CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Environmental Microbial Exposure and Protection against Asthma

Patrick G. Holt, D.Sc., and Peter D. Sly, D.Sc.

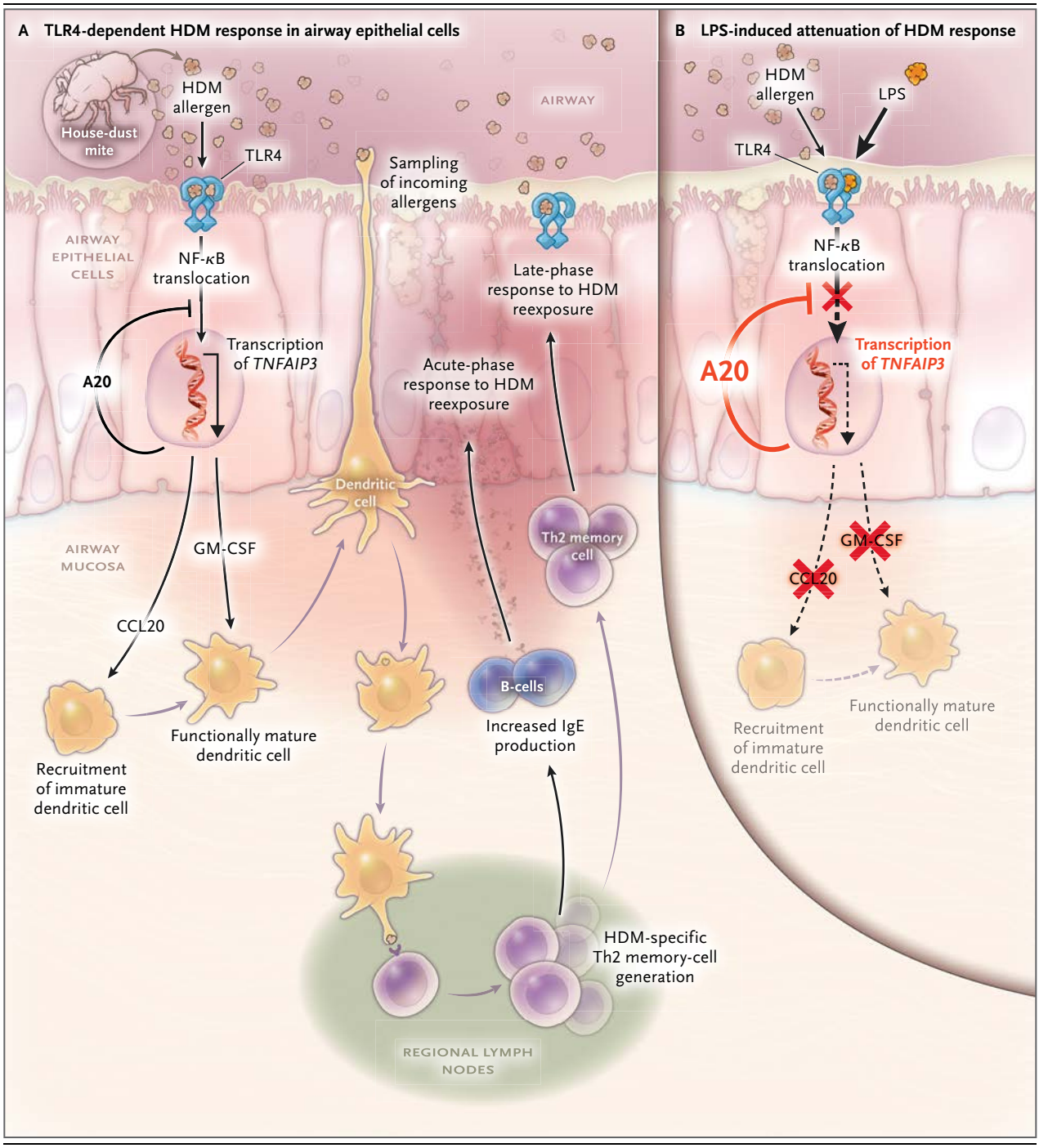
Despite the increasing availability of highly specific T-helper type 2 (Th2) antagonists, atopic asthma remains a major cause of morbidity among children and adults and may require lifelong treatment. Prospective birth-cohort studies have shown that asthma usually begins to develop during infancy or soon after. Increasingly, interest is turning toward the prevention of asthma through the targeting of early-stage progression during childhood.[1] Accordingly, international attention has been drawn to the epidemiologic dissection of the "experiment of nature" involving the natural resistance to asthma and allergy among children born into traditional dairy-farming households in northern Europe.[2] The identification of microbe-rich animal-barn dust that contains high levels of bacterial lipopolysaccharides as one of the prime mediators of this protective effect reflects the findings of earlier studies, in which an inverse relationship has been reported between levels of lipopolysaccharides in household dusts and the prevalence of allergy in urban children. These findings have fueled studies of the mechanisms underlying this relationship in order to develop asthma-preventive therapeutics.

Schuijs et al.[3] recently described a study designed to elucidate the molecular mechanisms underpinning the barn-dust effect. Focusing on the atopic component of asthma, they showed that a key initial step in the process of sensitization to the house-dust mite is the allergen-induced activation of airway epithelial cells mediated by toll-like receptor 4 (TLR4), which elicits secretion of the chemokine CCL20 and granulocyte–macrophage colony-stimulating factor (GM-CSF), both of which are required for the recruitment and functional maturation of mucosal dendritic cells (Fig. 1). These cells are responsible for local immune surveillance. A subset of these cells mediates the transport of inhaled allergen to regional lymph nodes, where the allergen primes specific Th2 memory cells to produce IgE the next time they are exposed to the allergen (e.g., a house-dust mite). Another subset of the cells responsible for local immune surveillance resides in the airway mucosa and activates incoming Th2

Figure 1 (facing page). Suppression of the Inflammatory Response through Exposure to House-Dust Mites.
Sensitization to the house-dust mite (HDM) and the ensuing expression of HDM-induced, T-helper type 2 (Th2)–mediated airway inflammation can be amplified by the activation of innate immune responses to the allergen in airway epithelial cells, which is triggered by toll-like receptor 4 (TLR4) (Panel A). TLR4 signaling results in the activation of nuclear factor κB (NF-κB), which in turn activates epithelial genes (e.g., *TNFAIP3*) that encode proinflammatory mediators, including chemokine CCL20 and granulocyte–macrophage colony-stimulating factor (GM-CSF). These mediators are responsible for the recruitment and functional maturation of mucosal dendritic-cell precursors. At baseline, mucosal dendritic cells have a rate-limiting effect on the capacity to generate and express local T-cell immunity. In response to these epithelial signals, the local dendritic-cell network up-regulates the priming and activation of T cells, facilitating the expression of potent asthma-associated, Th2-dependent inflammatory responses in the airways if HDM exposure continues. An internal negative feedback loop — involving coexpression of the NF-κB attenuator A20, encoded by *TNFAIP3* — operates to modulate the production of GM-CSF and CCL20. However, at baseline the levels of A20 expression may be insufficient to effectively shut down these HDM responses. In the model described by Schuijs et al. (Panel B),[3] concomitant exposure to bacterial lipopolysaccharides (LPS) (or other microbial-derived TLR4 ligands) up-regulates A20 expression to levels that suppress this HDM-induced pathway, effectively truncating the epithelial-cell–driven amplification loop.

memory cells, which in turn secrete cytokines that drive the airway inflammation that is characteristic of the late-phase asthmatic response.

Consistent with the findings of earlier epidemiologic studies,[4] Schuijs et al. observed in their study in mice that airway exposure to lipopolysaccharides before or during allergen exposure markedly attenuates the asthmalike pattern of proinflammatory responses to inhaled house-dust mites, but they also went on to link this effect to a reduction in the activation of local dendritic-cell functions mediated by CCL20 and GM-CSF. They further showed that exposure to lipopolysaccharides suppresses the responsive-



ness of airway epithelial cells to TLR4-induced activation by the house-dust–mite allergen and that this suppression is dependent on the attenuation of signaling by nuclear factor κB (NF-κB). The authors attributed the protective effect of lipopolysaccharides to an increase in the synthesis of the enzyme A20, an attenuator of NF-κB, in airway epithelial cells. (A20 is encoded by *Tnfaip3*.)

Follow-on experiments involving cultures of the human bronchial epithelial air–liquid interface revealed a similar inverse association between lipopolysaccharide-mediated stimulation of GM-CSF production and *TNFAIP3* activation. This association was further examined in a case–control study in which investigators observed a relative deficiency in the lipopolysaccharide-mediated production of A20-specific mRNA in airway epithelial cells from persons with asthma as compared with healthy controls. To cap the "case for the prosecution," the authors observed a positive association between a polymorphism in *TNFAIP3* and susceptibility to asthma in the population of GABRIELA (Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community [GABRIEL] Advanced Study), a study that focuses on the effects of farm environments on asthma and allergy in children from central Europe.

Collectively, these findings represent an impressive body of circumstantial evidence that supports the idea that the production of A20 by airway epithelial cells plays an important role in protecting against the development of asthma, implicating A20 as a potential therapeutic target. Schuijs et al. added a note of caution to their discussion because of experiments involving mice in which the expression of A20 was selectively knocked down in pulmonary epithelial cells.[3] The investigators observed that while the effects of the knockdown abrogated lipopolysaccharide-mediated protection against the manifestations of asthma induced by the house-dust mite, they observed only partial suppression of CCL20 and GM-CSF production, suggesting the existence of other molecular mechanisms through which lipopolysaccharides suppress asthma.

This apparent heterogeneity is not unexpected and reflects the complex nature of the causal pathways that are being reported in the literature on asthma, especially in relation to the role of environmental microbial stimuli in these processes. In particular, it has become evident that atopy-associated inflammatory pathways triggered by aeroallergens may interact with host responses to common respiratory viral infections in early life, leading to great increases in the risk of asthma.[1] Moreover, these interactions are in turn modulated by the balance between bacterial pathogens and commensals present in the upper airways at the onset of infection.[5] What's more, the diversity of both bacterial and fungal species present in barn dust has been identified as a major determinant of the asthma-protective activity of the barn-dust agent,[1] suggesting the involvement of multiple mechanisms triggered by microbial pattern-recognition receptors. All that being said, the possibility that *TNFAIP3* and its product, A20, may be part of a final common pathway that mediates these effects in airway epithelial cells merits more detailed investigation.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From the Department of Cell Biology, Telethon Kids Institute, Perth (P.G.H.), and Queensland Children's Medical Research Institute, Brisbane (P.D.S.) — both in Australia.

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