Killing Two Birds with One Stone: Mold-induced Pulmonary Immune Responses and Arterial Remodeling

Molds are ubiquitous because they have simple requirements to thrive. First, their required substrate is cellulose, found in plaster board, newspapers, corn husks, or cellulose ceiling tiles. Second, they require moisture from either high humidity or water intrusion into buildings. Finally, molds need warm temperatures. Thus, almost every home has a potential for mold contamination.

"Killing two birds with one stone" is a common expression. The meaning is clear. Why not achieve two objectives at once with a single action? In this issue of the *Journal*, Croston and colleagues (pp. 563–576) show both pulmonary arterial remodeling and a mixed T-helper cell type 1 (Th1)/Th2 T-cell response to two different strains of the toxic mold *Stachybotrys chartarum* (1). Their project was designed to explore pulmonary immune responses to mold. When the authors examined mouse lungs exposed to mold, they also discovered remodeling of pulmonary blood vessels. The authors achieved two objectives simultaneously. They enlarged our understanding of the mechanisms mold uses to induce pulmonary inflammation. They also generated ideas about the development of pulmonary arterial hypertension and the possible role of mold in altering pulmonary vascular smooth muscle.

In humans, mold spores and fragments are major factors in damp building–related illness, a type of sick-building syndrome (2). Many patients with damp building–related illness develop persistent pathologies, including idiopathic environmental intolerance (IEI), sometimes characterized as multiple chemical sensitivity. People with IEI are often sensitive to a wide array of substances, such as fragrances, petroleum, and household cleaning products, as well as to fungal products at concentrations far lower than those that affect others. Reactions include bronchitis, headaches, skin rash, confusion, fatigue, and muscle and joint pain (3). Patients with IEI typically report a notable initiating exposure, which is frequently due to living in a moldy home or working in a moldy office (4). Subsequently, low-level exposures to mold or other airborne contaminants trigger symptoms even after the initiating event.

The pathogenesis of IEI is controversial but likely involves activation of the immune system. Asthma is common in people with IEI, with 71% of sufferers diagnosed with asthma compared with 27% of people without IEI, suggesting an allergic aspect (4). Trichothecene mycotoxins produced by *S. chartarum* are candidates for initiating the immune responses that lead to IEI and other persistent responses to mold exposure.

Future studies connecting mold to pulmonary vessel remodeling should emphasize characterizing mechanisms. Do mold-exposed cells die by necrosis or apoptosis? Apoptosis is increasingly recognized as an important mechanism in environmental exposures (5–7). An emerging topic in lung biology and respiratory diseases is the role of rare cells. Studies of individual cells have revealed that even in apparently uniform cell layers such as the respiratory epithelium, cells that are few in number may have critical physiological and pathogenic roles, such as ionocytes, tuft cells (Fox11 positive) (8), and neuroendocrine cells.

Responses to mold are influenced by the genetics of both mold and mice. Rosenblum Lichtenstein and colleagues (9–11) showed that different strains of mice respond differently to mold. Croston and colleagues show that repeated exposures to two different strains of *S. chartarum* lead to different patterns of inflammation and arterial remodeling (1). Both mold strains induced a mixed Th1/Th2 response after 13 weeks of biweekly exposure, but only strain A showed evidence of arterial remodeling and a marked Th2 response after 4 weeks of biweekly exposures. Notably, strain A was more fragmented and contained smaller particles (0.5–2-μm diameter) than strain B. Strain A was also higher in the trichothecene verrucarol. Further research is needed to determine if the particle size or mycotoxin content or both are responsible for the more rapid immune response and arterial remodeling.

In the study by Croston and colleagues (1), the mice showed no arterial remodeling or immune response to heat-inactivated control spores. This is surprising, given that macrocyclic trichothecenes cause inflammatory responses in the absence of spores (12, 13). Follow-up studies should evaluate whether macrocyclic trichothecenes such as verrucarol survive the heat inactivation protocol. On the one hand, if macrocyclic trichothecenes are destroyed by heat inactivation, this may suggest best practices in mold remediation. On the other hand, this may represent trichothecene-induced immune evasion (14).

A strength of the article by Croston and colleagues is the integration of proteomic and microRNA analyses to assess changes in gene regulation. They report upregulation of genes involved in tissue remodeling and inflammation, consistent with their cellular and histological data. The proteomic data strongly support their assertion that there is a mixed Th1/Th2 response to strain A. The microRNA data are consistent with the proteomic analysis and the changes in inflammatory mediators. Similarly, the microRNAs are consistent with the infiltration of eosinophils into the lung.

Mold has become a growing concern as a consequence of climate change. We have become more vulnerable to water damage. We see more intense hurricanes, and they move more slowly and thus drop larger quantities of water in a given location (e.g.,

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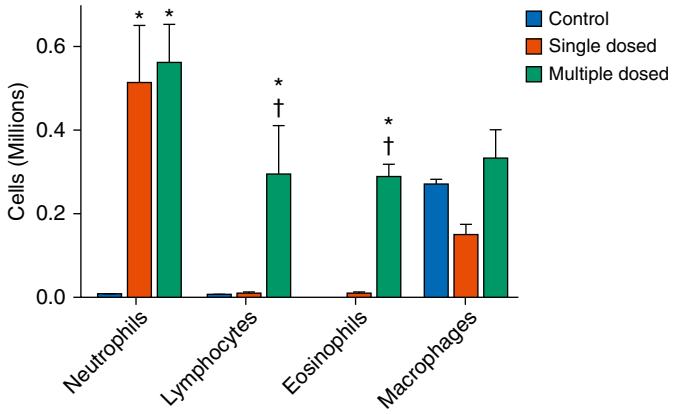

Figure 1. The effects of mold exposure on cells in BAL fluid. Repeated exposures of mice to *Stachybotrys chartarum* shift the immune response from type 1 to type 2. Mice intratracheally instilled with *S. chartarum* weekly for 7 weeks (multiple dosed), with saline instilled weekly for 6 weeks followed by a single intratracheal instillation of *S. chartarum* in the seventh week (single dosed), and with saline instilled weekly for 7 weeks (control). BAL analysis was performed 24 hours after the last instillation. *Adjusted false discovery rate (FDR) q value less than 0.05 versus control; †adjusted FDR q value less than 0.05 versus single-dosed mice; two-way ANOVA. Data are mean (±SE); $n = 10–12$. Adapted by permission from Reference 11.

>60 inches of rain fell near Houston, TX, during Hurricane Harvey in 2017 [15]). Aggravating these changes in weather are lax building restrictions and their enforcement. Houses are built in river flood plains or on coastal barrier islands. Measuring the acute and chronic effects of mold provides ammunition for rational regulations governing where and how homes and offices are built, together with their design, maintenance, and insurance.

Mold antigens and macrocyclic trichothecenes may elicit immune responses after repeated exposures (10, 11). Importantly, the Croston paper describes repeatedly exposed mice. The repeated exposures and duration of this study (biweekly exposures for 13 wk) emphasize that chronic exposures to *S. chartarum* have very different consequences from acute exposures. Our mouse experiments (*see* Figure 1) compared naive mice with multiple-dosed mice.

Given the compelling images of pulmonary arterial remodeling that Croston and colleagues report (1), future studies should measure quantitative parameters characteristic of pulmonary arterial hypertension, including vessel wall and smooth muscle layer thickness and right heart hypertrophy. Croston and colleagues not only demonstrate the course of pulmonary inflammation but also show progressive pulmonary arterial remodeling. Both observations should be pursued further. ■

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