express increased SERPINB4 in response to Th2 cytokines. Our study has demonstrated a key role for Serpinb3a in regulating mucus production. Excessive mucus production is a key pathologic feature of asthma and contributes to plugging of small airways. Current asthma therapies primarily target inflammation and bronchoconstriction. SERPINB4 may be an important new target for therapeutic intervention to specifically target mucus production, and may have some anti-inflammatory effects as well. There are several other conditions, such as viral infections^{35, 36}, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF)²⁶, in which mucus accumulation significantly contributes to the disease phenotype. Given our observations in asthma, Serpinb3a may regulate mucus production in these other conditions as well.

Key Messages

- The serine protease inhibitor Serpinb3a contributes to mucus production in a HDM model of asthma
- Expression of the transcription factor SPDEF, critical for goblet cell hyperplasia, is attenuated in the absence of Serpinb3a
- Serpinb3a contributes to IL-13 induced increases in AHR and inflammation

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

WT wild type

HDM	house dust mite
APTI	airway pressure time index
AHR	airway hyperresponsiveness
BALF	bronchoalveolar lavage fluid
IP	intraperitoneal
IT	intratracheal
CLCA3	calcium activated chloride channel 3
SPDEF	SAM pointed domain containing ETS transcription factor
FOXA3	forkhead transcription factor A3
SERPINB4	human SERPINB4 protein
SERPINB3	human SERPINB3 protein
Serpinb3a	mouse Serpinb3a protein
SERPINB4	human SERPINB4 gene
SERPINB3	human SERPINB3 gene
Serpinb3a	mouse Serpinb3a gene
Serpinb3b	mouse Serpinb3b gene
Serpinb3c	mouse Serpinb3c gene
Serpinb3d	mouse Serpinb3d gene

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Figure 1.

Generation and characterization of Serpinb3a null mice. A) Schematic of the mouse and human SCCA locus (grey arrows indicate pseudogenes). B). Schematic of the targeting vector (top), wild type (middle) and recombinant construct (bottom). B= BamH1 and E= EcoR1 restriction sites. The probe used for Southern blotting is indicated in red. (C) Genotyping by Southern blotting of BamH1-digested genomic DNA. The 2 kbp recombinant (red arrow) and 4.5 kbp wild-type alleles (black arrow) are indicated. The 6.5 kbp band is from wild-type Serpinb3b (green arrow). (D) Expression of Serpinb3a (top), Serpinb3b (middle) and β-actin (bottom) in wild type (WT) and Serpinb3a null (KO) mice. (E) % B and T cells in the spleen. (F) % B and T cells in the lymph node.

Figure 2.

Serpinb3a null mice show attenuated APTI following HDM challenge with no effect on sensitization (A) APTI measurements, (B) HDM specific IgG1 levels, (C) HDM specific IgE levels in plasma, (D) % B cells, and (E) % CD3⁺ T cells in the lymph node. Experiments include 6–8 mice per group. P values are shown only for statistically significant differences. The horizontal bar for each group represents the mean.

Figure 3. Serpinb3a null mice show no effect on inflammation following HDM challenge. (A) Differential cell counts in BALF, (B) H&E staining of airways, Experiments include 6–8 mice per group. P values are shown for statistically significant differences. Data shown are the mean $\pm$ SD. In panel B, the pictures were taken at 200x magnification and the inset panel was taken at 1000X magnification.

Figure 4. Decreased mucus production and goblet cell hyperplasia in Serpinb3a null mice following HDM challenge. (A) Representative images of Muc5AC staining (200X magnification). (B) Quantitation of Muc5AC staining. (C) Representative images of CLCA3 staining. (D) Quantitation of CLCA3 staining. In (B) and (D) Black bar = WT+HDM; White bar = KO+HDM. In B n=10 for WT+HDM and n=12 for KO+HDM. In D, n=9 for WT+HDM and n=10 for KO+HDM. Frequency distribution across the groups were significantly different between the WT and KO mice for both Muc5AC (p-val=0.005) and CLCA3 (p-val=0.003).

Figure 5. Decreased SPDEF expression in Serpinb3a null mice following HDM challenge. (A) SPDEF staining in WT and Serpinb3a null mice following PBS or HDM challenge. A representative image at 200X magnification from each treatment is shown. (B) Quantitation of SPDEF staining. Black bar = WT+HDM; White bar = KO+HDM.. n=13 for WT+HDM and KO+HDM.. Frequency distribution across the groups were significantly different between the WT and KO mice (p-val<0.001).

Figure 6.

The asthma phenotype is attenuated in the Serpinb3a null mice following IL-13 treatment. (A) APTI measurements (n=11–13 per group). (B) Differential BALF cell counts (n=11–13 per group). P values are shown only for statistically significant differences. (C) Representative images of Muc5AC staining (200X magnification).

Figure 7. SERPINB4 expression is induced by IL-13. (A) Quantitative PCR for SERPINB4 in primary bronchial epithelial cells from controls and asthmatics (6 subjects per group) treated with IL-13 for 24 hours. Data are presented as a fold increase in SERPINB4 expression in IL-13 treated cells compared to paired untreated controls. (B) SERPINB4 expression after treatment with IL-4, IL-13, TNF- α , or IFN- γ . Data shown are representative of 3 independent experiments (each done in triplicate). The horizontal bar for each group represents the mean in panel A. In panel B, data shown are the mean $\pm$ SD.