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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[1–4]. 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[5]. 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[6–8]. 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 p(H1N1)09, as well as by zoonoses such as severe acute respiratory syndrome and Middle East respiratory syndrome[9–11].

The only currently licensed and generally available vaccines against respiratory viruses are for influenza virus, and even these are suboptimal[12]. 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[13]. 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[14,15].

Immunological protection against specific strains of influenza virus can persist for many years[16], and infections with rhinovirus also confer durable serotype-specific protection[17]. 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[18]. 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[19]. 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

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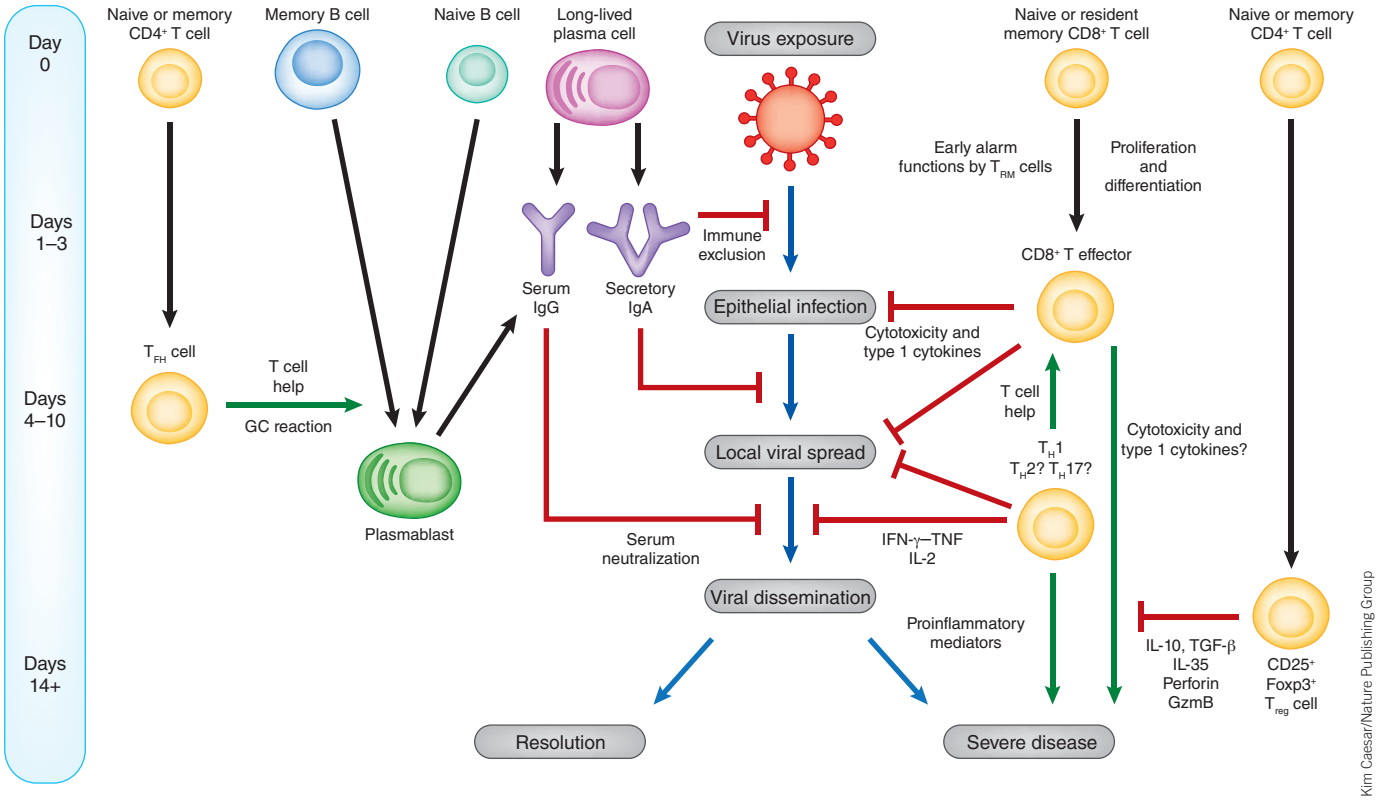

Figure 1 The roles of adaptive T cells and B cells in respiratory viral infection. During acute respiratory viral infection, humoral and cell-mediated immunity act at different points in time to limit disease. Mucosal IgA generated during previous encounter with virus can prevent or limit infection. IgG in the lungs can limit more severe disease. T cells are beneficial in terms of eliminating virus-infected cells; they coordinate a regulated immune response and, as T_{FH} cells, promote high-affinity durable antibodies. Failure to control viral dissemination can lead to severe disease. T_{reg} cells restrain effector responses through various mechanisms, including suppressive cytokines (IL-10, IL-35 and TGF-β) and possibly active killing via perforin and granzyme B (GzmB). An overexuberant or poorly regulated immune response can also lead to damaging immunopathology.

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)[20–22]. In the event of infection, mechanisms to restore epithelial integrity and lung function are also rapidly engaged. For example, innate lymphoid cells are involved not only in the generation of proinflammatory cytokines but also in airway remodeling and tissue repair following infection with influenza virus[23].

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[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[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[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[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[31]. Ligation of these leads to a signaling cascade that results in the upregulation of type I and III interferons that trigger the inflammatory response, contribute to the differentiation of cells of the adaptive immune system and promote an antiviral state locally[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[33]. PRR ligation and cytokine signaling promote DC maturation that enables the induction of adaptive immune responses via antigen presentation and

costimulation[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-γ (IFN-γ), with its various immunostimulatory effects and polarization of incoming T cells to a more antiviral, cytolytic T helper type 1 (T_H1) response[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[37] but also provide the costimulatory signals needed to induce a program of proliferation and differentiation into mature B cells and T cells[38], which enables the early control and clearance of infection, followed by contraction and the establishment of durable memory that protects against future infections.

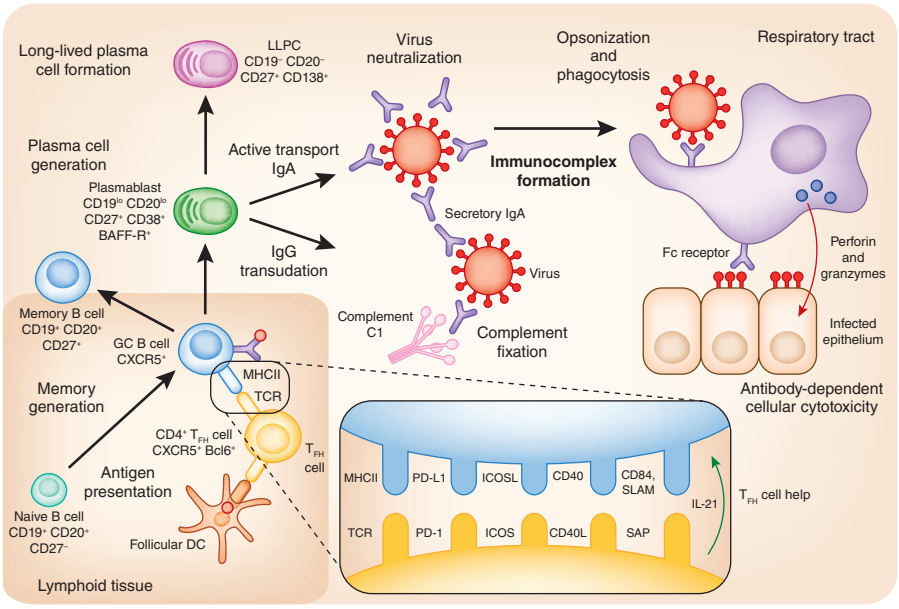

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Figure 2 B cells and antibody in viral infection. Following antigen recognition, B cells differentiate with the help of cognate $CD4^+$ T_{FH} cells to form antibody-secreting plasma cells and memory B cells. Class switching to IgG and IgA is followed by the secretion of large amounts of high-affinity antibody that can directly neutralize virus, block entry of the virus into the cell, fix complement, promote phagocytosis and allow antibody-dependent cellular cytotoxicity. Once antigen is cleared, a subset of plasma cells differentiate into a long-lived phenotype and migrate to survival niches in the respiratory tract and bone marrow. LLPC, long-lived plasma cell; MHCII, major histocompatibility complex class II.

B cells and antibodies in the respiratory tract

The best defined correlate of protection in almost any infectious disease is antibody[39]. Although correlation is not proof of causation, it is highly plausible 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*[40]. Functional neutralizing antibodies to respiratory viruses (including influenza virus and RSV) can be measured by plaque-reduction assays, although such methods require standardization[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[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[46] (**Fig. 1**). Mucosal antibodies, particularly those in the upper respiratory tract, are mainly in the form of locally produced dimeric secretory IgA[47], and IgA-deficient mice are highly susceptible to influenza virus[48]. IgA may also confer some cross-reactive protection against different strains of influenza virus[49], but rapid mutation of viral HA and neuraminidase (NA) proteins allows this virus to evade the immune system. Although titers of circulating antibodies can correlate with protection against infection, this may be because they reflect local protective IgA; IgG also is thought to have a direct role in defense of the lower respiratory tract, where it is more abundant than in the nasal mucosal fluid[50]. In RSV disease, this is illustrated by the clinical efficacy of the humanized mouse monoclonal IgG1 antibody palivizumab, the administration of which reduces hospitalization of premature infants by up to 78% but does not reliably prevent nasal infection[51].

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[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[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[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[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 (T_{FH} 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[61]. 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[62]. TLR signaling is crucial in this process, with TLR9 and TLR10 on B cells potentially directly promoting immunoglobulin production[63]. 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[64]. 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[65].

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[66]. B cell memory is particularly important for responses to respiratory viruses, to which people are commonly exposed multiple times throughout their lives. Classically, recurrent encounter with viral antigens should lead to boosting of antibody titers that ultimately leads to complete protection. However, respiratory viruses have evolved diverse ways of evading host immunity. These strategies include the aforementioned mutation of genes encoding the surface antigenic determinants HA and NA of influenza virus, or the reassortment of gene segments that leads to wholesale replacement of these by variants from zoonotic strains to which human populations have no preexisting immunity[67]. Similarly, rhinoviruses have diversified into over 150 distinct serotypes against which cross-protection is weak or nonexistent[68]. In contrast, RSV is relatively well conserved, with only the attachment G glycoprotein displaying substantial antigenic diversity[69]. Despite the fact that the other major RSV surface antigen, F protein, is more highly conserved across RSV strains and F protein–specific antibodies are easily detected in all adults, recurrent infection with RSV occurs throughout life even in the absence of any immunological defect[70]. This might be explained by the expression of various proteins with immunomodulatory potential that can affect immunological memory, including the nonstructural proteins that interfere with type I and III interferons and a fractalkine-like motif in G protein[71,72]. The reasons underlying the remarkably rapid decrease in protective immunity after infection with RSV and the short half-life of RSV-specific antibodies remain unknown but probably explain the lifelong susceptibility to recurrent infection with this virus[73].

T_{FH} cells

T_{FH} cells were first described as a $CD4^+$ T cell subset in the tonsils specialized to help the generation and maintenance of high-affinity B cells and antibody responses[58]. T_{FH} cells express a characteristic set of markers, including the chemokine receptor CXCR5 (to allow homing to the GCs, where they chiefly reside), PD-1, ICOS, CD28, CD40L, SAP and their lineage-specifying transcription factor Bcl-6. Their localization in GCs allows close contact with proliferating B cells and follicular DCs. T_{FH} cells can deliver signals to promote 'preferential' selection, with further differentiation allowing B cells to out-compete low-affinity suboptimal clones[74]. As well as participating in direct cell-to-cell signaling, T_{FH} cells produce IL-21, which promotes isotype switching to IgA and enhances Bcl-6 expression in B cells, thus augmenting memory generation[75]. T_{FH} 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 T_{FH} cell responses[18]. Although there are as yet no data on T_{FH} cells in human infection with RSV, it is possible that a defect in T_{FH} cell help (such as impaired IL-21 signaling) might contribute to the poor longevity of RSV-specific antibody responses.

The differentiation pathways of T_{FH} cells are not fully understood. In common with GC B cells, T_{FH} 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 T_{FH} 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 T_{FH} cell phenotype[76]. However, the key triggers for differentiation into the T_{FH} cell phenotype remain controversial. $CD4^+$ T cells exhibit a high degree of plasticity, and conversion between T_{FH} cells and other subsets of helper T cells (including T_H1, T_H2 and T_H17 cells) has been described in various systems depending on the environmental context, cytokine milieu and infection type. *In vitro*, T_{FH} 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*[77]. However, several studies suggest that the lineage 'decision' between T_H1 cells and T_{FH} cells occurs early during the activation of naive $CD4^+$ T cells and that the T_{FH} cell phenotype is maintained stably into the memory phase[78]. This may be due to persistent antigen availability, with longer engagement of the T cell antigen receptor promoting the T_{FH} cell fate over the T_H1 lineage fate[79]. Conversely, in a mouse model of infection with influenza virus, IL-2 has been shown to polarize $CD4^+$ T cells to a T_H1 phenotype and away from the T_{FH} cell phenotype, with defective GC B cell responses when systemic IL-2 is administered[80].

In human studies, limitations on accessing lymphoid tissue have driven a search for T_{FH}-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[81]. These T_{FH}-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[82,83]. Their exact relationship to T_{FH} 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 T_{FH} 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 T_{FH} cells is clear. Additionally, novel intracellular pathways are increasingly recognized as regulating the differentiation and function of T_{FH} cells and therefore affecting B cell fate. For example, the microRNA cluster miR-17~92 is required for the differentiation of T_{FH} cells and their migration into B cell follicles. Moreover, inactivation of miR-17~92 impairs the differentiation of T_{FH} 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 T_{FH} 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 uninflamed 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_H2 cells and T_H17 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_H1 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 those 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 $\alpha_E\beta_7$ (identified by antibodies to α_E (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 predisposes $CD8^+$ 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 $CD11b^{hi}$ 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 $\alpha_V\beta_8$ on DCs (or

specific microbial proteases such as influenza virus neuraminidase[112]), which triggers release of the active form of TGF-β1. This acts on naive CD4$^+$ T cells to inhibit the differentiation of high-affinity T_H1 or T_H2 cells and (with IL-2 and retinoic acid) promotes the differentiation of induced T_{reg} cells. In the presence of IL-6, TGF-β drives the differentiation of regulatory T_H17 cells[113] (**Fig. 3**).

The lungs of influenza virus–infected mice contain abundant T_{RM} cells that are responsible for heterosubtypic immunity and are present for up to 7 months[114]. The mechanisms underlying the subsequent decrease in the frequency of T_{RM} cells are unclear but might possibly include the insufficient constitutive expression of TGF-β in the respiratory tract. It is also intriguing that lung T_{RM} cells selectively express the interferon-induced transmembrane protein IFITM3, which is defective in some cases of severe infection with influenza virus[115]. Lung T_{RM} 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 T_{RM} cells[116].

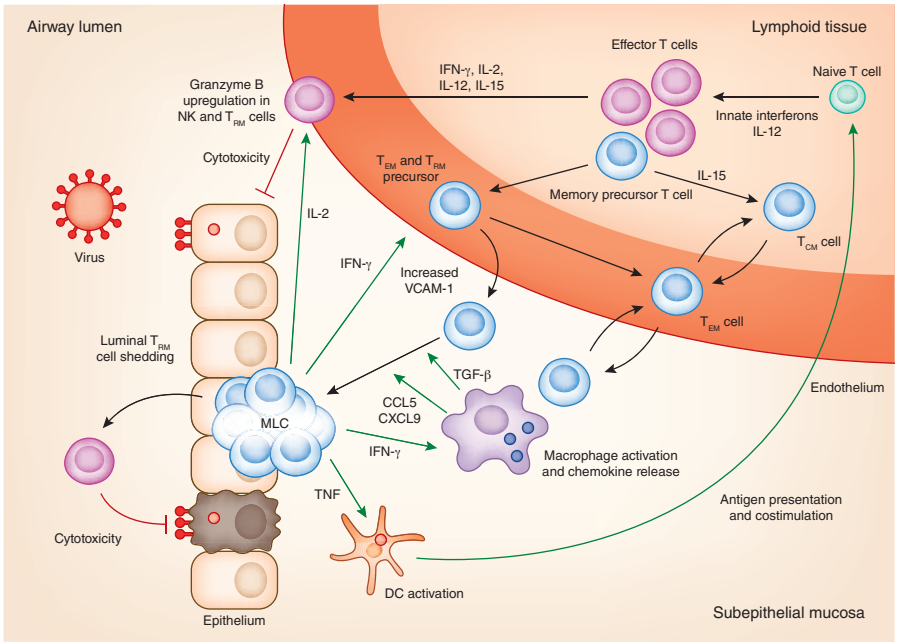

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Figure 3 Maintenance of the tissue localization and function of T_{RM} cells in the mucosa. Mucosal lymphocyte clusters (MLCs) are formed by T_{RM} cells after viral infection and act as sensory cells as well as early effector cells during reencounter with pathogens. They are maintained by upregulation of IFITM3, which allows T_{RM} cells to resist apoptosis during viral infection. Basal levels of IFN-γ production by T_{RM} cells activates tissue macrophages that release CCL5 and CXCL9, which keep the T_{RM} cells *in situ* and prevent the migration of cells from the mucosal lymphocyte clusters into the lumen of the airway. 'Polyfunctional' production of IL-2 by T_{RM} cells activates granzyme B production in natural killer (NK) cells and lymphocytes, whereas tumor-necrosis factor (TNF) activates DCs that carry antigen to regional nodes, thereby initiating the activation of T cells. T_{CM} cell, central memory T cell; T_{EM} cell, effector memory T cell.

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 cells[117]. This has been described as an immunological or cytokine 'storm', in which abundant cells or inflammatory mediators contribute to disease[118,119].

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 virus[90] (**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 cytokines[122]. RSV also encodes an NS1 that suppresses type I interferons, and there is evidence that RSV G protein contains a region that has structural similarity to the chemokine fractalkine and thus promotes inappropriate inflammatory responses[72,114,123,124].

Although it has long been assumed that immunological factors have a central role in the pathogenesis of RSV and influenza virus disease, this assumption has been challenged by the findings that severe disease is characterized by inadequate (rather than excessive) adaptive immune responses and robust viral replication[125]. How viral and host factors interact to predispose certain people to these aberrant responses and why the normal regulatory mechanisms fail has been discussed elsewhere[18].

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 antigens[126], low-affinity and poorly neutralizing antibodies[127] that caused the deposition of inflammation-promoting immunocomplexes[128], aberrant skewing of CD4$^+$ T cells toward a T_H2 response[128,129], and a lack of induction of T_{reg} cells and their recruitment to the lungs[130]. 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 T_H17 cells and cytokines have a role in immunopathology[129,131,132].

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 mechanism[133]. 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 T_{reg} cells are now well recognized as important modulators of multiple immune mechanisms that prevent autoimmunity as well as immunopathology in response to viral infection[18]. Interestingly, they have also been found to promote T_{FH} cell differentiation by limiting the availability of IL-2, which can suppress T_{FH} cells[134] (**Fig. 1**). T_{reg} cells are characterized by expression of the transcription factor Foxp3 and make up 5–10% of all CD4$^+$ T cells. T_{reg} cells that

differentiate in the thymus act mainly to maintain tolerance to self antigens. 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[135]. They also have a role in controlling vaccine-enhanced disease following vaccination with FI-RSV, for which transfer of conventional $CD4^+$ T cells augments disease, whereas recruitment of T_{reg} cells to the lungs ameliorates it[130]. Thus, the unregulated promotion of proinflammatory 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[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[137].

For RSV, vaccine development has been slowed by concerns about vaccine-enhanced immunopathology and inadequately defined correlates 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[138]. Therefore, a substantial clinical need remains for the development of vaccines, antiviral agents and immunomodulatory therapeutics based on increased understanding 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[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[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 increasingly 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 problematic 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 respiratory tract are constantly improving, which provides hope that the rational development of vaccines and therapeutics will continue to accelerate.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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