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Advancing Lung Immunology Research

An Official American Thoracic Society Workshop Report

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Abstract

The mammalian airways and lungs are exposed to a myriad of inhaled particulate matter, allergens, and pathogens. The immune system plays an essential role in protecting the host from respiratory pathogens, but a dysregulated immune response during respiratory infection can impair pathogen clearance and lead to immunopathology. Furthermore, inappropriate immunity to inhaled antigens can lead to pulmonary diseases. A complex network of epithelial, neural, stromal, and immune cells has evolved to sense and respond to inhaled antigens, including the decision to promote tolerance versus a rapid, robust, and targeted immune response. Although there has been great progress in understanding the mechanisms governing immunity to respiratory pathogens and aeroantigens, we are only beginning to develop an integrated understanding of the cellular networks governing tissue immunity within the lungs and how it changes after inflammation and over the human life course. An integrated model of airway and lung immunity will be necessary to improve mucosal vaccine design as well as prevent and treat acute and chronic inflammatory pulmonary diseases. Given the importance of immunology in pulmonary research, the American Thoracic Society convened a working group to highlight central areas of investigation to advance the science of lung immunology and improve human health.

Keywords: allergy and immunology; mucosal immunity; lung diseases

Contents

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Overview

In this workshop report, we have chosen four main areas we believe are central to the advancement of lung immunology, including airway and lung immune sensing, tissue-resident immune memory, age-related changes in lung immunity, and advancing human experimental systems. In each area, we outline our current working models, including areas of uncertainty and technical limitations, and pose remaining questions for future investigation. Our goal is to provide a framework for greater cross-disciplinary investigation and the development of novel tools and techniques in lung immunology research. The key points of this workshop are as follows:

- The mechanisms whereby the airways and lungs sense and respond to inhaled pathogens, particulate matter, and allergens remain incompletely defined. Specifically, the cross-talk between various sensors, including the airway epithelium, peripheral nervous system, stromal cells, immune cells, and local microbiota, remains unclear. An integrated model of airway and lung immune sensing will require novel experimental approaches and greater collaboration among scientists with distinct areas of expertise.
- After inflammatory responses within the airways and lungs, local immune memory persists, with significant implications for host protection and pulmonary diseases. However, the mechanisms whereby protective or pathogenic immune memory persists in the lungs remain unclear. Specifically, how innate immune cells and structural cells within the lung are trained by previous inflammation and how adaptive immune cells are instructed for tissue residency in various contexts remains poorly defined. Furthermore, the niches and signals supporting mucosal immune memory are incompletely understood. Developing novel mucosal vaccine platforms as well as targeted therapies for inflammatory pulmonary diseases will require defining immune memory at the tissue level.
- There are marked differences in airway and lung immunity across the human life course. For example, early life is associated with an increased risk for allergic asthma, whereas extremes of age are risk factors for severe pneumonia. Older individuals and the very young exhibit marked differences in lung immunity and repair after injury. Defining the mechanisms whereby the lung microenvironment and immune responses change over the life course will be critical to reduce morbidity and mortality from pulmonary diseases.
- Although model systems have been tremendously valuable for elucidating lung immunology, our ultimate goal is to define the rules of human lung immunology *in vivo*. Improving animal models to better recapitulate human biology and developing novel *ex vivo* and *in vivo* human experimental approaches, such as human challenge studies, will be needed to advance our understanding of human lung immunology.

Introduction

The main function of the mammalian lungs is to perform gas exchange, which exposes the host to a myriad of inhaled antigens, including particulate matter, allergens, and pathogens (1). The immune system plays an essential role in protecting the host from respiratory pathogens, but a dysregulated immune response during respiratory infection can impair pathogen clearance and potentially lead to immunopathology (2). Furthermore, inappropriate immunity to inhaled antigens can lead to inflammatory pulmonary diseases, such as asthma and hypersensitivity pneumonitis (3). A complex network of epithelial, neural, stromal, and immune cells has evolved in the airways and lungs to sense and respond to inhaled antigens, including the decision to promote tolerance versus a rapid, robust, and targeted immune response. Although there has been great progress in understanding the basic immunological rules and mechanisms governing the host response to various respiratory pathogens and aeroantigens, we are only beginning to develop an integrated understanding of the cellular networks and niches governing tissue immunity within the lungs. Furthermore, there is a growing appreciation of how the local microbiota, previous inflammation, immune system development, and aging influence lung immunity. An integrated, systems-level model of airway and lung immune responses in various contexts will be necessary to improve mucosal vaccine design as well as prevent and treat acute and chronic inflammatory pulmonary diseases.

Methods

To address critical areas of investigation in lung immunology, the American Thoracic Society (ATS) convened a panel of lung immunology experts. The workshop co-chairs (R.A.R., A.I.S., and B.D.S.) identified investigators based on their research expertise in lung immunology. All participants in the workshop submitted conflict-of-interest statements before the workshop was held. All participants disclosed industry relationships and other potential conflicts of interest, which were vetted and managed according to the rules of the ATS. The workshop proposed the following topic areas: airway and lung immune sensing, tissue-resident immune memory, age-related changes in lung immunity, and advancing experimental systems for human lung immunology. The workshop convened virtually on June 10 and 11, 2021. Workshop moderators (C.A.B. and W.P.D.) oversaw presentations by individual workshop participants that covered subtopics within each of the four general topic areas. After the presentations, there was open discussion of the topics, including addressing key questions and the need for new experimental tools and approaches. After the workshop was completed, presenters drafted narrative reviews covering each subtopic. Each author limited information and citations to published manuscripts. The workshop co-chairs (R.A.R. and B.D.S.) integrated and expanded the sections into a complete workshop report. Upon completion of a draft report, members of the workshop reviewed and edited the document before submission for publication.

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Toward an Integrated Model of Airway and Lung Immune Sensing

How do airway and lung sensors cross-talk to regulate an immune response?

The airways and lungs have evolved complex sensors capable of recognizing a broad range of noxious particles and respiratory pathogens (1). Airway epithelial cells and their associated tight junctions form a physical barrier, but they also perform critical immune sensing and effector functions and serve as an integrative hub of the airway immune system (4). The traditional model of the airway epithelium focused on its barrier role, with basal epithelial stem cells forming a platform on which other epithelial cells construct a protective mucociliary barrier. Specialized goblet cells produce mucus, a complex viscoelastic biopolymer, and ciliated epithelial cells synchronously sweep mucus in a distal-to-proximal fashion, promoting mucociliary clearance that protects against noxious particles and pathogens (5). Beyond the barrier functions of mucociliary clearance, epithelial–immune cell interactions are critical in the initiation, progression, and resolution of immune responses to both pathogens and allergens (4). In humans, genetic defects in epithelial cell function contribute to a range of immune-mediated pulmonary diseases, due to either barrier dysfunction or altered immune signaling (6, 7). Studies using mouse models have dissected the immune functions of the airway epithelium and how interactions between the airway epithelium and cells of the immune system determine the response to pathogens and allergens (1, 4, 8). Airway epithelial cells express various pathogen recognition receptors and in response to respiratory infection secrete a broad array of cytokines, chemokines, antimicrobial peptides, danger-associated molecular patterns, and enzymes that directly promote pathogen clearance or induce a robust and targeted immune response (8). For instance, in response to respiratory viral infection, pathogen recognition receptor expression in the airway epithelium, such as RNA-sensing, Retinoic acid-inducible gene I-like receptors and TLRs (Toll-like receptors), induce production of type I and III IFNs, which inhibit viral replication and orchestrate immunity to enhance host defense (9–11). In contrast, the airway epithelium also plays an important role in inappropriate immunity to environmental antigens such as allergens. For example, TLR-4 and Protease-activated receptor 2 expression in the airway epithelium can be activated directly by allergens or via cleavage products of allergen proteases, leading to epithelial cell secretion of granulocyte-macrophage colony-stimulating factor, IL-1, and various alarmins such as IL-33 and thymic stromal lymphopoietin that promote allergic immunity (12, 13). Consequently, the airway epithelium coordinately performs barrier and immune sensing functions.

The development of single-cell RNA sequencing platforms and high-resolution imaging has dramatically expanded our view of the epithelial landscape to reveal a dynamic cellular structure that contains a wide variety of specialized cells contributing to both barrier defense and immunosurveillance (8). Landmark single-cell RNA sequencing studies in mice and humans characterized the composition and heterogeneity of airway epithelial cells, including Tuft, neuroendocrine, and ionocytes, which are continually and directly repleted by basal progenitors (14–16). Notably, although ionocytes are relatively rare in abundance, they are the major source of transcripts for the cystic fibrosis transmembrane conductance regulator and seem to play an important role in airway fluid and mucus physiology *in vivo*, underscoring that rare epithelial cell types contribute to airway homeostasis and response to stress (14–16). In addition, transcriptional profiling of the airway epithelium led to the discovery of "hillocks," which consist of Keratin-13–expressing cells arranged in discrete, stratified structures (15). Such hillock structures exhibit high turnover and expression of genes associated with squamous epithelial differentiation, cell adhesion, and immunomodulation (15). Last, although microfold cells (M cells) have been described in the intestine, where they function to endocytose and transport luminal antigens into the lamina propria, it is clear that M cells also exist in the airways of mice and humans, where their function is beginning to be defined (17–21). Adding to the heterogeneity of airway epithelial cells, there are distinct epithelial cell subtypes, including two goblet cell subsets as well as distinct tuft cell subsets (8, 15, 22). Beyond the airway epithelium, alveolar epithelial cells play an important role in host defense and the response to lung injury. Within the alveolar epithelium, alveolar type 2 (AT2) cells produce pulmonary surfactant to reduce surface tension, which prevents atelectasis and promotes gas exchange. Surfactant is enriched in the phospholipid dipalmitoylphosphatidylcholine (DPPC) as well as four SPs (surfactant-associated proteins), SP-A, SP-B, SP-C, and SP-D (23). DPPC and the hydrophobic SPs, SP-B and SP-C, lower surface tension at the air–liquid interface (23). In contrast, SP-A and SP-D are hydrophilic proteins that belong to a family of innate immune proteins termed collectins, which promote pathogen clearance and exhibit immunomodulatory properties (23, 24). In addition, AT2 cells serve as progenitor cells that slowly promote self-renewal and differentiate into AT1 cells (25). Upon lung injury, AT2 cells increase their proliferation to promote regeneration. Multiple groups have identified a unique transitional stem cell state promoting repair (26–28). Of note, cytokines such as macrophage-derived IL-1β can promote the transitional stem cell state, demonstrating important cross-talk between the local immune system and regeneration, a process that we are only beginning to define (25, 28). Consequently, single-cell transcriptional analysis has transformed our view of the airway and alveolar epithelium and significantly advanced our understanding of the development and heterogeneity of cell types and states. Moving forward, we need to define the significance of novel epithelial cell types and states in regulating homeostasis and airway immunity in various contexts and how these epithelial cells change phenotype and/or function during disease states and across the life course. Furthermore, although previously thought to be sterile, it is now well accepted that the epithelial surfaces of the respiratory tract are colonized by a complex and dynamic microbial ecosystem, termed the "lung microbiome," which plays an important role in both health and disease (29). Determining the role of the airway microbiome in regulating various airway epithelial cell functions and pulmonary disease remains a central area for future investigation.

Although it is clear that the airway epithelium continuously senses inhaled antigens, the peripheral nervous system, which densely innervates the airways, also plays an important role in aeroantigen

immunosurveillance and immune function. The vagus nerve innervates most visceral organs, with up to 20% of its termination being located within the airways (30). The vagus nerve dominates innervation of the airways with an additional small (~5%) contribution from lumbar neurons (31, 32). Vagal neurons serve various physiologic, homeostatic, and organ-monitoring functions. Similar to the airway epithelium, neurons are also heterogeneous in nature, and their biological role appears to be circuit dependent. For example, NPY_2R^+ vagal neurons control rapid and shallow breathing, $P_2RY_1^+$ neurons silence respiration and promote expiratory reflexes, and $PIEZO_2^+$ vagal sensory neurons, akin to the mechanosensing neurons of the skin, regulate apnea (33–35). A large portion of vagal neurons are also of sensory origin and express markers such as the heat-sensing ion channel TRPV1 (Transient receptor potential cation channel subfamily V member 1) and the voltage-gated sodium channel $Na_V1.8$ (36–38). These sensory neurons are high-threshold noxious stimuli detectors designed to limit tissue damage by detecting chemical, mechanical, or thermal threats and initiating protective airway reflexes such as coughing and bronchoconstriction (39).

Numerous findings support an important role for the peripheral nervous system in regulating immunity. Pathogens can directly activate nociceptor neurons, which regulate immune responses via locally released neuropeptides, promoting both pro- and antiinflammatory responses, depending on the context (40–48). Nociceptor neurons also play a critical role in regulating immunity to allergens. For instance, nociceptor neurons directly respond to allergens, producing neuropeptides that promote the function of type 2 innate lymphoid cells (ILC2s), induce mast cell degranulation, and promote the trafficking of type 2 conventional dendritic cells (cDC2) to the draining lymph node, driving $CD4^+$ T-helper cell type 2 (Th2) differentiation (48–54). Furthermore, $TRPV1^+$ vagal neurons regulate inflammatory cell recruitment to the airways during allergic disease as well as promote mucin production (55–57). Together with the direct sensing of pathogens and allergens, neurons receive inputs from immune mediators that regulate their function. For instance, nociceptor neurons respond to inflammatory cytokines such as IL-4 as well as antibodies (58, 59). Specifically, $TRPV1^+$ sensory neurons express the high-affinity IgE receptor FcεR1 and respond to IgE–allergen immune complexes by releasing the neuropeptide SP (Substance P), which, in turn, amplifies Th2 cell production of IL-5 and IL-13 (58, 60–62). Albeit at lower surface amounts than in Th2 cells, B cell subtypes also express SP receptors. When coexposed to IL-4 and LPS, SP-stimulated B cells showed enhanced formation of antibody-secreting cells and IgE release (63). In response to allergen challenges, the genetic ablation and pharmacological silencing of vagal nociceptors decreases IgE production as well as inflammatory cell infiltration, demonstrating that IgE-sensing neurons amplify the lung humoral immune responses, highlighting a novel nociceptor–B cell circuit (63). Notably, the airway nervous system changes during development, influencing airway immunity. During postnatal development in mice and humans, sympathetic nerves undergo a dopaminergic-to-adrenergic transition. In young mice, allergen exposure induces dopamine release, directly promoting Th2 cell differentiation via dopamine receptor 4, enhancing allergic immunity, and suggesting a mechanism whereby young children are more likely than adults to develop allergic asthma (64). Furthermore, airway nerves can change in the context of chronic inflammation. For example, patients with asthma have airways that are hyperinnervated, display an increased sensitivity to air irritants, and exhibit higher levels of airway neuropeptides (65). Together, these features indicate excessive lung nociceptor activity in the context of allergic asthma, and, as such, a TRPA1 antagonist is now in clinical trials for allergic asthma (66–68). Consequently, there is a complex cross-talk between the airway nervous and immune systems, which interact through shared receptors, cytokines, and neuropeptides (69). Of note, although much of the work on the cross-talk between the peripheral nervous system and immune system has focused on allergic inflammation, type 2 immunity plays an important role in host defense against toxins and toxin-producing pathogens (70–74). For instance, IgE and mast cells contribute to host defense against toxin-producing bacteria such as *Staphylococcus aureus* (74). Defining the cross-talk between the nervous and immune systems in the lung in various contexts represents an important area of investigation to promote host defense while limiting immunopathology.

Together with the airway epithelium and peripheral nervous system, the mononuclear phagocyte (MP) system plays a central role in immune sensing and responses (75). The MP system in the lungs is composed of tissue-resident alveolar macrophages, interstitial macrophages (IMs), dendritic cells (DCs), and monocytes (75). Of note, humans have similar MP subsets in the lungs and lymph nodes compared with mice (76, 77). Resident macrophages can be divided into two categories: tissue-resident macrophages that only exists in one organ and express a unique transcriptional profile (i.e., alveolar macrophage) and IMs, which are macrophage subtypes present in many organs sharing a common transcriptional profile. In the murine lung, there are at least two IMs, $Lyve1^+Folr2^+$ IMs that express high levels of CD206 with gradient expression of Timd4 (T cell immunoglobulin and mucin domain containing 4), and $Lyve1^-Folr2^-$ IMs expressing low levels of CD206 and high levels of MHCII (Major Histocompatibility complex class II), CD11c, and CCR2 (78). These two IMs appear to have distinct locations and functions. $Folr2^+$ IMs surround the blood vessels, whereas $Folr2^-$ IMs colocalize with nerves (79–81). Functionally, $Folr2^+$ IMs display classical macrophage characteristics based on transcriptome expression, phagocytosis, and slow replenishment rates by circulating monocytes, whereas $Folr2^-$ IMs, although displaying macrophage properties, have a higher turnover rate, expressing proinflammatory mediators and monocytic and DC-like genes and properties (78). Circulating monocytes traditionally have been viewed as precursors to tissue-resident macrophages. However, we now know that monocytes continuously traffic through nonlymphoid and lymphoid tissues, where they survey the environment. Unless there is a macrophage niche to fill, steady-state monocytes do not readily differentiate into self-renewing, tissue-resident macrophages (82, 83). During inflammation, monocytes can differentiate into inflammatory and resolving macrophages, which display distinct properties from resident macrophages (84–86). DCs are potent antigen-presenting cells that link innate and adaptive immunity. In contrast to tissue macrophages, peripheral conventional DCs acquire antigens, traffic through lymphatic vessels to draining lymph nodes, and present

exogenous antigens to cognate T cells, inducing adaptive immunity (87–91). The induction of naive T cells into effector T cells is mainly attributed to two conventional DC subtypes, cDC1 and cDC2, with further subdivision of cDC2 states during inflammation (92–95). The various lung MP cells, including subsets of macrophages, tissue monocytes, and migratory DCs, are capable of directly sensing pathogens via a diverse array of pattern recognition receptors (96, 97). In addition, lung MP cells can respond to epithelial cell–derived alarmins and neuropeptides (8). Future research is needed to define how cells of the MP system cross-talk with various immune sensors in the airways and lungs, including the epithelium and peripheral nervous system, during homeostasis and in response to various stimuli *in vivo*.

Although single-cell sequencing technologies have allowed for a detailed delineation of cell heterogeneity among airway and lung immune sensors, we are only beginning to integrate such complexity into working models of lung immunity *in vivo*. For the lung immunology community, it will be essential to rigorously define how the heterogeneous immune sensors recognize and respond to epithelial barrier breach by respiratory pathogens or noxious aeroantigens. How do the specialized subsets of airway epithelial cells, neurons, immune cells, and other structural cell types coordinately respond in specific contexts; how are the output signals integrated; and how do immune sensors cross-talk to regulate an immune response *in vivo* (Figure 1)? An integrative model of airway and lung immune sensing has great therapeutic potential. For instance, an optimal mucosal vaccine platform will likely benefit from regulating the airway epithelial and sensory neuronal responses. In addition, targeting airway immune sensors may hold therapeutic potential for inflammatory pulmonary diseases. For instance, targeting nociceptive neuronal cross-talk with proallergic cytokines and/or IgE may improve allergic inflammation in asthma. Murine models have been, and will continue to be, critical for advancing our understanding of airway and lung immune sensors *in vivo*. Specifically, genetic approaches in mice will continue to be a mainstay approach for mechanistic studies. Developing novel Cre-drivers that can specifically target unique cell populations will be required to interrogate the heterogeneous cell populations defined by single-cell sequencing approaches. Furthermore, as outlined below, it will be vital to create better models and systems for investigating human lung immunity. Such innovations will require multidisciplinary approaches and, consequently, greater cross-disciplinary collaborations.

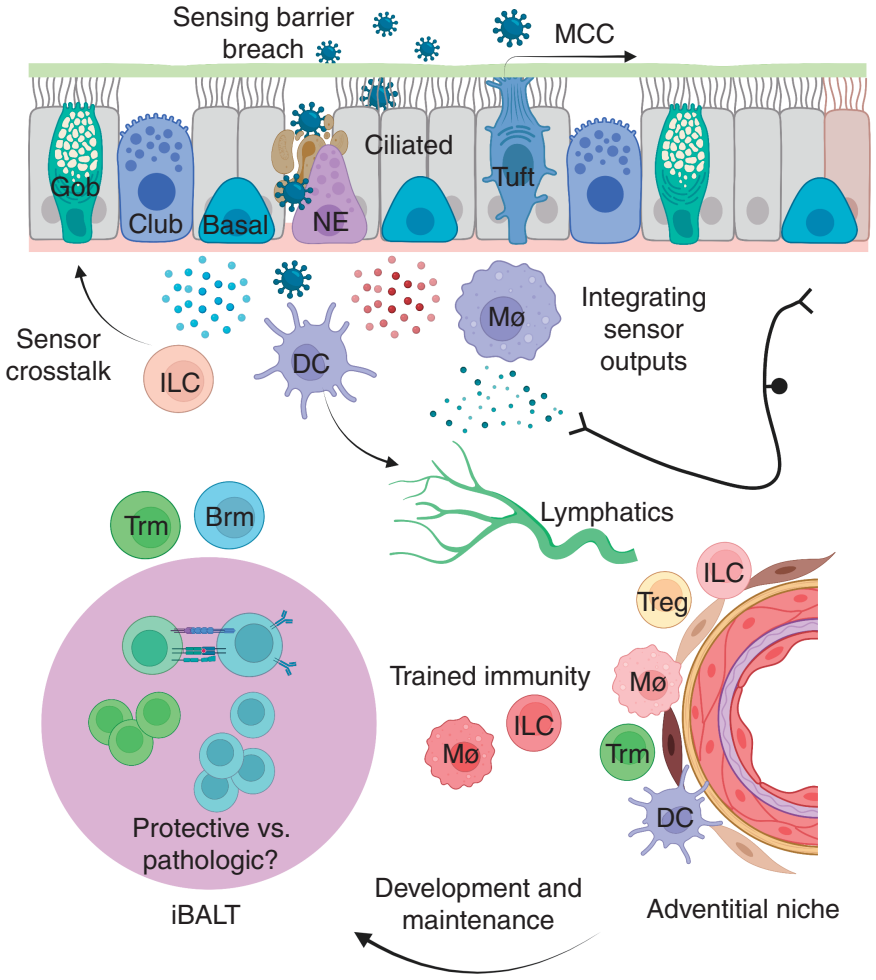

Figure 1. Model of airway sensing and memory. The airway epithelium consists of heterogeneous cell types that perform barrier functions, including mucociliary clearance, as well as immunosurveillance. Barrier function breach, such as occurs with respiratory pathogens, is sensed by specific epithelial cell subsets and other sensors, including neurons and innate immune populations. Defining how various immune sensors respond and integrate signals in distinct contexts to initiate an immune response represents a critical area of investigation. After an immune response, inflammatory memory is retained in various cell populations and niches, including tissue-resident memory T and B cells in adventitial niches or inducible bronchus-associated lymphoid tissue, which can promote host protection or immunopathology. The signals regulating the development and maintenance of tissue-resident memory remain an active area of investigation. Illustration created in Biorender.com. Brm = tissue-resident memory B cell; DC = dendritic cell; Gob = goblet cell; iBALT = inducible bronchus-associated lymphoid tissue; ILC = innate lymphoid cell; MCC = mucociliary clearance; Mϕ = macrophage; NE = neuroendocrine cell; Treg = regulatory T cell; Trm = tissue-resident memory T cell.

Defining Tissue-Resident Immune Memory within the Airways and Lungs

How do the airways and lungs remember previous inflammatory responses?

Together with the airway and lung immune sensors and their associated effector functions outlined above, the lungs contain a variety of tissue-resident ILCs and innate-

like, unconventional T cells such as iNKT (Invariant natural killer T), γδ T cells, and mucosal-associated invariant T cells (98, 99). ILCs and unconventional T cells can directly respond to exogenous antigens and/or respond to signals from the airway epithelium, peripheral nervous system, stromal cells, or other immune cells to play important protective and pathologic roles in the lungs (98, 99). For instance, ILC2s are regulated by alarmins such as IL-33, IL-25, and thymic stromal lymphopoietin, as well as neuropeptides such as neuromedin and calcitonin gene–related peptide, and promote type 2 immunity as well as tissue repair in the context of injury or infection (51–54, 99, 100). In addition, over the last decade, it has become well established that a subset of memory T and B cells establish residency in peripheral tissues to orchestrate local recall responses (101–103). The discovery of tissue-resident memory T (Trm) and B (Brm) cells has revealed that nonlymphoid tissues can be imprinted with antigen-specific immune memory, transforming our view of immune memory. Given the critical role of memory T and B cells in host protection against pathogens as well as driving inflammatory diseases, there has been great interest in defining the biology of Trm and Brm cells *in vivo*.

Landmark studies using viral infection models in mice demonstrated that a subset of effector $CD8^+$ T cells establish long-term residency in previously inflamed peripheral tissues and offer superior protection to viral rechallenge (104–106). Trm cells exhibit a distinct phenotype and transcriptional program from their circulating counterparts (101). Within the lungs, $CD8^+$ Trm cells specific for respiratory pathogens such as influenza not only offer protection against recurrent infection but also provide subheterotypic immunity, leading to significant interest in the development of mucosal vaccines capable of promoting Trm development (107–109). In addition, in various murine models of pulmonary infection or mucosal vaccination, multiple distinct $CD4^+$ T cell subsets, including Th1, Th17, and T follicular helper cells, establish tissue residency and play an important role in local immunity and host protection (110–115). In human lungs, both memory $CD8^+$ and $CD4^+$ T cells with a Trm phenotype have been described (116–122). Although Trm cells enhance host protection against pathogens, it is clear that they can also mediate immunopathology. In models of inflammatory pulmonary diseases such as asthma, allergen-specific memory Th2 cells establish residency within the lungs and play an important role in driving recurrent allergic airway disease (123–125). Furthermore, a subset of individuals with steroid-resistant asthma exhibit a Th1-biased inflammatory profile in the airways with high-dimensional profiling of airway immune cells demonstrating the presence of IFNγ-expressing $CD8^+$ and $CD4^+$ Trm cells (126–128). Trm cells regulate barrier immunity by rapidly responding to cognate antigen, producing inflammatory cytokines that can quickly enhance local innate and adaptive immunity (129–133). Furthermore, Trm cells cross-talk with structural cells, including the airway epithelium, which can enhance host protection to infection but also promote immunopathology (125, 129, 134, 135). Beyond Trm cell biology, Brm cells persist within the lungs and enhance airway antibody production upon antigen reexposure (122, 136–140). Consequently, the development of distinct subsets of Trm and Brm represents an important mechanism whereby the airways and lungs remember previous inflammatory insults.

In light of the importance of Trm and Brm cells to immunity, the signals instructing tissue residency represent an area of active investigation. To establish residency, Trm and Brm cells upregulate tissue-retention receptors and downregulate tissue egress molecules (101). In $CD8^+$ T cells, the transcription factors Runx3, Hobit, and Blimp-1 drive the tissue-residency program (141). Although there are certain features shared by all Trm cells (e.g., low expression of tissue egress molecules), recent studies in $CD8^+$ Trm cells have revealed significant Trm cell heterogeneity within and across organs, demonstrating that there may be distinct pathways to Trm development in various contexts (142, 143). Furthermore, although inflamed tissues clearly provide signals to regulate Trm development, several recent studies have suggested that T cells are programmed for Trm fate during initial priming via instructive signals within the lymph node (144). During priming of naive $CD8^+$ T cells, a population of $DNGR\text{-}1^+$ (dendritic cell NK lectin group receptor-1) DCs exhibit an enhanced capacity of cross-presentation, allowing exogenously acquired antigens to be loaded on MHCI and presented in the draining lymph node. In a murine model of influenza infection, depletion of $DNGR\text{-}1^+$ DCs minimally impacted the effector $CD8^+$ T cell response but had a dramatic effect on $CD8^+$ Trm cell development within the lungs (145). Additional studies have suggested that naive $CD8^+$ T cells can also be preconditioned for Trm fate or instructed for tissue residency during early T cell priming, before significant clonal expansion (144, 146, 147). Whether similar preconditioning or early instructive signals within lymph nodes promotes a tissue-residency program for $CD4^+$ T cell or B cell responses remains to be determined. Defining potential preconditioning or early instructive signals as well as fate-determining signals within inflamed tissues that promote tissue residency has significant implications for mucosal vaccine development as well as novel therapeutic approaches for inflammatory diseases.

There is great interest in defining the lung niches supporting tissue-resident lymphocytes *in vivo*. Broadly, immune niches in nonlymphoid tissues can be characterized as *1*) epithelial and subepithelial niches; *2*) mesothelial or capsular boundary niches; *3*) parenchymal niches; and *4*) adventitial niches (148). Recently, adventitial stromal niches, which include the outmost layer of the airways and blood vessels, have been shown to be critical locations regulating immunity (148–150). Adventitial stromal niches are the site of diverse immune cells, including IMs, DCs, mast cells, ILCs, innate-like or unconventional T cells, regulatory T (Treg) cells, as well as Trm cells (148–150). In addition, these adventitial locations are enriched in lymphatics, neurons, and a variety of specialized stromal cells, which cross-talk with immune populations during homeostasis and immunity (148, 150–152). The unique immune and nonimmune cells that compose adventitial stromal niches, including their heterogeneity and immune regulatory functions in various contexts, remain an ongoing area of investigation (148).

In terms of Trm localization, after influenza infection, $CD8^+$ Trm cells within the lungs persist in adventitial niches with features of tissue repair that have been termed repair-associated memory depots (153). Interestingly, Trm cell durability seems to vary between the upper and lower airways. For instance, $CD8^+$ Trm cells generated after influenza infection in mice appear to persist for longer within the nasal mucosa than in the lower airways, where an attrition over

time is well described (107, 154–156). Whether a unique adventitial niche promotes greater Trm maintenance in the upper airway requires further investigation. In the context of chronic inflammation, adventitial domains give rise to tertiary lymphoid structures, such as inducible bronchus-associated lymphoid tissue (iBALT) (150). Certain types of $CD4^+$ Trm cells have been shown to persist in iBALT (115, 157, 158). In addition, although organ transplant studies reveal that Trm cells can persist for years, studies in mice and humans have suggested that Trm cells may be capable of egress from peripheral tissues, presumably via adventitial lymphatics, and join the circulating memory T cell compartment, while maintaining features of the Trm program and exhibiting a predilection to return to the tissue of origin (159–162). The local environmental signals dictating persistence, death, and egress over time represent an active and important area of study. For instance, lymphatic endothelial cells can acquire and maintain antigen for prolonged periods of time *in vivo*, which influences memory T cell biology (163, 164). Whether such antigen "archiving" occurs in adventitial niches or iBALT to regulate Trm cell biology remains unclear. In terms of Brm cell biology, the niches for Brm cell persistence appear different depending on the model. After influenza infection, Brm cells persist in iBALT (113, 114, 136). In contrast, after pneumococcal pneumonia in mice, Brm cells seem to predominantly persist independently of mature iBALT, but rather within small clusters in adventitial niches of bronchovascular bundles (137).

iBALT is a well-characterized immune niche within the lungs, defined by the presence of distinct T and B cell areas, which are interspersed with conventional dendritic cells, follicular dendritic cells, and stromal cell networks, and is generally located near airways or blood vessels (165, 166). In some instances, such as infection with *Mycobacterium tuberculosis*, the formation of iBALT structure is protective and is beneficial to the host (167–169). In contrast, under conditions of persistent exposure to antigens during chronic inflammation, including allergic inflammation or autoimmunity, iBALT can mediate immunopathology (170–172). Although some of the early molecular signals that mediate formation of iBALT structures have been identified, including expression of CXCL13, IL-17, and IL-22, the functional role of iBALT toward a protective or pathogenic function within the lungs remains unclear (169, 173, 174). For example, differences in the type and duration of inflammation may result in distinct roles of iBALT structures *in vivo*. Although iBALT formation may serve an immediate protective solution for pathogen control, if left unresolved and upon chronic exposure to antigen, it may result in long-term foci of immunopathology. Furthermore, the flavor of T cells associated with protective versus pathological iBALT may be distinct; although Th1 and Th17 responses appear to be involved in protective iBALT, prolonged Th17 and Th2 responses may be associated with immunopathologic responses (170, 174–177). For instance, persistent Th17 cell responses and iBALT structures are associated with airway pathology in chronic obstructive pulmonary disease (172). Defining the features and mechanisms regulating protective versus pathologic iBALT has important therapeutic implications. For instance, mucosal vaccine platforms that drive potential targets, such as IL-17, IL-22, lymphotoxin-α, and the associated chemokines CXCL13 and CXCL12, has the potential to induce iBALT, providing enhanced tissue-resident host defense. However, pathways associated with protective and pathogenic iBALT formation may overlap significantly, underscoring the need to define the unique features and functions of iBALT in distinct contexts. Last, delineating how epithelial, stromal, and nervous system inputs regulate iBALT development, persistence, and function remains important. These new avenues of research could open up a new field of host-directed therapeutics that specifically target iBALT for improved protection against pathogens and inhibition of inflammatory pulmonary diseases.

Together with tissue-resident adaptive immune memory, ILCs and unconventional T cells as well as other innate immune cells can acquire and maintain memory-like properties, a process termed "innate training." Innate training is characterized by a nonspecific (antigen-independent) enhancement in cell responsiveness to activating signals (178). Although the mechanisms of innate training are cell-type and context specific, the operational pathways generally involve metabolic and epigenetic reprogramming after stimulus exposure (178). For instance, after activation, ILC2s acquire memory-like properties, including enhanced responsiveness to alarmins, which are driven by epigenetic changes that promote a poised effector program (179). ATAC-seq (Assay for transposase-accessible chromatin with sequencing) analysis demonstrated that memory-like ILC2s possess altered gene accessibility, which is driven by Bach2 (BTB Domain And CNC Homolog 2) and AP1 (Activator protein 1) motifs, and include a "preparedness" program that allows activation to previous subthreshold stimulation (179). In addition to innate training of immune cells, growing evidence suggests that structural cells, including the airway epithelium, can acquire and retain inflammatory memories that regulate immune responses. Last, the peripheral nervous system plays an important role in regulating immunity, but neuronal memory-regulating tissue immunity remains a largely unexplored area. The interested reader is referred to the excellent reviews on these topics (178, 180). An integrated understanding of how the lungs "remember" and "forget" inflammatory experiences is a critical area of investigation. How do Trm cells, Brm cells, and other forms of resident memory develop and persist in the airways and lungs? How do specific adventitial niches change after inflammation, such as giving rise to iBALT, to permanently change the immune function of the lungs (Figure 1)? Addressing such questions will be needed to intelligently and effectively regulate airway and lung immune memory to prevent and treat pulmonary diseases.

Delineating Age-related Changes in Lung Immunity

How is lung immunity altered by aging?

There are marked differences in airway and lung immunity across the human life course. Early life is a critical period in immune education in which individuals are exposed to new environmental antigens and pathogens. Growing evidence suggests that exposures *in utero* and during early life, including the acquisition of the lung microbiome, can have long-term influences on immunity (29, 181, 182). Early life is associated with an increased risk of allergic

asthma, which coincides with a Th2 bias in the developing lung and can be influenced by early environmental exposures (183, 184). For example, during the perinatal period in mice, there is an accumulation of type 2 innate immune cells within the lung that occurs in an IL-33–dependent manner, and postnatal lung DCs are efficient at inducing Th2 immunity (183). Furthermore, as outlined above, sympathetic innervation in the lung exhibits a dopaminergic-to-adrenergic transition during the postnatal period, and dopamine signaling in $CD4^+$ T cells promotes Th2 cell differentiation and susceptibility to allergic inflammation (64). These physiological differences also increase the risk of lower respiratory tract infection (pneumonia) due to both bacteria and viruses during early childhood. Indeed, pneumonia is responsible for more than 6 million deaths per year in children <5 years of age, and ~1 million of these deaths occur in neonates (infants >28 d of age) (185, 186). Introduced above, the Th2 bias present early in life results in dysregulated Th1- and Th17-coordinated protection from pathogens (187). Although the function of infection-induced iBALT in children remains a topic of controversy (188), it is notable that children with severe respiratory infection carry a high incidence of iBALT and that iBALT appears in the lungs of nearly all late-term fetuses that miscarry after pulmonary infection–related amnionitis (189–191). Finally, although influenza pneumonia has classically demonstrated a J-shaped mortality curve across the life course, children with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection uncommonly develop severe pneumonia (192). Analysis of upper respiratory tract samples obtained from children and adults with viral infection suggests that age-related differences in IFN responses explain differences in disease severity between children and adults with SARS-CoV-2 pneumonia compared with pneumonia due to other pathogens (193, 194).

Older age is also a dominant risk factor for many other lung diseases, including pneumonia (195). Indeed, pneumonia, which is most often due to viral pathogens, represents the leading cause of death from an infectious disease (196, 197). Influenza viruses cause 300,000–650,000 respiratory deaths per year, principally among people >65 years old (198). Recently, the coronavirus disease (COVID-19) pandemic has highlighted the dramatic association between age and the severity of viral pneumonia–induced lung injury (199, 200). As the number of adults >65 years old will exceed 2 billion by 2050, these epidemiologic observations have prompted an examination of how the immune system changes with age to render older individuals more susceptible to pneumonia (201).

Although a full accounting of age-related immune system alterations is beyond the scope of this ATS Document, we will focus on the alterations in lung immunity associated with advancing age. Changes in both innate and adaptive immunity result in the lungs of older individuals exhibiting increased basal secretion of inflammatory cytokines, decreased pathogen-induced T cell cytokine production and cytolytic function, and increased neutrophil-mediated tissue injury (202). Older people have increased levels of proinflammatory cytokines—including IL-1β, IL-6, IL-8, and TNF-α—in their circulation and lung tissue. Advancing age is also associated with decreased production of naive T and B cells. This decreased production limits the diversity of the adaptive immune pool, which becomes progressively hypofunctional over time. As discussed below, the tissue-protective and reparative functions of lung immune cells are also diminished with aging (203).

The cumulative effect of these age-related alterations in immune cell phenotype and function is decreased resilience to the stress of pneumonia, which results in increased mortality and often heralds the compounding multimorbidity and functional limitation observed among older survivors of severe respiratory infection (204–210). A complete view of cellular and molecular mechanisms underlying age-related immune system dysfunction in the lung remain unclear, although aging hallmarks—including epigenetic alterations, mitochondrial dysfunction, cellular senescence, and other phenomena—have been proposed as causal mediators (211). Identifying cell-nonautonomous versus cell-autonomous mechanisms of age-related immune system dysfunction provides a tractable framework to study and address the biology of aging in a translational context. Importantly, murine models of viral pneumonia and aging recapitulate many features of human disease (199, 212, 213). Here, we highlight recent studies of the effect of aging on critical cell types involved in the host response to experimental viral pneumonia–induced lung injury—alveolar macrophages, tissue-resident $CD8^+$ T cells, and Treg cells.

Alveolar macrophages are responsible for launching and modulating the host immune response to respiratory viral pathogens. A recent study found that cell-nonautonomous alterations in the aging lung microenvironment govern the dysfunctional response of alveolar macrophages to viral lung injury (214). Alveolar macrophages acquired transcriptional programs aligned with the age of the microenvironment, irrespective of their age at the time of heterochronic (age-mismatched) adoptive transfer. Interestingly, heterochronic parabiosis experiments revealed that age-related alterations in alveolar macrophage phenotype and function are independent of circulating factors or cells. The authors went on to suggest that hyaluronan, which is increased in the aged lung microenvironment, drives a hypoproliferative state in macrophages. This study highlights the cell-nonautonomous role that the aging lung microenvironment plays in driving immune system alterations over the life course.

After the initiating inflammatory events mediated mostly by myeloid cells, the adaptive T cell response to viral infection is critical in viral clearance and in coordinating resolution of inflammation and repair of the damaged parenchyma. Tissue-resident $CD8^+$ T cells represent a key cell type in providing adaptive antiviral immunity. Interestingly, a recent study demonstrated that tissue-resident $CD8^+$ T cells contribute to persistent lung pathology in aged hosts (215). Unlike the data in alveolar macrophages discussed above, adoptive transfer experiments revealed that the age-related dysfunction of tissue-resident $CD8^+$ T cells is cell autonomous, as transfer of tissue-resident $CD8^+$ T cells from aged lungs were unable to induce heterologous protective immunity. Depletion of tissue-resident $CD8^+$ T cells mitigated postviral lung fibrosis in aged, but not young, mice. Altogether, these findings support a cell-autonomous role for tissue-resident T cells in driving age-related lung pathology after viral pneumonia.

$Foxp3^+$ (Forkhead Box P3) Treg cells are a subset of $CD4^+$ T cells possessing immune-suppressive functions that maintain self-tolerance and dampen overexuberant immune system activation (216, 217). *Foxp3* gene mutations in mice result in the scurfy phenotype, characterized by severe multiorgan lymphoproliferative inflammation (218, 219).

Human *FOXP3* mutations lead to a similar presentation: the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (220). Beyond their canonical suppressive function, investigators have identified emerging tissue-protective and tissue-reparative roles for Treg cells, mediated in part by the generation of proepithelial growth molecules such as amphiregulin (221). A recent study demonstrated that aged Treg cells are hypofunctional in their ability to suppress inflammation and promote repair in a murine model of influenza pneumonia (222). The authors found that in heterochronic adoptive Treg cell transfer experiments, aged Treg cells exhibit a cell-autonomous impairment in their ability to resolve lung inflammation and orchestrate lung parenchymal repair after influenza pneumonia. These results demonstrate that Treg cells retain a memory of their age even when transferred into an age-mismatched environment. A corollary concept is that the aged lung microenvironment retains its ability to undergo Treg cell–mediated repair after virus-induced lung injury. Mechanistically, the authors found evidence for both a loss of reparative function and a gain of deleterious inflammatory function within Treg cells as a function of age. Specifically, amphiregulin, among other reparative molecules, was upregulated to a greater extent by the lung Treg cells of young mice than aged mice after influenza. Simultaneously, aged $Foxp3^+$ T cells generated inflammatory cytokines (e.g., IFN-γ and IL-17A) and expressed canonical T helper transcription factors (e.g., TBX21 [T-Box Transcription Factor 21] and RORγt [retinoic acid-related orphan receptor-γt]). DNA methylation, a hallmark age-related epigenetic alteration, governs Treg cell phenotype and function in mice and humans across the life course (212, 223–226). Interestingly, genome-wide DNA methylation profiling and computational analyses determined that age-related epigenetic alterations explained the loss of reparative transcriptional programs after influenza infection (222). In summary, this work demonstrates how aging drives cell-autonomous dysfunction of Treg cells in mediating immune homeostasis and promoting tissue repair after viral lung injury. Defining the mechanisms that promote age-related alterations in the lung microenvironment remains a critical area of investigation in lung immunology. Delineating how the airway and lung immune sensors as well as tissue-resident immune cells and niches change with aging will have significant clinical implications in various pulmonary diseases.

Advancing Human Models of Lung Immunity

How can we improve our ability to model human lung immunity?

Animal models have been an essential tool to define lung immunity *in vivo*, allowing for the development of novel therapies for human pulmonary diseases. Although certain features of human disease can be recapitulated in murine models, there are important aspects of human biology that are distinct in mice. For instance, the transcriptional signature of Th2 cells in humans and murine models of allergic disease are similar (227). In contrast, a population of $CD45RA^+$ effector memory $CD8^+$ T cells, which represent a substantial fraction of the memory $CD8^+$ T cell compartment in humans, do not exist in mice (228). Together with differences within the immune system, there are notable differences in airway structure and airway epithelial cell composition between humans and mice (229). In humans, pseudostratified epithelium containing basal cells extends to the terminal bronchioles, with the respiratory bronchioles exhibiting a simple, cuboidal epithelium lacking basal cells. In mice, a pseudostratified epithelium with basal cells is restricted to the trachea, with the intrapulmonary airways exhibiting a simple columnar epithelium without basal cells and lacking respiratory bronchioles. Consequently, it is important for investigators using murine models to be aware of such limitations when addressing specific questions.

Given the power of murine experimental systems, there is great interest in modifying murine models to more accurately recapitulate features of human biology. For instance, it is now clear that mice maintained in standard laboratory conditions ("specific pathogen–free" [SPF]) exhibit a drastically different microbiome and immune profile from mice with diverse microbial exposures throughout life (230). Wild or pet-store mice (i.e., "dirty mice") exhibit immune responses with greater similarity to humans than SPF mice, resulting in significant interest in using dirty mice as a better model for human immunity (230). For example, compared with SPF mice, laboratory mice cohoused with pet-store mice exhibit an immune response upon influenza vaccination that better recapitulates the response in humans (231). Specifically, cohoused mice exhibited a dampened vaccine-induced humoral response, resulting in poorer control upon influenza challenge (231). Consequently, mice with a diverse history of microbial exposure potentially offer a better preclinical system for vaccine testing than SPF mice. Beyond dirty mice, genetically modified mice have been used to improve *in vivo* experimental systems. For example, mice modified to express human ACE2 (Angiotensin Converting Enzyme 2) have allowed for the study of SARS-CoV-2 infection *in vivo*, including using mice with humanized Ig genes to generate human antibodies against the SARS-CoV-2 spike protein (232–234). A wide variety of humanized murine models have been developed to advance preclinical studies, and innovations in this area have the potential to further leverage experimental techniques in mice to gain insight into human biology (235).

The lack of a murine model can significantly limit scientific progress in biomedical research, including lung immunology. For example, for >100 years, eosinophilic inflammatory diseases such as asthma, chronic rhinosinusitis with nasal polyps, and allergic bronchopulmonary aspergillosis have been associated with extracellular deposits of morphologically diverse crystals. First described by Charcot followed by Leyden in the 19th century, Charcot-Leyden crystals (CLCs) are composed of Gal10 (galectin-10), which is one of the most abundant proteins in human eosinophils (236–239). However, mice lack an ortholog of Gal10, and, as a result, it had remained unclear whether CLCs regulate disease or represent a bystander phenomenon during eosinophilic inflammation *in vivo*. The administration of recombinant CLCs to mice has revealed these crystals to be important regulators of immunity (238). Specifically, administration of Gal-10 crystals, but not soluble protein, to the airways of mice significantly enhanced inflammatory cytokine production, including IL-1β, IL-6, and TNF-α (238). Furthermore, CLCs enhanced neutrophil, monocyte, and dendritic cell recruitment to the lungs as well as enhanced mucus production by airway epithelial cells (238). CLC treatment also promoted antigen-specific T cell responses and increased antigen-specific antibody

responses *in vivo* (238). Of note, type 2 immunity in mice also triggers protein crystal formation, which is due to crystallization of two closely related chitinase-like proteins, Ym1 (Chil3) and Ym2 (Chil4) (239). Defining the biology of CLC and pseudo-CLC (Ym1 and Ym2 crystals) and their function in type 2 immunity is an ongoing area of investigation. Such work underscores the importance of defining distinct features of murine and human lung immunity, as it allows for the development of novel murine models to uncover new biology relevant to humans.

Although murine models have been critical experimental systems for mechanistic studies, there is great interest in developing human *ex vivo* models, allowing for experimental approaches that cannot be easily performed *in vivo*. The advent of air–liquid interface cultures represented an important breakthrough that allowed the investigation of differentiated human airway epithelial cells *in vitro* (240). Various methods have been devised to advance the air–liquid interface culture system, such as organ-on-a-chip models and precision-cut lung slices (PCLS) (240, 241). Efforts to culture primary cells from healthy donors and patients with pulmonary disease at air–liquid interface have progressed, with the development of protocols to induce self-assembled organoids as well as biological and bioengineered scaffolds. The latter incorporate biodynamic forces to mimic air flow and stretch to better simulate the *in vivo* environment. However, as of yet, few of these systems reflect the heterogenous epithelial cell communities that are emerging from large-scale sequencing studies described above, and most do not recapitulate the cross-talk between the airway epithelium, peripheral nervous system, MP system, as well as other cell types that occurs *in vivo*. PCLS are an attractive model and can use both healthy and disease-specific tissue (241). However, PCLS eliminate the natural innervation of the airways, and care must be taken given heterogeneity between slices from different locations, particularly in disease states. Consequently, although these approaches are very promising and will likely play an important role, none can completely recapitulate the complexity of human lung biology *in vivo*. Obtaining samples from the airways and lungs of humans is an essential tool for understanding lung disease but requires careful attention to the safety of research participants as well as novel technologies to extract as much information as possible from limited samples (242).

Research bronchoscopy facilitates direct sampling of the airways and has become an indispensable tool for the study of human lung immunology. BAL fluid, endobronchial brushings or biopsies, and transbronchial biopsies can be collected during bronchoscopy and used for qualitative and quantitative measurements of structural and immune cells as well as mediators, mucus, and features of tissue remodeling. Research bronchoscopy has been most extensively used in volunteer participants with chronic lung diseases (e.g., asthma, chronic obstructive pulmonary disease, interstitial lung disease) or with chronic exposures (e.g., cigarette smoke) and has substantially contributed to our understanding of the mechanisms that contribute to these disorders (243–246). Research bronchoscopy has also increasingly been applied to acute lung diseases, including acute respiratory distress syndrome and SARS-CoV-2 infection (128, 213, 247, 248). This approach has significant advantages, including the ability to measure dynamic immune responses over time and to link these responses to clinical outcomes. Limitations include heterogeneity in the inciting cause as well as the presence of coexisting conditions or treatments that can alter lung immune responses.

Airway challenge (or provocation) models have been used since the 1980s and are a powerful tool for studying dynamic lung immune responses *in vivo* (249, 250). These models allow for control over the timing and intensity of exposure, can be safely performed in both healthy volunteers and participants with chronic lung disease, and are generally well tolerated (251–254). During airway challenge, an experimental reagent is delivered to the upper or lower airways to mimic environmental exposures, infections, or asthma exacerbations. Experimental reagents can be delivered to the whole lung through the use of an exposure chamber or via nebulization. Alternatively, bronchoscopy can be used to administer reagents to one or more segments of the lung, allowing for more precise localization and dosing while limiting the total amount of lung exposed. Airway challenge is often coupled with pulmonary function testing or novel imaging modalities to enable correlation between the immune response and lung structure and function (255, 256).

Airway challenge models have been most extensively used to study mild to moderate allergic asthma. Allergen- or virus-induced asthma exacerbations can be simulated by exposure of the lower airways to allergen or inoculation of rhinovirus in the nose (257, 258). In addition, some study designs use sequential challenges, for example administration of diesel exhaust or ozone before allergen challenge, to understand how environmental exposures alter airway immune responses (259, 260). These studies have contributed to our understanding of the key cell types and mediators involved in asthma exacerbations, their kinetics, and the relationship between inflammation, tissue remodeling, and airway hyperresponsiveness. Importantly, challenge models have also been successfully used to predict the response to pharmacologic interventions, thereby directly contributing to the development of new treatments for asthma (261, 262). In addition, human challenge studies with respiratory viruses have revealed novel biology that could not have been replicated with other human model systems. For instance, although reinfection with a respiratory virus can be partially explained by waning adaptive immune memory, a recent study using nasal administration of respiratory syncytial virus demonstrated that neutrophilic inflammation in the airways at the time of pathogen exposure predisposes the host to symptomatic infection (263). Furthermore, a human influenza challenge study demonstrated unique, innate-like features of lung-resident $CD8^+$ T cells during influenza infection (264).

Although research bronchoscopy and airway challenge models have proven to be a powerful tool for understanding the *in vivo* airway immune response, one of the limitations has been the relatively low number of cells recovered. These challenges are now being addressed by methodologies for high-dimensional profiling of single cells, including RNA and ATAC sequencing, mass cytometry, and spectral flow cytometry. Single-cell approaches have greatly facilitated the study of rare cell types and are helping to unravel heterogeneity within immune cell subsets recovered from the airway (265, 266). The application of multiomics approaches and newer techniques, such as spatially resolved transcriptomics, to research bronchoscopy has the potential to increase the resolution with which we can study airway immune responses *in vivo*. Finally,

Table 1. Outstanding Questions and Challenges in Airway and Lung Immunology

Airway/lung immune sensing
• How do novel epithelial cell types and states regulate airway immunity?
• How do airway epithelial cells, neurons, stromal cells, and immune cells cross-talk to regulate immunity?
• How does the microbiome influence airway and lung immunity?
Tissue-resident immune memory
• When and how is the tissue-residency program instructed in various adaptive lymphocyte populations?
• What are the niches supporting tissue-resident immune memory in various contexts?
• How does innate training integrate with Trm and Brm cells to imprint inflammatory memory at the tissue level?
Age-related changes in lung immunity
• Which features of the aging lung microenvironment are causal in driving cell-nonautonomous alterations in immune cells?
• What are the cell-autonomous pathways that drive age-related immune cell dysfunction?
Advancing human experimental systems
• How can animal models be improved to better recapitulate human airway and lung immunity?
• How do we improve human *in vitro* and *ex vivo* experimental systems?
• How do we improve human *in vivo* models, such as challenge studies, and enhance high-dimensional profiling of the recovered cells?

Definition of abbreviations: Brm = tissue-resident memory B cell; Trm = tissue-resident memory T cell.

machine learning approaches hold potential to classify disease states and reveal novel patterns present in high dimensional data sets (267, 268).

Conclusions

In this workshop report, we have discussed four areas of investigation that we believe are critical to advance our understanding of lung immunology, including outlining important questions and challenges (Table 1). Although this report cannot be exhaustive, we believe the themes discussed above represent central topics of inquiry. Although there has been tremendous progress in defining the biology of airway and lung immunity, it has become increasingly clear that developing more integrative models will require the development of new tools and great multidisciplinary collaborations. Specifically, improving murine and human models and leveraging novel single-cell technologies will be necessary. In addition, multi-investigator collaborations among immunologists, epithelial and stromal cell biologists, neuroscientists, computational biologists, and clinicians, among other disciplines, will be necessary to develop new models and novel therapeutic approaches to prevent and treat inflammatory pulmonary diseases. ■

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