REPOSITORY APPENDIX

Subjects

Exclusion criteria were: (1) use of oral, nasal, or IV steroids as well as nebulized or inhaled steroids with a face mask within the past 14 days; (2) nasal malformations, tumors, or nasal obstruction that precludes sampling; (3) bleeding diathesis; (4) co-morbid lung condition; (5) history of discharge home after birth from the NICU or nursery on supplemental oxygen; (6) dependence on oral steroids or an immunosuppressive agent for a medical condition other than asthma; or (7) history of a congenital cardiac anomaly and/or heart lesion requiring medication or surgery. Patients were discharged home when: (1) oxygen saturations greater than or equal to 91% on room air for at least 6 hours; (2) no evidence of respiratory distress; and (3) symptom control was achieved with albuterol nebulizer treatments a maximum of every 4 hours x 2.

Quantitative real time-PCR analyses

Gene-specific primers were designed by using Primer-BLAST software (National Center for Biotechnology Information, NCBI), in which at least one intron was spanned in the genomic sequence to ensure that mRNA-derived products were amplified and the contamination of genomic products was minimized. The sequences of primers for the target genes are listed in Repository Table E1. One to two μg of total RNA was used for cDNA synthesis per sample (SuperScript II cDNA synthesis kit, Invitrogen). RT-PCR analysis was conducted with the iCycler (Bio-Rad, Hercules, CA) by using the iQ SYBR Green Supermix Taq polymerase mix (Bio-Rad). The amount of double-stranded DNA product was indicated by the intensity of SYBR Green fluorescence and measured at the end of each extension cycle. The results were expressed as average fold changes in gene expression relative to the housekeeping gene *GAPDH*.

DNA isolation, bisulphite treatment, and pyrosequencing analysis

Genomic DNA was extracted from nasal epithelial samples using the Allprep DNA/RNA Micro Kit (Qiagen) according to the manufacturer's protocol. 200ng DNA from each sample was bisulphite modified using the EZ DNA Methylation Kit (Zymo Research). For pyrosequencing, a two-step PCR reaction was performed using primer pairs designed to amplify the target region specifically, with the reverse primer biotinylated. Primer sequences used for the bisulphite pyrosequencing reactions are listed in Repository Table E2. The chromosomal coordinates in the University of California at Santa Cruz February 2009 human genome assembly for each CpG site were shown. The annealing temperature used for both PCR reactions was 50°C. Pyrosequencing analysis was conducted using a Pyromark Q96 MD (Qiagen) at CCHMC Pyrosequencing Lab for Genetic and Epigenetic studies, and the DNA methylation percentage was determined by Qiagen Pyromark CpG software 1.0.11 (Qiagen).

Experimental Asthma Model

VNN1$^{-/-}$ mice were kindly provided by Dr. Ruslan Medzhitov at Yale School of Medicine with permission from Dr. Franck Galland at Université de la Méditerranée, France[E1]. Age- and sex-matched wild-type BALB/c mice were purchased from Harlan Laboratories (Indianapolis, IN). All mice were housed in a specific pathogen-free environment in the animal facility at Cincinnati Children's Hospital Medical Center. All animal procedures and protocols were approved by the Animal Care and Use Committee at the Cincinnati Children's Hospital Research Foundation.

Mice were exposed to intranasal doses of HDM (20 ug in 50 ul saline) or saline (0.9% NaCI, 50 ul; control group) 3 times a week for 3 weeks as previously described[E2]. Mice were treated with intra-peritoneal dexamethasone (3mg/kg in dimethyl sulfoxide, (DMSO)) or DMSO (100 ul) for the last 5 days of the 3-week model. Airway hyperresponsiveness (AHR) was assessed 24 hours after the last HDM challenge using a flexiVent system (SCIREQ, Montreal, QC, Canada) as previously described[2]. Bronchoalveolar lavage fluid (BALF) was collected, processed, and inflammatory cells were quantified analyzed as previously described[E2].

References

E1. Pitari G, Malergue F, Martin F, et al. Pantetheinase activity of membrane-bound Vanin-1: lack of free cysteamine in tissues of Vanin-1 deficient mice. FEBS letters 2000;483:149-54.

E2. Brandt EB, Kovacic MB, Lee GB, et al. Diesel exhaust particle induction of IL-17A contributes to severe asthma. The Journal of allergy and clinical immunology 2013;132:1194-204 e2.

Repository Table E1. Primer sequences used for qRT-PCR

Gene	Accession	Sense	Anti-sense
SRGN	NM_002727	CCTGGTTCTGGAATCCTCA	TCGAACATTGGTCCTTTTTCTT
SOD2	NM_001024465	TTACAGCCCAGATAGCTCTT	ATGGCTTCCAGCAACTC
HCK	NM_001172133	TCTGCATCCCTGGTGTGTAA	AAGTTGATGGCTTCAGGAG
VNN1	NM_004666	CTCAGTGGCACTTTCGG	CAACCTCCCAAACAGAGTTAC
GAPDH	NM_002046	GGGGAAGGTGAAGGTCGGAGTCA	AGCCTTGACGGTGCCATGGAAT

Repository Table E2. Primer sequences used for bisulphite pyrosequencing.

		Sense	Antisense
PCR	Internal	AGGTGTTGTTTTTTTAATTATATTA	[a]5Biosg/CTTAACTCCAAAAAAATTCACTTCC
	External	TTTAAAGATGGTTTTTAATGTTTTATTG	CCCAAAATCTCTTTCACAAAACTAC
		Chromosomal coordinates[b]	**Sequence**
Sequencing	CpG1, 2	chr6: 133036726 and 133036717	AGGTGTTGTTTTTTTAATTATATTA
	CpG3	chr6: 133036652	GTTGTTTGGTTTTAAGGGAATTTAG
	CpG4, 5	chr6: 133036574 and 133036546	TTGGGTAATAATTAATGAGGTTTTG

[a] 5'-biotin. [b] The chromosomal coordinates of each CpG site were retrieved from University of California at Santa Cruz February 2009 human genome assembly.