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Environmental exposures and innate immunity in the lung

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The lung serves a vital role in immunity by sensing and responding to inhaled particles and pathogens. A properly functioning respiratory immune system will “send out an alarm” only for truly harmful substances (e.g. viable microbes) but not to otherwise harmless encounters (e.g. allergens). Why this process breaks down in asthma and other immune-mediated diseases is unknown. During human evolution, early hominids were exposed to airborne particulates in the form of wind-blown dusts, volcanic ash, and pollens (some of which are quite ancient ¹). Thus the capacity for the respiratory tract to handle inhaled particulates is a primitive feature and likely evolved over millions of years. However, the appearance of combustion derived carbon-rich particles during the Industrial Revolution, and more recently engineered nanoparticles in the 20th century, are very recent phenomena, and it is increasingly apparent that pulmonary defense mechanisms do not know how to handle these more modern insults.

Pulmonary defense mechanisms can be compartmentalized into innate versus adaptive components. The innate immune system is positioned at the interface between the host and the external environment, and includes both structural lung epithelial cells and hematopoietic cells. Innate immune responses are characterized by rapid onset and lack of memory. Among hematopoietic innate immune cells, dendritic cells (DC) are intimately associated with epithelial cells and are one of the key sentinels that sense inhaled particles and pathogens. Since DC are particularly effective at antigenpresentation to T cells, they provide a key link between the innate and adaptive immune system. Dendritic cells receive cues from neighboring epithelial cells that influence their activation state, and subsets of dendritic cells exist at mucosal surfaces that have specialized function ².

In this issue of the *Journal*, five articles address different aspects of how the lung innate immune system senses and responds to different environmental exposures including inhaled toxicants. These articles were written by authors with complementary expertise, and include a critical review of literature in this fast moving field together with thoughtful analysis of relevance for human health and disease. Several articles cover areas of controversy including seemingly protective effects of “harmful” exposures, which is particularly informative.

Astrakianakis and Murray (JEIT-D-12-00017) review the fascinating observation that whereas occupational exposure to endotoxin (or lipopolysaccharide, LPS) is associated with the increased risk of certain lung diseases (e.g. chronic obstructive pulmonary disease, COPD), endotoxin exposure is paradoxically also associated with lower risk of lung cancer.

Exposure to endotoxin occurs in farming environments, and is known to affect the risk of atopic diseases³. The authors propose an interesting hypothesis that endotoxin might promote a beneficial anti-tumor immune response via Lipid A, tumor necrosis factor (TNF)- α , IL-12 and other mediators, although these relationships are neither simple nor monotonic. The authors speculate that these mediators may induce anti-tumor immune responses involving natural killer (NK) cells and other immune pathways. One of the challenges in this area relates to difficulties in modeling real world occupational exposures over years in high-risk workers using in vitro or animal models. These challenges are not unique to endotoxin exposure, and apply to any model of environmentally mediated lung disease. As the authors note, it is difficult to extrapolate data obtained from clinical protocols using LPS to treat established cancers to the epidemiologic evidence indicating that endotoxin has a protective effect on cancer development long-term. Future studies and animal models that faithfully recapitulate the complexities of cancer development in humans will provide new insights into these interesting relationships.

One important determinant of the outcome of environmental exposures on lung immune responses is the age of the host at time of encounter. A fascinating new and emerging area of lung biology indicates that exposures early life (including during development in utero) can have a long-lasting impact on lung structure and function and influence susceptibility to diseases later in life. The mechanisms by which early life exposures can have such long-lasting effects, some of which may even be trans-generational, are poorly understood.

Ramsey, Foong, and Zosky (JEIT-D-12-00019) provide a timely review of this topic and describe how early-life exposures can influence lung-development leading to long-lasting changes in structure, and also influence the cellular composition of the lung leading to alterations in immune responses. It is worth remembering that unlike intestinal epithelial cells, which undergo very rapid turnover, the lung epithelium is much more quiescent⁴. Consequently, environmental exposures that do not induce cell injury or death can potentially affect lung innate immune responses by affecting long-lived epithelial cells.

Several environmental exposures have been shown to have a long-lasting effect on lung development including hyperoxia, cigarette smoke, nutrition deficiencies and air pollution^{5, 6}. Ramsey and colleagues specifically focus on arsenic toxicity and vitamin D deficiency. The authors document the tragic events occurring in Chile in the 1950's, during which early life arsenic poisoning resulted in severe and sometimes fatal lung fibrosis in children. Exposure in utero appeared to be particularly important and conferred risk not only for lung cancer but also for bronchiectasis in adults. The authors review recent studies using mouse models documenting how arsenic exposure during development can affect not only somatic growth and lung size, but also alter lung composition by inducing smooth muscle growth cells and epithelial cell differentiation. The later process involves alteration in the expression of key genes in epithelial cells (e.g. CLCA3 and CFTR) that presumably affect airway surface liquid composition and mucociliary clearance.

Vitamin D deficiency is currently being intensively studied because of the pleiotropic effects of this vitamin on development and immunity. Low vitamin D levels are widely assumed to be a risk factor for asthma, and the authors summarize this fast-growing area including the controversies in the field given the results from a vitamin D supplementation study in

Finland showing increased prevalence of asthma and atopy later in life⁷. Ongoing clinical trials of vitamin D supplementation that are currently underway should provide much needed clarity in this area. Like arsenic, vitamin D deficiency could affect lung structure later in life by delaying or retarding somatic growth. The authors summarize recent research in mice uncovering specific effects of vitamin D deficiency on alveolar epithelial cell differentiation, surfactant gene expression, and lung alveolarization. Interestingly, vitamin D also can regulate airway smooth muscle cell growth, suggesting that early-life vitamin D deficiency may be a risk factor for asthma since increased airway smooth muscle mass is an early feature of asthma⁸.

The molecular mechanisms by which the lung innate immune system senses foreign and dangerous inhaled microbes is a rapidly growing area of research. Different receptor families that recognize conserved microbial epitopes have been uncovered, including the Toll-like receptor (TLR) and intracytoplasmic helicases. The recent recognition of an entirely new class of receptors termed the “inflammasome” was a major breakthrough in the field. Inflammasomes are comprised of multiple components and result in activation of the IL-1 family of cytokines including IL-1b and IL-18. **Ather, Martin, Ckelss, and Poynter (JEIT-D-13-00001)** provide a detailed and thoughtful review of the potential role of inflammasome-dependent signaling in environmentally mediated lung diseases. The precise mechanisms by which inflammasomes are activated intracellularly are complex, and involve sensing of cellular redox status including reactive oxygen and nitrogen species. The author's state-of-the-art review summarizes the role for inflammasomes in asthma, redox regulation, and in the response to nitrogen dioxide, cigarette smoke, diesel exhaust particles, silica and asbestos exposure, engineered nanoparticles, metabolic syndrome and trauma. Important recent research indicates a crucial role for inflammasomes in the initiation of allergen-specific immune responses in the airway in asthma. One important observation is that requirement for inflammasomes in the initiation of immune responses to model allergens depends on the route of sensitization and nature of allergens and adjuvants used (as summarized in Table 1 of Ather et al.). Consequently at present it is difficult to predict with certainty whether a given immunization scheme will or will not involve NLRP3 or other inflammasome components. The authors review some of the controversies indicating that alum, a widely used adjuvant, can induce immune responses in both inflammasome-dependent and independent pathways. They mention key studies from the Poynter lab have defined the importance of inflammasome-dependent pathways in IL-1-dependent Th17 generation in asthma models in particular^{9, 10}. Th17-driven allergic immune responses are characterized by airway neutrophilia and glucocorticoid unresponsiveness, emphasizing the clinical relevance of these findings.

Thompson, Sayers, Glista-Baker, Shipkowski, Taylor, and Bonner (JEIT-D-13-00003) review the effects of nanoparticles on innate immune responses in the lung. The authors focus on engineered nanomaterials (ENM), which are gaining in popularity in engineering, electronics, and medical applications, emphasizing pre-clinical studies investigating safety and toxicology. Key factors influencing the immunotoxicity of ENM include their size, aspect ratio, and deformability, such that rigid wall carbon nanotubes are generally more pro-inflammatory than other ENM. Once taken up by macrophages and other cells, CNT are

difficult if not impossible to digest and lead to frustrated phagocytosis and persistent cell activation. Some ENM may even translocate through the lung parenchyma and end up in the pleural space¹¹. This is strikingly similar to asbestos fibers, which have well-known effects in causing lung fibrosis and mesothelioma, and although certain ENM have been shown to cause lung fibrosis in animal models, less is currently known about their carcinogenicity. An important aspect of the review by Thompson et al. is the author's careful consideration of model system employed, including differences between bolus aspiration, which is convenient and widely used but highly non-physiological, vs. inhalation of ENM using physiological aerosol delivery methods. The authors conclude that inhalation of ENM has the potential to lead to lung fibrosis, exacerbate existing airway diseases, and potentially affect systemic immune responses (e.g. suppression of T cell activation¹²). In the airway, inhalation of ENM can lead to both Th2-type as well as Th17-type immune responses, some of which may involve inflammasome-dependent pathways described above. However, some of the controversies in these fields are also covered, including the observation that depending on ENM used and dosing schema, ENM (e.g. TiO₂ particles) can also suppress existing allergic airway disease. The authors conclude that ENM can either inducing or suppress pro-inflammatory signaling pathways in innate immune cells, determined by multiple factors including shape, elemental composition, and oxidant-generating potential.

In the final review, **von Garnier, Rothen-Rutishauser, and Blank (JEIT-D-13-00004)** describe the fate of inhaled nanoparticles and how this might impact lung antigen presenting cell (APC) function. APC's are an essential link between the innate and adaptive immune systems, because T cells recognize only small peptide fragments when presented in the context of major histocompatibility complexes. Consequently, the ability to uptake, digest, and present antigen fragments is an essential step in mounting protective T cell effector responses to pathogens. Although there are many potential candidates in the lung, the key “professional” APC's are dendritic cells (DC). DC are endowed with an array of pattern recognition receptors that help discriminate harmless from harmful exposures. Many different subsets of DC exist in the airway, and although this is one area of immunology where parallels between mouse and man break down because of differences in surface marker expression, several principles appear to hold true across species. For example, in the steady state, immature DC constitutively sample inhaled particulate antigens that cross the epithelial barrier and may transduce a tolerogenic signal. This is due to low-levels of co-stimulatory molecules and high levels of IL-10 expressed by these unactivated DC. However in response to tissue inflammation and/or cell damage, DC undergo a maturation process making them much better at stimulating naïve T cells to divide and proliferate. Subtle differences during DC activation can have long lasting effects on their ability to induce T cells to differentiate down different lineage including Th1, Th2, Th17 etc. Very strongly activated DC, which secrete IL-12 and related family members, are potent inducers of Th1 immunity. Exactly how DC become good at inducing Th2 or Th17 responses is not entirely clear, but it is now known that these are not simply “default” pathways but due to active environmental signals. Interestingly, some of these signals can include inhaled particulate matter including diesel exhaust particles^{13, 14}.

Von Garnier et al emphasize the physiological relevance of model systems, and make several important observations of significance for our understanding of lung immunology in general. Although it has been known for decades that the aerodynamic diameter of particles impacts their deposition in the respiratory tract, the fate of inhaled nanoparticles is more challenging to model. At the nanoscale, biophysical interactions become important and the authors point out how inhaled NP impact by diffusion and emphasize that the thermodynamic diameter (rather than aerodynamic diameter) is a critical factor impacting NP deposition. An interesting topic covered by the authors relates to the relationship between epithelial barrier integrity and NP deposition and translocation. Based on recent studies by this group (and others) the vast majority of inhaled nanoparticles will be taken up by alveolar macrophages and enter the lung parenchyma, and only a fraction will translocate into the airways¹⁵. This is important because airways are thought to be the sight of immune responses in asthma. The authors review the potential role of NP in the initiation and exacerbation of asthma, and mention different mechanisms by which NP activate their target cells, come of which are inflammasome-dependent. Another potential lung cell-type targeted by NP are mast cells, which are interdigitated throughout the respiratory epithelium and release a variety of proinflammatory mediators upon degranulation. Although some NP promote mast cell degranulation, the authors also cover paradoxical examples whereby polymeric NP could co-opt secretory machinery and in fact inhibit mast cell mediator release. The authors conclude that NP hold great potential for novel clinical applications, and that NP size, shape and charge could be exploited for different therapeutic or diagnostic purposes.

In conclusion, we hope this issue of the *JEIT* provides readers with a timely, comprehensive, but thought-provoking series of articles that not only summarize state-of-the-art research, but also provide a blueprint for future research. New insights into how the lung immune system senses and responds to inhaled environmental exposures will undoubtedly be forthcoming in the near future. The challenge now is to harness this new and exciting body of knowledge into applications that improve human health.

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