VDR activity even in subjects with asthma and with IL-13, highlighting retained functionality. Expression of Class I histone deacetylases 1–3 (HDAC) and overall HDAC activity were lower in IL-13–exposed ASM, but calcitriol enhanced HDAC expression/activity.

Conclusions: In asthmatic ASM, Vit D functionality is maintained, allowing calcitriol to reduce the procontractile and proremodeling effects of inflammatory cytokines, particularly IL-13, which is relevant to asthma. These findings highlight a potential role for Vit D in asthma pathogenesis, particularly in the context of airway structure and functional changes early in disease.

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Identifying Molecular Mechanisms of the Late-Phase Asthmatic Response by Integrating Cellular, Gene, and Metabolite Levels in Blood

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Rationale: Individuals with allergic asthma respond differently, but reproducibly, to allergen inhalation challenge. Some individuals develop an isolated early response (early responders) (ERs), whereas others go on to develop a late response (dual responders) (DRs). It is not understood why late responses do not develop in all sensitized individuals.

Objectives: The aim of this study was to identify blood biomarkers that can discriminate ERs and DRs using cellular frequencies and gene and metabolite expression from whole blood.

Methods: Thirty-two individuals participated in the allergen inhalation challenge as part of the AllerGen Clinical Investigator Collaborative. Fifteen participants were classified as ERs and 17 as DRs. Blood samples were collected before (pre) and 2 hours after (post) the allergen challenge. Cell counts were obtained using a hematolyzer, gene transcript relative levels using RNA sequencing, and metabolite concentrations using tandem mass spectrometry. An integrative ensemble algorithm that was based on canonical correlation analysis was used to classify ERs and DRs using all three data sets, adjusting for age and sex. The objective of this algorithm was to identify a correlated subset of molecules from each data set that best discriminated ERs from DRs. Gene set enrichment analysis was performed using Enrichr (Chen *et al., BMC Bioinformatics* 2013;128).

Measurements and Main Results: The pre-challenge multisignature classifier (error = 30%) outperformed the post-challenge multisignature classifier (error = 50%) in separating ERs from DRs. The cells selected in the prechallenge multisignature panel included eosinophils, lymphocytes, and neutrophils. The selected metabolites were enriched for glycerophospholipids. The subset of gene transcripts in the multisignature panel was enriched for the T-cell receptor and costimulatory signaling pathway ($P = 3.4 \times 10^{-6}$) (Wikipathways) and positive regulation of antigen receptor–mediated signaling pathway ($P = 5.7 \times 10^{-4}$) (GO Ontology).

Conclusions: This study provides a systems perspective on the deregulated molecular processes between early and dual responses in whole blood. The integrative biomarker analysis suggests that a molecular signature that is predictive of the late-phase response can be identified. The variability in the onset of the late response may explain the poor predictive performance of the postchallenge multiomic biomarker signature. Replication of the prechallenge biomarker signature in additional independent samples is required to validate this panel.

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Mucins and Their Sugars
Critical Mediators of Hyperreactivity and Inflammation

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Excessive mucus causes severe airflow obstruction in fatal asthma. It is also present in mild to moderate disease, but is poorly understood and treated. Mucus overproduction is associated with dysregulated expression of the mucins MUC5AC and MUC5B. Whereas increased MUC5AC is a consistent finding, MUC5B

varies—remaining stably produced in some patients but strongly repressed in others (>90%). Patients with lower MUC5B display worsened asthma phenotypes including airway hyperreactivity (AHR) to methacholine (MCh) and eosinophilic inflammation. To better understand the roles of mucins in asthma, we generated *Muc5ac* and *Muc5b* knockout ($^{-/-}$) mice. AHR to MCh was abolished in antigen-challenged *Muc5ac*$^{-/-}$ mice, due to prevention of heterogeneous mucous plugging that occurred in allergic wild-type mice during MCh-induced bronchoconstriction. Thus, in addition to the established role of smooth muscle–mediated airway narrowing, Muc5ac is an essential noncontractile AHR component. We also found that, unlike *Muc5ac*$^{-/-}$ mice, *Muc5b*-deficient mice were not protected from asthma phenotypes. Furthermore, whereas inflammation was unaffected by Muc5ac deficiency, it was exaggerated in the absence of Muc5b. On the basis of these differential effects, we are now determining how asthma phenotypes are regulated by mucin isoform specificity. Glycosylation is dramatically different: Muc5ac is heavily fucosylated whereas Muc5b is mainly sialylated. Fucosylation increases mucus viscoelasticity, and FUT2, the enzyme that catalyzes mucin α1,2-fucosylation, is associated with severe asthma exacerbation risk. Sialylation is required for binding to siglec (*sial*ic acid–binding *i*mmuno*g*lobulin-like *lec*tin) receptors on leukocytes. Eosinophils express Siglec-F (mouse) or Siglec-8 (human). Engagement by sialoside ligands induces eosinophil apoptosis, and Muc5b via sialylated termini that require the α2,3-sialyltransferase ST3Gal3 for synthesis binds Siglec-F and induces apoptosis in mouse eosinophils. Because Muc5b is required for host defense in mouse lungs, inhibiting MUC5AC while preserving or enhancing MUC5B functions may be effective for treating asthma.

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Amish and Hutterite Environmental Farm Products Have Opposite Effects on Experimental Models of Asthma

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The critical role of environmental exposures in asthma development is illustrated by asthma protection among European children raised on traditional farms, but specific protective exposures and the underlying mechanisms are unknown. Differences in asthma prevalence are recapitulated in two farming populations in the United States, the Indiana Amish and the South Dakota Hutterites, who share multiple lifestyles associated with asthma risk or protection. However, asthma prevalence is 2–3% among the Amish, who practice traditional farming, and 15% or higher among the Hutterites, who embrace modern farming.

To begin dissecting the mechanisms underlying asthma protection and risk among the Amish and Hutterites, aqueous extracts of dust collected from Amish or Hutterite homes were administered in an ovalbumin (OVA) model of experimental asthma. Amish dust extracts (DE) were sufficient to protect OVA-treated Balb/c mice from airway hyperresponsiveness and bronchoalveolar lavage (BAL) eosinophilia. BAL IL-13 and IL-5 were abrogated, whereas IL-17 and IFN-γ were unaffected. In contrast, Hutterite DE exacerbated OVA-induced airway hyperresponsiveness and did not affect BAL eosinophilia and cytokine production. Lung gene expression profiling revealed multipronged suppression of OVA-dependent pathways related to airway remodeling and epithelial, dendritic, alternative macrophage and Th2 cell activation in mice treated with Amish but not Hutterite DE.

Because treatment with Amish and Hutterite house DE recapitulates the asthma protection and risk profiles found in the two populations, we conclude that the different asthma prevalence among the Amish and Hutterites depends on distinct environmental exposures.

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DNA Methylation Changes in Nasal Epithelia Are Associated with Allergic Asthma in the Inner City

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