IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma

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Background: T_H2 cytokines are not responsible for the ongoing symptoms and pathology in children with severe therapy-resistant asthma (STRA). IL-33 induces airway hyperresponsiveness, but its role in airway remodeling and steroid resistance is unknown.
Objective: We sought to investigate the relationship between IL-33 and airway remodeling in pediatric patients with STRA.
Methods: IL-33 levels were quantified in neonatal mice given inhaled house dust mite (HDM), and the effect of blocking IL-13 on remodeling and IL-33 levels was assessed. HDM-induced allergic airways disease (AAD) in neonatal $ST2^{-/-}$ mice lacking the IL-33 receptor was assessed, together with collagen production after IL-33 administration. The effect of steroid therapy on IL-33 levels in patients with neonatal AAD was explored. IL-33 expression was quantified in endobronchial biopsy (EB) specimens from children with STRA and related to remodeling, and collagen production by airway fibroblasts from pediatric patients stimulated with IL-33 and budesonide was quantified.
Results: Blocking IL-13 after AAD was established in neonatal mice and did not reduce remodeling or IL-33 levels; airway hyperresponsiveness was only partially reduced. IL-33 promoted collagen synthesis both from asthmatic fibroblasts from pediatric patients and after intranasal administration in mice. Increased cellular expression of IL-33, but not IL-13, was associated with increased reticular basement membrane thickness in EB specimens from children with STRA, whereas remodeling was absent in HDM-exposed $ST2^{-/-}$ mice. IL-33 levels were maintained, whereas IL-13 levels were abrogated by steroid treatment in neonatal HDM-exposed mice and in EB specimens from children with STRA.
Conclusion: IL-33 is a relatively steroid-resistant mediator that promotes airway remodeling in patients with STRA and is an important therapeutic target. (J Allergy Clin Immunol 2013;132:676-85.)

Key words: *Asthma, pediatric, airway remodeling, steroid resistance, IL-33, therapy*

Children with severe therapy-resistant asthma (STRA) represent a small number of all asthmatic patients, yet they use a disproportionate amount of health care resources[1] and present a significant clinical challenge because they remain symptomatic despite maximal doses of conventional therapy.

The key pathophysiologic abnormalities of asthma include airway hyperresponsiveness (AHR), inflammation, and tissue remodeling, including increased thickness of the reticular basement membrane (RBM)[2] and increased smooth muscle mass.[3] We have shown that children with STRA have ongoing symptoms, abnormal lung function, and evidence of airway remodeling in the absence of the classical T_H2 cytokines (IL-4, IL-5, and IL-13) that are thought to mediate allergic disease in adults.[4] However, the high-dose maintenance steroid therapy that is prescribed in patients with severe disease might attenuate these relatively steroid-sensitive cytokines.[5-7] Critically, even in adults, severe asthma is recognized as being heterogeneous, with the T_H2 pathway being active in only select subgroups, as demonstrated by the variable efficacy of anti–IL-13 antibody therapy.[8] A significant limitation of current asthma therapies is their lack of effect on any features of airway remodeling, even though these structural changes contribute to disease chronicity. Specific steroid-resistant mediators driving airway remodeling in patients with STRA therefore require elucidation.

The importance of innate immune responses in asthma pathogenesis has become increasingly apparent,[9,10] with particular emphasis on the innate cytokine IL-33.[11] IL-33 is expressed in the epithelium and smooth muscle of adults with severe asthma.[12,13] It is also sufficient to initiate allergic airway responses in adult mice lacking T and B cells through the induction of IL-13–producing innate helper cells.[14,15] At present, little is known about the role of IL-33 in the pathophysiology of childhood asthma. Specifically, the contribution of IL-33 to airway remodeling and steroid resistance has not been investigated, although both are critical features of severe disease. Because the T_H2 cytokines were not predominant in our patients with STRA,[4] we hypothesized that IL-33 induces airway remodeling and is a steroid-resistant mediator in pediatric patients with STRA. We have investigated this using a neonatal mouse model of house dust mite (HDM)–induced allergic airways disease

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Abbreviations used
AAD: Allergic airways disease
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
EB: Endobronchial biopsy
HDM: House dust mite
RBM: Reticular basement membrane
STRA: Severe therapy-resistant asthma

(AAD) and endobronchial biopsy (EB) specimens and cultured airway fibroblasts from children with STRA. For the first time, we demonstrate the relationship between IL-33 levels and airway remodeling and identify IL-33 as a therapeutic target for severe steroid-resistant asthma.

METHODS

Animals and reagents

Female BALB/c mice and litters were maintained by in-house breeding in specific pathogen–free conditions and given food and water *ad libitum* (see the Methods section in this article's Online Repository at www.jacionline.org). Mice were matched for age and background strain. All procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986. Recombinant mouse IL-33 (50 μg/kg per mouse) for intranasal administration was purchased from eBioscience (Hatfield, United Kingdom). Anti–IL-13 antibody was a gift[16] and 10 mg/kg was administered intraperitoneally twice weekly. Relevant isotype-matched antibodies were used as controls. Budesonide Respules (Breath Ltd, Stevenage, United Kingdom) (0.25 mg/mL) were used for intranasal administration (0.6 mg/kg).

Allergen challenge

From day 3 of life, pups received either intranasal HDM extract (Greer, Lenoir, NC) or PBS 3 times per week, according to our published protocol.[17] For more information, see the Methods section in this article's Online Repository.

Measurement of AHR

Airways resistance was measured from 2 weeks of age by using the flexiVent small-animal ventilator (SCIREQ, Montreal, Quebec, Canada) according to our established protocols.[17] For more information, see the Methods section in this article's Online Repository.

Inflammation and cell recovery

Bronchoalveolar lavage (BAL) was performed with PBS through a tracheal cannula, as previously described.[17] Lavage fluid was centrifuged, and cell pellets were resuspended in 0.5 mL of complete medium. After lavage, 2 lobes from the right lung were dissected and processed, as previously published, to obtain lung cells.[17] The total cell yield of BAL and lung cells was quantified with a hemocytometer (Immune Systems Ltd, Devon, United Kingdom). For more information, see the Methods section in this article's Online Repository.

Quantification of cytokines

Lung tissue was homogenized, and the supernatant was collected (see the Methods section in this article's Online Repository). Cytokines were analyzed in lung homogenate supernatants. Paired antibodies for mouse IL-4 and IL-5 (PharMingen, San Jose, Calif), IL-13 and IL-25 (eBioscience), and IL-33 (R&D Systems, Minneapolis, Minn) were used in standardized sandwich ELISAs, according to the manufacturer's protocol.

Airway remodeling

Lung sections stained with Sirius red and Gordon and Sweet's silver stain, as previously described,[17] were used to quantify peribronchiolar collagen and reticulin levels, respectively, by using computer-aided image analysis (QWin version 3; Leica, Wetzlar, Germany). For more information, see the Methods section in this article's Online Repository.

RNA extraction and real-time PCR

Total RNA was extracted from 50 to 100 mg of lung tissue by using the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized from 1 μg of total RNA and analyzed by using PCR on a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, Calif). Reactions were run with TaqMan probes (Applied Biosystems, Foster City, Calif) and SsoFast Probe Supermix (Bio-Rad Laboratories). For more information, see the Methods section in this article's Online Repository.

Human data

Subjects. School-aged children (age, 6-16 years) with STRA[4] who were undergoing a clinically indicated bronchoscopy were recruited. Their clinical details have been published.[4] They were prescribed 800 μg/d or greater budesonide (or equivalent). Children with mild-to-moderate asthma (inhaled corticosteroids, <800 μg/d) and age-matched nonasthmatic control subjects having a bronchoscopy for another indication were also recruited. The study was approved by the institutional ethics committee, and written informed parental consent and child assent were obtained. For clinical details of all subjects, see the Methods section in this article's Online Repository.

Histopathology. EB specimens were processed to paraffin. Five-micrometer sections were stained with hematoxylin and eosin and used to assess morphology and quantify RBM thickness[4] and smooth muscle mass.[3] Sections were stained by using immunohistochemistry for IL-13 and IL-33. Human polyclonal rabbit anti–IL-13 antibody (PeproTech, Rocky Hill, NJ; dilution, 1:50) and Human Clone IL3305B mouse mAb (Alexis Biochemicals, Enzo Life Sciences, Farmingdale, NY) used at a dilution of 1:1000 in PBS and 1% BSA by using an avidin/biotin staining. Subepithelial cells were quantified and expressed per square millimeter of tissue.

Fibroblast culture and stimulation. Primary pediatric bronchial fibroblasts were isolated by outgrowth culture and used between passages 3 and 6. Fibroblasts were grown to 80% confluence. Recombinant human IL-33 was used between 10 and 100 ng/mL, and budesonide was used at a final concentration of 10^{-8} mol/L. In costimulations budesonide was added to cultures for 2 hours before the addition of rIL-33. Conditioned media were taken, and cell lysates were prepared in RIPA buffer. For more information, see the Methods section in this article's Online Repository.

Western blotting and densitometry. Cell lysates were cleared by means of centrifugation at 10,000*g* for 10 minutes. Five micrograms of protein per well in LDS sample buffer (Invitrogen, Carlsbad, Calif) was loaded onto 4-12% Bis-Tris gels (Bio-Rad Laboratories) and run at 150V in a Criterion Cell (Bio-Rad Laboratories). α-Smooth muscle actin primary antibody (ab5694; Abcam, Cambridge, United Kingdom) was added at 1:5000 dilution in blocking buffer and incubated overnight at 4°C. Horseradish peroxidase–conjugated anti-rabbit antibody (Cell Signaling Technology, Danvers, Mass) was used at 1:2000 dilution, and membranes were developed with ECL substrate (Pierce, Cheshire, United Kingdom) for film exposure (Amersham, Little Chalfont, Bucks, United Kingdom). For more information, see the Methods section in this article's Online Repository.

Soluble collagen measurement. Secreted collagen in conditioned media was assayed with the Sircol kit (Biocolor, Carrickfergus, United Kingdom), according to the manufacturer's instructions.

Statistical analysis

Nonparametric tests (Mann-Whitney *U* tests) were used to detect differences between groups by using GraphPad Prism 4 software (GraphPad Software, La Jolla, Calif), and statistical significance was accepted at a *P* value