Antiviral B cell and T cell immunity in the lungs

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Respiratory viruses are frequent causes of repeated common colds, bronchitis and pneumonia, which often occur unpredictably as epidemics and pandemics. Despite those decimating effects on health and decades of intensive research, treatments remain largely supportive. The only commonly available vaccines are against influenza virus, and even these need improvement. The lung shares some features with other mucosal sites, but preservation of its especially delicate anatomical structures necessitates a fine balance of pro- and anti-inflammatory responses; well-timed, appropriately placed and tightly regulated T cell and B cell responses are essential for protection from infection and limitation of symptoms, whereas poorly regulated inflammation contributes to tissue damage and disease. Recent advances in understanding adaptive immunity should facilitate vaccine development and reduce the global effect of respiratory viruses.

The lungs and gut are open doors to the environment, which puts them at special risk of attack by pathogens. Respiratory infections are second only to cardiovascular disease in their effect on global health and especially affect people at the extremes of age. Respiratory viruses cause a spectrum of illness, ranging from an asymptomatic state to life-threatening multi-organ failure, and impose a vast socioeconomic burden. The convergence of over 200 serologically distinct viruses into one ecological niche demonstrates both the unique vulnerability of the respiratory tract and the difficulty in making progress against such a diversity of pathogens.

Around 50% of hospital admissions for children and 22% of hospitalizations of adults with community-acquired pneumonia are associated with respiratory viruses, most commonly respiratory syncytial virus (RSV) and influenza virus, respectively. In addition, rhinoviruses (the classic common cold virus), human metapneumovirus, parainfluenza virus, adenoviruses and coronaviruses can sometimes cause serious disease both in healthy people and in those with underlying cardiorespiratory conditions. Viral infections can both precipitate and exacerbate asthma and chronic bronchitis, each of which are top-ranking noncommunicable disease of children and adults, respectively.

Despite an intensive and sustained research effort over many decades, respiratory viruses continue to decimate respiratory health. In addition to causing repeated sporadic and seasonal disease, they constantly threaten to emerge as epidemic or pandemic outbreaks that stretch the social and medical resources of even wealthy nations. This was clearly demonstrated by the pandemic of influenza A virus pH1N109, as well as by zoonoses such as severe acute respiratory syndrome and Middle East respiratory syndrome.

The only currently licensed and generally available vaccines against respiratory viruses are for influenza virus, and even these are suboptimal. The paucity of vaccines is due in part to the only limited understanding of immune responses that can provide protection against respiratory viral infection: in many cases, even fundamental correlates of protection have yet to be accurately defined, and the most appropriate antigens to which vaccines should be targeted remain unknown. Animal models are generally imperfect guides to human disease, and the populations at highest risk of severe infections (i.e., young children and elderly adults) are the most difficult to study. In addition, vaccines are often less effective in those with immature or senescent immune systems.

Immunological protection against specific strains of influenza virus can persist for many years, and infections with rhinovirus also confer durable serotype-specific protection. Respiratory viruses have evolved diverse strategies to evade control by the immune system, including vast antigenic variation. However, infections with RSV recur throughout life despite its relatively stable antigenicity, which suggests impairment of durable protective immune responses by mechanisms that are yet to be fully elucidated. Further understanding of the mechanisms underlying adaptive immunity and the ways in which they interact with respiratory viruses therefore continues to be an urgent priority.

Effect of the lung environment on adaptive immunity

The lungs have very large mucosal and gas-exchanging surfaces that are constantly exposed to the environment. The exchange of oxygen and carbon dioxide depends on preservation of the delicate anatomical structures of the lungs that are relatively intolerant of physical or inflammatory damage. Lung tissues therefore maintain a fine balance, tolerating nonpathogenic environmental encounters but mounting a vigorous and effective response to harmful organisms. Mechanisms that prevent over-exuberant responses to environmental antigens include expression...
of the ‘do not eat me’ signal molecule CD200, the mucin MUC-1 and surfactant proteins, along with the reduced expression of pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs)\textsuperscript{20–22}. In the surfactant proteins, along with the reduced expression of pattern-recognition receptors (PRRs). Furthermore, the types and abundance of bacterial and fungal species may well differentially influence the immune responses that species may well differentially influence the immune responses that are triggered by pathogens\textsuperscript{28}.

Although it was long believed to be essentially sterile, it is now clear that the lower respiratory tract is colonized by a variety of relatively fastidious organisms that are generally innocuous but may be necessary for the maintenance of mucosal defences\textsuperscript{24}. It is well known that in the gut, the intestinal microbiota influences local and systemic immunity, and it is becoming increasingly clear that this is also the case in the lungs\textsuperscript{25–27}. Although inflammation in response to commensal respiratory flora is tightly restrained, bacterial products maintain the basal activation state of lung epithelial cells through stimulation via PRRs. Furthermore, the types and abundance of bacterial and fungal species may well differentially influence the immune responses that are triggered by pathogens\textsuperscript{28}.

Since cells of the immune system do not traffic equally between peripheral blood and the lungs, such local factors confer additional levels of complexity on attempts to understand mucosal immune responses. This is especially true for humans, in whom direct sampling of respiratory tissues can be difficult or impossible and extrapolation from studies of peripheral blood may be misleading. As understanding of the interplay between innate immunity and adaptive immunity improves, knowledge of tissue-specific factors and local sampling will become increasingly important in defining determinants of infection risk and in vaccine development.

**Initiation of the immune response**

The specialized needs of the pulmonary environment dictate that highly compartmentalized and sequential immune responses are essential for minimizing loss of function during the inflammatory processes of antiviral defense. Respiratory viruses enter via the respiratory epithelium and need to circumvent intrinsic mechanisms of protection, including mucus and antiviral peptides, that can prevent initial attachment and viral entry\textsuperscript{29,30} (Fig. 1). If these defenses are breached, epithelial cells have a central role in initiating the immune response by recognizing viral components via PRRs such as TLRs and intracellular sensors, including RIG-I-like receptors\textsuperscript{31}. Ligation of these leads to a signaling cascade that results in the upregulation of type I and type III interferons that trigger the inflammatory response, contribute to the differentiation of cells of the adaptive immune system and promote an antiviral state locally\textsuperscript{32}.

Alveolar macrophages and dendritic cells (DCs) that patrol and probe the respiratory lumen sample their surroundings, picking up microbial components and contributing to secretion of the cytokines and chemokines that maintain or ‘step up’ immune responses\textsuperscript{33}. PRR ligation and cytokine signaling promote DC maturation that enables the induction of adaptive immune responses via antigen presentation and...
costimulation\textsuperscript{34,35}. Furthermore, inflammatory mediators recruit innate cells, including neutrophils and, critically, natural killer cells. These have the ability to kill virus-infected cells via perforin–granzyme–dependent mechanisms, and the type II transmembrane protein cytokine FasL via its receptor Fas (CD95), as well as the production of interferon-\(\gamma\) (IFN-\(\gamma\)), with its various immunostimulatory effects and polarization of incoming T cells to a more antiviral, cytolytic T helper type 1 (T\(_{H1}\)) response\textsuperscript{36}.

The responsiveness of airway epithelial and myeloid cells to the initial stages of viral infection is therefore of critical importance to the subsequent development of adaptive immunity. They not only are responsible for the recruitment of primary and secondary cells of the adaptive immune system to the site of infection\textsuperscript{37} but also provide the immunostimulatory signals needed to induce a program of proliferation and differentiation into mature B cells and T cells\textsuperscript{38}, which enables the early control and clearance of infection, followed by contraction and the establishment of durable memory that protects against future infections.

**B cells and antibodies in the respiratory tract**

The best defined correlate of protection in almost any infectious disease is antibody\textsuperscript{39}. Although correlation is not proof of causation, it is plausibly that antibodies at appropriate sites have a direct role in protection against both infection and systemic dissemination. Antibodies can neutralize infectivity directly by binding to viral surface proteins that are essential for entry of the virus into host cells (Figs. 1 and 2). This property underlies the most commonly used serological measure of immunity to influenza virus: hemagglutination inhibition. This detects hemagglutinin (HA)-specific antibody by its ability to block the HA receptor-binding site, which prevents the clumping together of red blood cells after exposure to influenza virus in vitro\textsuperscript{40}. Functional neutralizing antibodies to respiratory viruses (including influenza virus and RSV) can be measured by plaque-reduction assays, although such methods require standardization\textsuperscript{41–43}. In addition to neutralizing viruses, antibodies can also act through the ligation of Fc receptors to enable triggering of the complement cascade and antibody-dependent cell-mediated cytotoxicity\textsuperscript{44,45} (Fig. 2). The importance of these mechanisms is less well defined than is the importance of virus neutralization, but these mechanisms may be important adjuncts for viral clearance and can also be quantified. The different aspects of antibody-mediated effector function that these assays measure are therefore important considerations in determining correlates of protection.

Most antibody-mediated correlates of protection have been defined for serum, in which the main immunoglobulin isotype is IgG. However, peripheral blood is probably not the site at which antibodies are most important in preventing infection with respiratory viruses. Instead, exclusion by the immune system occurs mainly at the respiratory mucosa, where virus-specific antibodies act in concert with the physical barriers and antiviral substances secreted by respiratory epithelium\textsuperscript{46} (Fig. 1). Mucosal antibodies, particularly those in the upper respiratory tract, are mainly in the form of locally produced dimeric secretory IgA\textsuperscript{47}, and IgA-deficient mice are highly susceptible to influenza virus\textsuperscript{48}. IgA may also confer some cross-reactive protection against different strains of influenza virus\textsuperscript{49}, but rapid mutation of viral HA and neuraminidase (NA) proteins allows this virus to evade the immune system.

The virus-specific B cell response is generated mainly in lymphoid tissues, either in regional draining lymph nodes or in mucosa-associated lymphoid tissue in the nose or bronchus\textsuperscript{52}. Tertiary lymphoid aggregates such as inducible bronchus-associated lymphoid tissue form during respiratory infection of mice; these aggregates preferentially promote IgA+ B cells and can be responsible for more persistent responses but have been difficult to demonstrate in the human airways\textsuperscript{53}. Early antibody production does occur locally under the influence of the B cell–trophic factors BAFF and APRIL, produced by DCs, but B cells generated in this way are short-lived and do not undergo affinity maturation\textsuperscript{54,55}.

The classic differentiation pathway of B cells is T cell dependent and results in the production of high-affinity antibody-secreting cells and memory B cells. Mature DCs migrate to secondary and tertiary lymphoid tissues, carrying virus-derived antigens, which are presented to B cells that migrate through the nodal tissue (Fig. 2). The recognition of antigen begins a program of differentiation that causes migration to the edge of the lymphoid follicle and proliferation. Contact with CD4+ helper T cells, which recognize cognate antigen presented by the B cell via major histocompatibility complex class II, leads to immunoglobulin isotype class switching from IgM to IgG or IgA and proliferation\textsuperscript{56,57}. This occurs through interactions of the costimulatory receptor CD40
with its ligand CD40L and cytokine signaling via interleukin 4 (IL-4), IFN-γ and (particularly in the case of IgA) transforming growth factor-β (TGF-β). Short-lived plasmablasts are formed at this stage, while some B cells progress to participate in the germinal center (GC) reaction, where somatic hypermutation and affinity maturation occur, promoted by specialized CD4+ follicular helper T cells (Tfh cells) that express the costimulatory receptor ICOS, the costimulatory molecule PD-1, IL-21 and other markers driven by the transcriptional repressor Bcl-6 (refs. 58–60). Following multiple rounds of selection, high-affinity antibody-secreting cells are generated, as are non–antibody-secreting memory B cells. The former are responsible for the abrupt increase in antibody titers that occurs relatively early during infection, but this population contracts following antigen clearance, leaving a small number of long-lived plasma cells. These cells migrate to survival niches that include the bone marrow and respiratory mucosa, and they are responsible for the maintenance of long-term antibody titers. TLR signaling is crucial in this process, with TLR9 and TLR10 on B cells potentially directly promoting immunoglobulin production. Adjuvants can enhance antibody production via similar B cell–intrinsic mechanisms, with ‘imprinting’ of B cells leading to upregulation of the Bcl-6 homolog Zbtb20 and promoting the long-term survival of plasma cells. The number and persistence of plasma cells are thus determined by a sequence of fate ‘decisions’ that depends on the integration of a variety of signals: the immediate choice between the proliferation or death of perifollicular B cells in the first 3 days after antigen encounter; differentiation into short-lived extra-follicular plasma cells or participation in the GC reaction; positive selection of the affinity of the B cell receptor for viral antigen (rather than self antigen) for the generation of B cell clones of increasingly higher affinity; and the long-term survival versus death of the resultant antibody-secreting cells.

Memory B cells are poised to generate a greater and more rapid secondary response on reencounter with antigen. The secondary activation of memory B cells leads to early proliferation and the production of antibodies, whereas a subset of these cells go on to participate once again in the GC reaction for further affinity maturation and replenishment of the memory pool. The fate of these memory B cells is probably determined at an early stage, with markers (including CD80 and PD-L2) defining subsets that are to undergo early differentiation into plasmablasts or those that reenter the GC. B cell memory is particularly important for responses to respiratory viruses, to which people are commonly exposed throughout life even in the absence of any immunological defect. This may be due to persistent antigen availability, with longer engagement of the T cell antigen receptor promoting the Tfh cell fate over the Tfh1 lineages. TFH cells express a characteristic set of markers (including CD80 and PD-L2) defining the ‘decision’ between the proliferation or death of perifollicular B cells versus their localization in GCs allowing close contact with proliferating B cells and follicular DCs. TFH cells produce IL-21, which promotes isotype switching to IgA and enhances Bcl-6 expression in B cells, thus augmenting memory generation. TFH cells are therefore essential for durable protective antibody responses and are potentially key to the efficient generation of vaccine-induced antiviral immunity. In some respiratory infections, such as infection with RSV, impairment of antigen presentation to T cells or inhibition of type I interferon signaling can have a role in modulating Tfh cell responses. Although there are as yet no data on Tfh cells in human infection with RSV, it is possible that a defect in Tfh cell help (such as impaired IL-21 signaling) might contribute to the poor longevity of RSV-specific antibody responses.

The differentiation pathways of Tfh cells are not fully understood. In common with GC B cells, Tfh cells express Bcl-6, and expression of this factor in both cell types is required for the GC reaction to occur. Bcl-6 expression in Tfh cells suppresses the expression of other lineage-specific transcription factors and removes the effect of microRNAs that suppress ICOS, PD-1 and CXCR5, thus promoting the Tfh cell phenotype. However, the key triggers for differentiation into the Tfh cell phenotype remain controversial. CD4+ T cells exhibit a high degree of plasticity, and conversion between Tfh cells and other subsets of helper T cells (including Th1, Th2 and Th17 cells) has been described in various systems depending on the environmental context, cytokine milieu and infection type. In vitro, Tfh cells can be induced to express type 1, 2 and 17 cytokines with the appropriate polarizing signals and, in some animal models, can also be shown to express these in vivo. However, several studies suggest that the lineage ‘decision’ between Tfh1 cells and Tfh2 cells occurs early during the activation of naive CD4+ T cells and that the Tfh2 cell phenotype is maintained stably into the memory phase. This may be due to persistent antigen availability, with longer engagement of the T cell antigen receptor promoting the Tfh cell fate over the Tfh1 lineages. Conversely, in a mouse model of infection with influenza virus, IL-2 has been shown to polarize CD4+ T cells to a Th1 phenotype and away from the Tfh cell phenotype, with defective GC B cell responses when systemic IL-2 is administered.

In human studies, limitations on accessing lymphoid tissue have driven a search for Tfh-like cells in peripheral blood. In subjects receiving the inactivated influenza vaccine, CXCR5+CD4+ T cells, when cultured with autologous B cells in vitro, help the differentiation of plasmablasts and production of influenza virus–specific antibodies. These Tfh-like cells also express ICOS, CXCR3 and IL-21 and seem to represent an early population in peripheral blood that correlates with subsequent response to vaccination. Their exact relationship to Tfh cells in GCs remains unclear; they do not express Bcl-6, and their function in vivo is unknown.

Therefore, the type and quality of interactions between Tfh cells and B cells probably have an important role in supporting plasma cell
longevity and antibody persistence. Although studies investigating this in humans are limited, the potential translational effect of understanding the role of TFH cells is clear. Additionally, novel intracellular pathways are increasingly recognized as regulating the differentiation and function of TFH cells and therefore affecting B cell fate. For example, the microRNA cluster miR-17–92 is required for the differentiation of TFH cells and their migration into B cell follicles. Moreover, inactivation of miR-17–92 impairs the differentiation of TFH cells, the formation of GCs and the secretion of high-affinity antibodies, while T cell–specific transgenic expression of miR-17–92 enhances the generation of TFH cells and leads to fatal autoimmunity,84,85. This is an area of innovative research that shows particular promise for the improvement of vaccine design.

Tissue-resident memory T cells

As well as contributing to protection through helping the B cell response, cell-mediated immunity may directly contribute to viral clearance. T cells are abundant at the lung mucosa, with an estimated 1 × 10^10 T cells in the uninfamed human lung (comparable to the total number in blood). Virus–specific T cells increase in frequency and number during infection,86–88, and mice with T cell deficiencies show delayed virus elimination and impaired generation of antibodies to influenza virus and RSV,89,90. Children with T cell immunodeficiencies are also unable to clear some respiratory viruses.91

Since T cells recognize mainly relatively conserved internal viral proteins, they can potentially mediate cross-protection against secondary infections with serologically distinct virus strains. This has been demonstrated in experimental human models of infection with influenza virus, in which preexisting CD4+ T cells in peripheral blood correlate with diminished susceptibility to natural or experimental infection with influenza A virus under circumstances when specific antibody is absent.92 Preexisting cross-reactive CD8+ T cells have also been shown to reduce the severity of symptoms during natural infection with influenza virus.93 However, although live attenuated vaccines against influenza virus can induce CD8+ T cells directed against conserved epitopes, there is little clinical evidence that they confer substantial heterosubtypic protection.94 Less is known about the role of T cells in protection against other respiratory viral diseases, but there are probably many commonalities.17,95,96. It is not clear that these circulating cells themselves protect against viral disease; they correlate with protection, possibly because they reflect local pulmonary T cells that actually mediate viral clearance.

Paralleling the initiation of B cell responses, T cells are primed by antigen carried to draining lymph nodes by activated DCs.97 Following engagement of the T cell antigen receptor and appropriate costimulatory signals, T cells undergo a program of differentiation that rapidly commits them to the formation of an enlarged effector pool. CD8+ T cells acquire cytolytic activity and upregulate chemokine receptors that allow them to migrate to inflamed sites, where they detect virus-infected cells by binding to viral peptides presented in the context of major histocompatibility complex class I. CD4+ T cells recognize viral peptides presented by major histocompatibility complex class II–bearing cells and differentiate, depending on the environmental context and strength of interaction, into a variety of helper T cell subsets that make specific cytokine combinations. Although in certain circumstances T effector 1 and T effector 17 cells can be induced (and potentially have a role in immunopathology), the protective response to respiratory viruses is typically dominated by IFN-γ and is therefore biased toward a T effector 1 response, which promotes cytolytic activity and viral clearance.18,98,99. It is notable that the rapid proliferation of cells occurs in the local nodes rather than the lung itself, possibly to remove intense activation of the immune system from the delicate respiratory structures.

Following clearance of virus, the effector T cell populations contract, leaving long-lived memory T cells that, like memory B cells, are ready to combat secondary infection. Diversity of signal intensity, antigen availability, costimulatory signaling and the cytokine milieu leads to a heterogeneous population of memory T cells that can be categorized into functional subsets in both animal models and humans according to their expression of surface markers.100 Until recently, two major subsets of memory T cells had been recognized: central memory T cells that express the lymph node–homing receptor CD62L and chemokine receptor CCR7, which allows them to circulate between blood and lymphoid tissues; and effector memory T cells that do not express these receptors and display heightened effector ability. Together, these populations have the ability to self-renew and, on reencountering their cognate antigen, proliferate rapidly while upregulating cytotoxic and other effector molecules.

Although some memory T cells circulate through peripheral tissues, adoptive transfer and parabiosis studies indicate that many T cells in nonlymphoid organs do not regularly appear in the blood or lymphoid tissues. Initially described in gut, this additional memory T cell subset, resident memory T cells (T RM cells), is also found at other sites.101 Unlike central memory and effector memory T cells, T RM cells seem to be restricted to tissues, including the gut, genital mucosa, skin and lungs (Fig. 3). These sites are major portals of pathogen entry at which specialized memory T cells might provide early innate-like cell–mediated protection. Certainly, T RM cells are capable of rapid upregulation of effector molecules, have innate-like sensing functions and can contribute to protection and heterosubtypic immunity against infection of mice with influenza virus.102–104

T RM cells share similarities with effector memory T cells, which suggests that both might be derived from a common precursor. However, T RM cells from various sites share a transcriptional profile that distinguishes them from other memory T cell subsets, which suggests that these cells are programmed by local anatomical or environmental cues.105 Most commonly, they express the C-type lectin CD69 (normally a marker of recent activation) and the integrin α4β7 (identified by antibodies to α4 and β7 (CD103)). In addition, the collagen-binding integrin VLA-1 is important in retaining CD4+ T RM cells in the lungs.106

The developmental pathway for lung T RM cells remains to be elucidated but seems to require a sequence of differentiation steps. In the draining lymph nodes, antigen presentation by CD103+ DCs predetermines CD8+ T cells to effector differentiation, with the ability to enter inflamed tissues via the expression of such receptors as CCR5 and downregulation of CD62L.107 This contrasts with CD11b+ DCs, which ‘preferentially’ prime central memory T cell–like cells. In addition, effector T cells destined for the lungs can express a combination of chemokine receptors, including CXCR3 and CCR4 (refs. 108,109). In the genital mucosa of mice, macrophages make the chemokines CCL5 and CXCL9, which attract and retain T RM cells.110 Following the migration of T RM cells to sites of infection, the strength of signaling via the T cell antigen receptor and availability of additional signals via costimulatory receptors and cytokines (such as IL-12) drive differentiation toward a terminally differentiated short-lived effector state or (later in the course of infection) toward memory precursor cells.111 Environmental cues probably govern commitment to the T RM cell phenotype; for example, CD103 is not expressed on herpes simplex virus–specific CD8+ T cells until they have been present in infected skin for a certain length of time.105 Important among local signals is TGF-β, produced by regulatory T cells (T reg cells) and, to a lesser extent, activated CD4+ T cells in the respiratory epithelium. Upon recognition of antigen presented by DCs, CD4+ T cells secrete the ‘latent’ form of TGF-β1; this dimer of TGF-β1 and the latency–associated protein LAP interacts with integrin α5β1 on DCs (or
specific microbial proteases such as influenza virus neuraminidase112), which triggers release of the active form of TGF-β1. This acts on naïve CD4+ T cells to inhibit the differentiation of high-affinity Th1 or Th2 cells and (with IL-2 and retinoic acid) promotes the differentiation of induced Treg cells. In the presence of IL-6, TGF-β drives the differentiation of regulatory Th17 cells113 (Fig. 3).

The lungs of influenza virus–infected mice contain abundant TRM cells that are responsible for heterosubtypic immunity and are present for up to 7 months114. The mechanisms underlying the subsequent decrease in the frequency of TRM cells are unclear but might possibly include the insufficient constitutive expression of TGF-β in the respiratory tract. It is also intriguing that lung TRM cells selectively express the interferon-generated transmembrane protein IFITM3, which is defective in some cases of severe infection with influenza virus115. Lung TRM cells that lack IFITM3 are more susceptible to infection by influenza virus and undergo selectively depletion during subsequent infections, which suggests that IFITM3 expression promotes the survival of TRM Cells116.

Immunopathology and immunomodulation by adaptive immunity

Although respiratory viruses can have direct cytopathic effects on lung tissues, disease can also be attributable to overexuberant immune responses that cause tissue damage while destroying virus-infected cells117. This has been described as an immunological or cytokine ‘storm’, in which abundant cells or inflammatory mediators contribute to disease118,119.

In viral bronchiolitis (most often due to RSV), infiltration of inflammatory cells, edema and tissue necrosis are thought to lead to respiratory failure due to airflow obstruction and alveolar collapse (atelectasis)120,121. Following infection of mice with RSV, depletion of either CD4+ T cell populations or CD8+ T cell populations typically leads to a reduction in disease severity but also impairment in the clearance of virus90 (Fig. 1). However, in severe respiratory viral infection, it seems likely that runaway activation of adaptive immunity (either through dysregulation of the immune system or driven by reduced viral control by host immunity) is a major component. For example, highly pathogenic strains of influenza virus, including recently emerged avian H5N1 strains that have crossed over into human populations, encode NS1 proteins that not only interfere with type I interferon activity but also are associated with increased release of inflammatory cytokines122. RSV also encodes an NS1 that suppresses type I interferons, and there is evidence that an NS1 that suppresses type I interferons, and there is evidence that

In the 1960s, a formalin-inactivated vaccine against RSV (FI-RSV) was assessed in trials of children and not only failed to induce protective immunity but also caused vaccine-enhanced disease on subsequent natural exposure to RSV. Several mechanisms may have contributed to this effect, including the generation carbonyl side chains on viral antigens126, low-affinity and poorly neutralizing antibodies127 that caused the deposition of inflammation-promoting immunocomplexes128, aberrant skewing of CD4+ T cells toward a Th2 response128,129, and a lack of induction of Treg cells and their recruitment to the lungs130. Inappropriate generation of helper T cell subsets might also be a factor in neonates, whose adaptive immunity tends toward type 2 responses, and in bronchiolitic children, in whom it has been suggested that Th17 cells and cytokines have a role in immunopathology129,131,132.

As well as the various intrinsic mechanisms by which the lungs restrain inflammation, T cells themselves regulate the adaptive immune response so that a balance between tissue damage and clearance of the virus is achieved. Following migration to an inflamed site, effector CD8+ T cells upregulate IL-10, which leads to overall dampening of the adaptive response through a negative feedback mechanism133. T cells also upregulate inhibitory receptors such as PD-1; signaling through these receptors suppresses effector function and, once antigen has cleared, rapid contraction of B cell and T cell populations occurs by apoptosis.

In addition, specialized Treg cells are now well recognized as important modulators of multiple immune mechanisms that prevent autoimmunity as well as immunopathology in response to viral infection18. Interestingly, they have also been found to promote Th17 cell differentiation by limiting the availability of IL-2, which can suppress Th17 cells134 (Fig. 1). Treg cells are characterized by expression of the transcription factor Foxp3 and make up 5–10% of all CD4+ T cells. Treg cells that
differen
tiate in the thymus act mainly to maintain tolerance to self anti-
gens. In contrast, inducible T_{reg} cells are generated following exposure
to cognate antigen in the context of cytokines such as TGF-β. Following
infection with RSV, activated T_{reg} cells accumulate in the mouse lungs
and reduce disease severity, suppressing antigen-specific effector CD8^{+}
T cells.\textsuperscript{135} They also have a role in controlling vaccine-enhanced disease
following vaccination with Fl-RSV, for which transfer of conventional
CD4^{+} T cells augments disease, whereas recruitment of T_{reg} cells to the
lungs ameliorates it.\textsuperscript{130} Thus, the unregulated promotion of proinflam-
matory adaptive immune responses by vaccination may not be wholly
desirable, and the role of immunomodulatory mechanisms in other
respiratory viral infections will need to be considered in more detail.

Harnessing adaptive immunity for vaccines and therapeutics
Vaccines and specific therapies against respiratory viral pathogens are
essentially limited to influenza virus, although vaccines against adenovirus
are available for military use in the USA. Although inactivated vaccines (IIV)
and live attenuated vaccines (LAIV) against influenza virus are well established in most vaccination programs, they remain
suboptimal in terms of both immunogenicity in many populations and
the requirement for regular re-vaccination to overcome variation
in influenza virus strains. Both IIV and LAIV must be reformulated
each year, and unexpectedly poor matching of vaccine with circulating
strains of influenza virus can reduce efficacy to less than 50% even in those with healthy mature immune systems.\textsuperscript{136} While LAIV is delivered
intranasally and has been shown to induce a mucosal immune response
more akin to natural infection, there is no demonstrated effect of LAIV
on heterosubtypic immunity. Furthermore, LAIV is generally ineffective
in older adults in whom preexisting immunity blocks local infection and
the vaccine-induced immune response.\textsuperscript{137}

For RSV, vaccine development has been slowed by concerns about
vaccine-enhanced immunopathology and inadequately defined cor-
relates of protection, as well as the specific difficulties of inducing appropriate
immune responses in infants. Candidate vaccines for other
respiratory viruses, including parainfluenza virus, have been held back
for both scientific reasons and economic reasons.\textsuperscript{138} Therefore, a sub-
stantial clinical need remains for the development of vaccines, antiviral
agents and immunomodulatory therapeutics based on increased under-
standing of immune and pathological responses of the respiratory tract.

Advances in defining critical protective epitopes have revitalized some
areas of the development of vaccines against respiratory viruses. The
finding of cross-reactive HA stem–binding antibodies following infection
with influenza virus and vaccination suggests that heterosubtypic
immunity might be achieved via antibody-mediated mechanisms as well
as via T cells.\textsuperscript{139} Elucidation of the structural conformations of the RSV
F protein have revealed novel epitopes that might allow better targeting
of antibody responses to infectious virions.\textsuperscript{140} Further understanding
of linear and conformational epitopes of respiratory viruses is necessary
so that both cellular immunity and humoral immunity can be brought
into vaccine-induced protection.

Conclusion
Immune responses are tightly regulated in the respiratory tract. The
distinction between adaptive immunity and innate immunity is increas-
ingly blurred; not only do local innate signals profoundly influence
the pattern and intensity of acquired responses, but also T cells and B
cells can assume functions classically associated with innate immunity.
Mucosal immune responses are highly localized, and the direct study
of lung cells, tissues and fluids (rather than the finding of correlates in
the peripheral blood) is therefore essential. This is particularly problem-
atic in humans, for whom much remains to be learned about immune

responses in high-risk populations (such as young children and elderly
adults). However, techniques that allow direct sampling of the respira-
tory tract are constantly improving, which provides hope that the ratio-
nal development of vaccines and therapeutics will continue to accelerate.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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