Protective and Harmful Immunity to RSV Infection

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Abstract

Respiratory syncytial virus (RSV) is an exceptional mucosal pathogen. It specializes in infection of the ciliated respiratory epithelium, causing disease of variable severity with little or no direct systemic effects. It infects virtually all children by the age of three years and then repeatedly infects throughout life; this it does despite relatively slight variations in antigenicity, apparently by inducing selective immunological amnesia. Inappropriate or dysregulated responses to RSV can be pathogenic, causing disease-enhancing inflammation that contributes to short- and long-term effects. In addition, RSV's importance as a largely unrecognized pathogen of debilitated older people is increasingly evident. Vaccines that induce nonpathogenic protective immunity may soon be available, and it is possible that different vaccines will be optimal for infants; older children; young to middle-age adults (including pregnant women); and elderly persons. At the dawn of RSV vaccination, it is timely to review what is known (and unknown) about immune responses to this fascinating virus.

INTRODUCTION AND CLINICAL BACKGROUND

Soon after its discovery in 1956, respiratory syncytial virus (RSV) was recognized as a leading global cause of respiratory disease in infants. It especially afflicts children in the first six months of life; it is the commonest cause of childhood acute respiratory infection and the single major cause of hospitalization during infancy. Most children are infected by RSV at least once before the age of two years (1). In most cases RSV infection results in only mild disease, but in some, RSV can cause bronchiolitis and viral pneumonia, an intense inflammatory response in the lower airways (1, 2).

With the advent of PCR-based diagnostics, RSV is increasingly appreciated as an important pathogen in at-risk adults, including frail, elderly persons and immunocompromised persons (**Figure 1**). Although rarely lethal in otherwise healthy people, it is an important cause of death in resource-poor settings, ranking below only pneumococcal pneumonia and *Haemophilus influenzae* type B as a cause of serious respiratory childhood infection. It is estimated that there are about 34 million new RSV lower respiratory tract infections (LRTIs) each year in children younger than five years, and that 99% of the childhood global deaths caused by RSV infection are in developing countries (3). In a prospective study of 84,840 Argentinian infants between 2011 and 2013, 65% of those with severe LRTIs were infected with RSV, accounting for 57% of fatal LRTIs (4).

Given this perpetual global toll and the fact that there are currently no specific treatments, new ways to prevent, diagnose, and treat RSV disease clearly have great potential to improve global

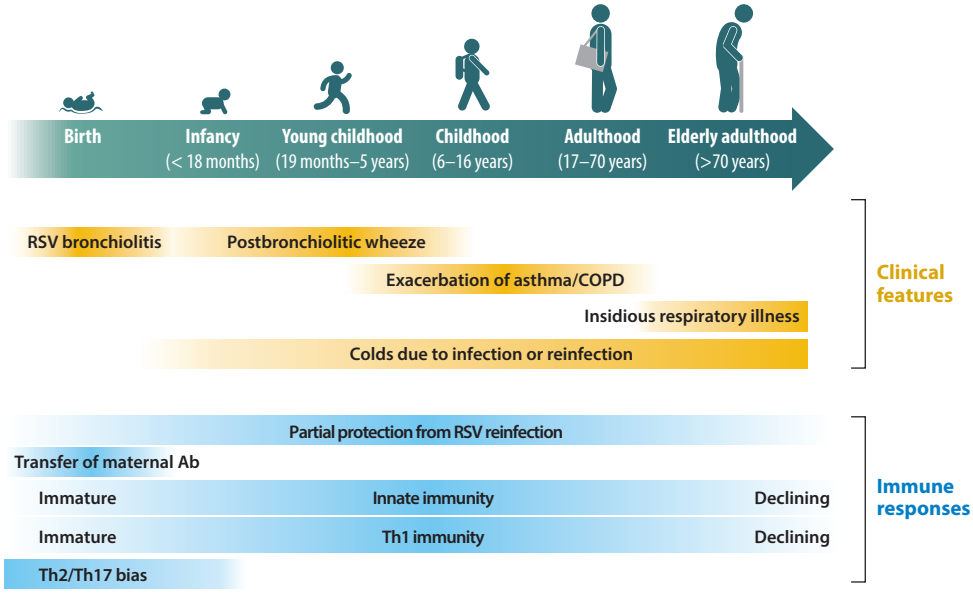

Figure 1

Age is a major determinant of RSV disease. First infections typically occur in the first RSV season encountered by a child after maternal antibody titers have declined; this is the time of greatest risk of severe lower airway disease, which may be followed by postbronchiolitic wheeze in later childhood. Immune responses mature in the first and second year of life, with more efficient innate immune responses, acquisition of protective Th1 immunity, and a relative decline in Th2 and Th17 responses. Repeated infections with RSV occur throughout life but in healthy adults only cause common colds. However, in those with respiratory conditions such as asthma or chronic obstructive pulmonary disease (COPD), RSV may precipitate exacerbations. Immunity tends to decline in old age, with most RSV deaths occurring in frail elderly persons.

health. There is now considerable optimism that progress in immunology and virology will lead to new approaches to prevention and therapy.

One fundamental question is what drives disease in infants: Is it high viral load, an excessive host response, or both? In some clinical studies, high viral load is associated with more severe disease and longer hospitalization (5, 6), and biopsy samples from children who die of severe RSV disease have a relative paucity of lymphocytes in the airways (7, 8). The high incidence of severe RSV disease and abundant viral shedding in immunocompromised children again indicates that high viral load can drive disease (9). In addition, human T cell responses peak only late in primary infection, after viral load has passed its peak and during recovery, suggesting they are unlikely to be the cause of pathology (10). Evidence of this sort suggests that some infants with severe disease mount a weak, delayed, and ineffective immune response to RSV that poorly controls viral replication compounded by immaturity of the neonatal immune system (11).

However, viral load may not be the only factor that drives disease. In some cases, the host response to RSV may be described as overexuberant, inappropriate, or dysregulated (**Figure 2**; 12). For example, some studies of children with severe or fatal bronchiolitis describe lung inflammation with a pronounced monocytic, T cell, and neutrophilic infiltrate (13) and an abundance of inflammatory mediators in the airway fluids (14–17), and many animal studies of RSV disease highlight the role of the excessive host response in causing disease (18). To reconcile these two views, it is evident that viral load is necessary to drive acute disease, the severity of which then depends on the immune and inflammatory response in the airway wall. The relative importance of viral load and inflammation to the pathogenesis of bronchiolitis is variable in individual cases of disease.

In addition to acute disease, RSV bronchiolitis is associated with long-term respiratory problems, especially persistent or recurrent wheezing and asthma. In a study of 90,341 children born between 1995 and 2000, 18% had bronchiolitis needing medical attention. Many went on to be diagnosed with asthma, with bronchiolitis involved in about one-third of cases (19). In a highly cited series of reports, Sigurs et al. followed up infants hospitalized with RSV bronchiolitis in their first year of life, comparing them to matched controls without early respiratory problems. At age 18 years, children who had had bronchiolitis showed an increased prevalence of asthma (39% versus 9%), clinical allergy (43% versus 17%), and atopic sensitization (41% versus 14%) compared with controls, leading to the conclusion that the risk of asthma increases with the severity of infant bronchiolitis (20).

RSV disease therefore poses many interesting and important immunological questions: It is not especially diverse antigenically, so how does it repeatedly reinfect with apparent ease? What are the mechanisms by which acute infections with a transient virus limited to the respiratory epithelium cause long-term pulmonary effects? Why are the very young and the very old so vulnerable, and what are the protective immune responses that should be induced by vaccines targeted to specific risk groups?

We describe what is known about immunity to RSV infection and address these issues in turn. **Figure 3** summarizes the different components of immune responses to RSV, and **Figure 4** depicts the timing of events in different situations.

INNATE DEFENSES

Mucus, Surfactants, and Antimicrobial Peptides

Respiratory mucus traps airborne particles that may carry infection, but excessive mucus secretion during infection may lead to airway plugging (21). RSV infection promotes mucin production via

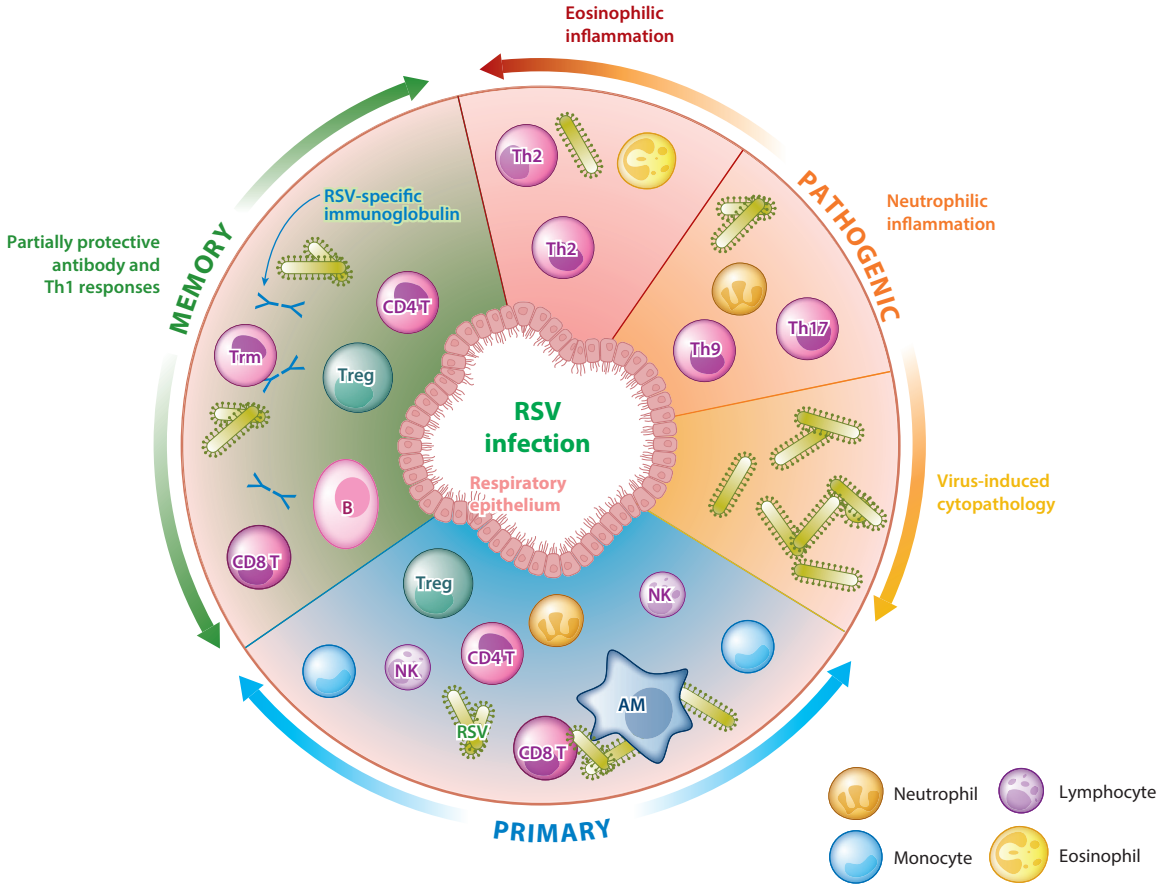

Figure 2

The spectrum of immune responses during RSV infection. Protective defenses against primary RSV infection include innate responses from resident airway cells [(e.g., epithelial cells and alveolar macrophages (AMs)] and recruited cells (e.g., neutrophils, monocytes, and NK cells) and antimicrobial secreted proteins. In established infection, adaptive immune responses assist viral clearance and result in partially effective immune memory. $CD4^+$ and $CD8^+$ resident memory T cells (Trms) and local IgA production provide partial protection against reinfection. Pathology can be driven by viral load but can also be caused by overexuberant host responses insufficiently modulated by regulatory T cells (Tregs). Immunopathogenic responses are probably associated with Th17-, Th2-, and (possibly) Th9-polarized adaptive immunity and lead to neutrophilic and/or eosinophilic inflammation. Vaccine augmentation caused by formalin-inactivated preparations is thought to be Th2 related and associated with poorly neutralizing antibody responses.

F protein–mediated enhancement of EGFR (epidermal growth factor receptor) phosphorylation (22). Certain RSV isolates are more mucogenic than others, the commonly used laboratory adapted A2 strain being a relatively weak mucin inducer (23). RSV can also cause ciliary dyskinesia (24), which, together with loss of ciliated cells, may result in impaired airway clearance and mucus obstruction.

Recent studies have also focused on ancient arms of innate immunity such as the antimicrobial peptide cathelicidin/LL-37, which has antiviral effects and inhibits epithelial cell infection by RSV in vitro and in mice. Higher preexisting nasal levels of LL-37 are also associated with protection following human experimental challenge (25). In addition, surfactant proteins can bind directly to RSV F protein (26) and enhance clearance of RSV in mice (27). Infants with severe RSV disease

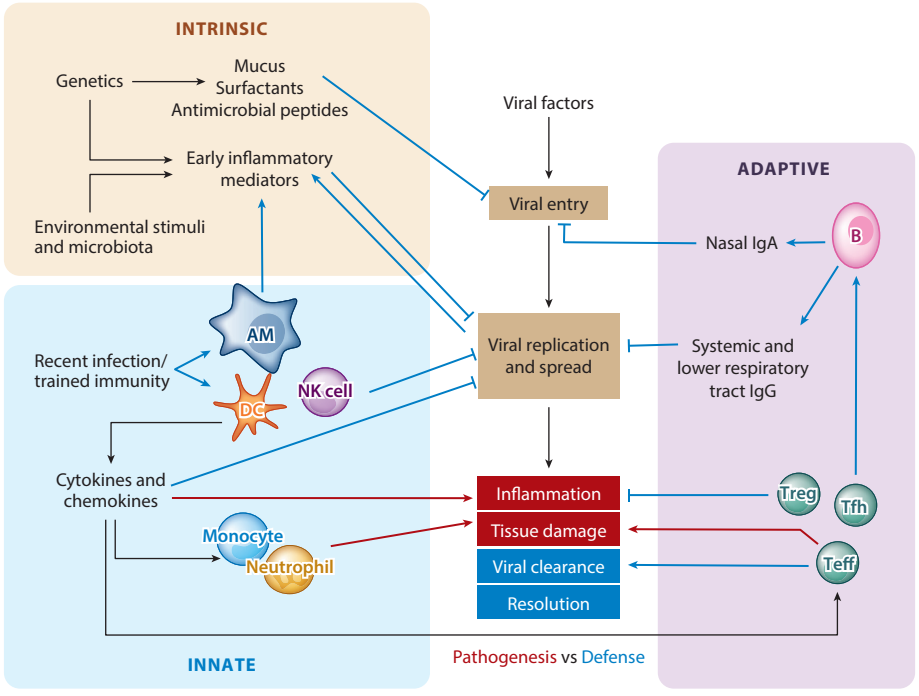

Figure 3

Pathways leading to antiviral defense and pathology. Viral entry and infection of the respiratory epithelium is blocked by the presence of specific antibodies, mucus, antimicrobial proteins, and inflammatory mediators produced early in infection. This initial phase is influenced by genetic factors, environmental stimuli, the resident respiratory microbiome, and infection history. Innate responses by resident airway cells, macrophages, and NK cells impede viral replication and spread to other parts of the respiratory tract. T cell responses are important for viral clearance and disease resolution but may be associated with inappropriately polarized responses and immunopathology. During secondary infection, tissue resident memory T cells and locally produced IgA may inhibit initial viral entry and replication, constrain infection to the upper airway and promote rapid resolution. Abbreviations: AM, alveolar macrophage; DC, dendritic cell; NK, natural killer; Teff, effector T cell; Tfh, T follicular helper cell; Treg, regulatory T cell.

have reduced levels of surfactant (28), and polymorphisms in surfactant genes are associated with disease severity (29, 30).

Resident Airway Cells and Early Cytokine Production

RSV mainly infects ciliated respiratory epithelial cells by binding of the attachment protein G to CX3CR1, present on the apical surface of ciliated cells and especially on the cilia themselves (31). Cellular entry is then dependent on the fusogenic capacity of the F protein, which is essential for infectivity. It is only weakly cytopathic, causing comparatively little cell lysis in human airway epithelial cells (32). However, it may undergo cell-to-cell transmission in infected airways and fuse cells to form syncytia, which is mediated by the F protein and small GTPase RhoA (33). The ability to form syncytia varies from one strain to another (A2 being relatively nonsyncytiogenic).

RSV infection triggers several different pattern-recognition receptors (PRRs), including cytosolic RIG-I-like receptors (RLRs) that signal via the adaptor protein MAVS (34–36). It also

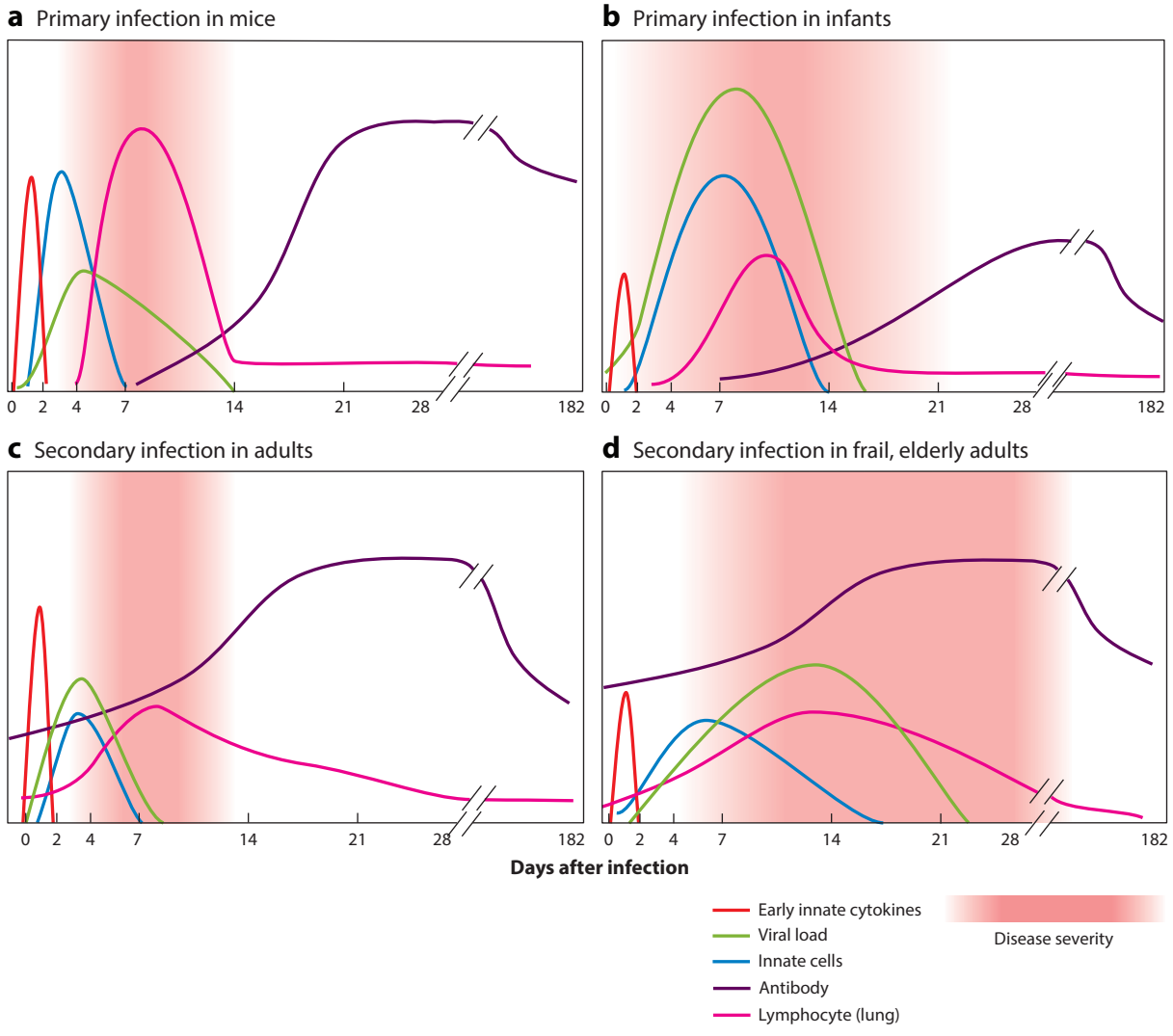

Figure 4

The time course of viral replication, disease, and immune responses after RSV infection. The timing and sequence of events are critical in understanding RSV disease in different situations. (*a*) Primary infection in mice. Early (innate) cytokine and chemokine production by resident airway cells occurs within the first 48 h of infection. This draws in innate cells (e.g., neutrophils, natural killer cells, and monocytes), which peak around 2–4 days after infection. Local adaptive immunity develops at the time of peak viral load and is associated with both virus clearance and disease. Mice develop a partial protective response to reinfection. (*b*) Primary infection in infants. Both innate and adaptive immunity are impaired, allowing the development of a high viral load associated with severe disease. RSV actively inhibits protective immune responses, and immunological memory is short-lived. (*c*) Secondary infection in adults. Adults have all been infected many times with RSV and have varying levels of circulating and airway IgG/IgA, which affords partial protection against reinfection and RSV common colds. Again, protective immunity is transient and incomplete, and antibody levels decline rapidly back to steady state. (*d*) Secondary infection in frail, elderly adults. Diminished innate and adaptive effector functions allow the insidious development of prolonged and severe disease. Note that the figure is illustrative and based on our current presumptions (data are incomplete, especially for humans).

triggers several Toll-like receptors (TLRs), and in mice, TLR2, 3, 4, and 7 are all involved in initiation of immune responses against RSV (37, 38).

Interferons have long been known to restrict the replication of viruses in cell culture through their effect on interferon-stimulated gene (ISG) upregulation. Viral sensing triggers interferons, and polymorphisms in type I interferon genes or genes of the type I interferon receptor signaling pathway have been reported to affect the risk of bronchiolitis (39, 40). Type I interferons induce an antiviral state in neighboring cells via induction of numerous ISGs, some of which amplify inflammatory responses after RSV infection (34, 41) by activation of dendritic cells (DCs), natural killer (NK) cells, and T cells (42). This occurs not only with live virus but also with defective viral particles, which may stimulate type I interferon production to an even greater extent (43).

The production of interferons and other innate mediators from infected epithelial cells has a crucial role in the subsequent course of RSV infection. Type III interferon production by the epithelium also induces an antiviral state and limits viral replication (44), whereas the type I interferon IFN-β may additionally promote production of the B cell survival factor BAFF by the respiratory epithelium (45).

In addition to innate responses occurring in epithelial cells, alveolar macrophages (AMs) may play an important part in initiation of responses in the distal airways. AMs are important for clearing debris and for lung homeostasis (46) and are ideally placed to sense viruses. In mice, they limit viral replication and trigger early innate immune responses to RSV (47–49), using cytosolic PRRs to detect RSV, and are an important source of type I interferons and other cytokines and chemokines (34, 50), leading to cellular recruitment to the infected respiratory bronchiole.

Recruited Innate Cells

The immediate response by epithelial cells and AMs induces a cascade of chemotactic factors that recruit a series of other innate (and later adaptive) immune cells. Plasmacytoid and conventional DCs (pDCs and cDCs) are recruited to the nasal mucosa of children with RSV infection, and their numbers remain elevated for several weeks after infection (51). In mice, pDCs are protective against pathology during RSV infection (52, 53), their activation being regulated by interaction with epithelial cells (54). Activation of DCs during RSV infection is partly dependent on autophagy (55–57) and is regulated by epigenetic modulation of gene transcription (58). However, inflammatory DCs upregulating PD-L1 appear to limit immunopathology by interaction with T cells expressing PD-1 (59).

Neutrophils are the predominant cell type in airway secretions from infants with bronchiolitis and are prominent in the lungs of RSV-infected mice given large inocula (34) or in those with heightened $CD8^+$ T cell responses (60). It is not clear whether they are beneficial or detrimental, or if they just reflect lung injury. In mice, monocytes infiltrate the lungs shortly after the neutrophils and peak at day 2 after infection. These cells seem to contribute to viral control (34) but probably also to tissue damage, as has been observed during influenza virus infection (61, 62).

NK cells have an important antiviral effect during RSV infection. They kill infected cells and promote Th1 responses by producing IFN-γ (63); their recruitment and activation is enhanced by AMs (48). γδ T cells have been shown to contribute to IL-17 production (64), their depletion attenuating RSV-induced inflammation and disease severity in mice (65), and NKT cells may contribute to IL-4 production during murine RSV infection (66). Thus, the recruitment of innate cells contributes to a complex network of pro- and anti-inflammatory signals that both helps to clear infection and sets the environment for subsequent adaptive immunity.

ADAPTIVE IMMUNITY

During viral infection, professional APCs (primarily cDCs) are responsible for presenting peptide antigens formed via proteasomal degradation of extracellular antigens and intracellularly generated antigens in the context of MHC class II and class I, respectively (67).

T cells are essential for resolution of acute infection and for virus-specific immunological memory. In most animal models, RSV induces a typical antiviral adaptive immune response, with resolution of primary infection resulting in high titers of virus-specific antibodies and large numbers of antigen-specific T cells. This limits infection during secondary infection, so that reinfection leads to only low levels of transient virus replication with little associated disease except under circumstances in which narrowly focused immunity enhances disease severity.

In humans, recurrent symptomatic infections occur throughout life even in healthy older children and young adults. As in animal models, secondary infection is characterized in most cases by reduced viral load and attenuated lung involvement. Prolonged or persistent RSV infection is seen in children with T cell immunodeficiency, emphasizing the importance of T cells in clearing virus from the respiratory tract.

$CD4^+$ T Cells

In mice and cotton rats, both $CD4^+$ and $CD8^+$ T cells are important in elimination of virus from the respiratory tract, but they also play a part in causing immunopathology during RSV infection (68, 69). $CD4^+$ T cells are essential for supporting an efficient host response, helping the generation of high-affinity antibodies by B cells and optimal $CD8^+$ T cell memory. However, they also have direct antiviral effector functions, and inappropriate activation of the $CD4^+$ T cell responses may contribute to acute RSV disease and also to vaccine-enhanced pathology.

Infecting mice with recombinant vaccinia viruses (rVVs) expressing single RSV proteins induces remarkably specific patterns of $CD4^+$ T cell priming associated with contrasting patterns of immunity and immunopathology. For example, infecting BALB/c mice via the skin with rVVs expressing RSV's attachment protein G induces very strong Th2 responses and lung eosinophilia during subsequent intranasal RSV infection, an effect that depends on $CD4^+$ T cells making IL-4 and IL-13. By contrast, rVV-F induces a response that is more Th1 directed, with neutrophilia and no eosinophilia. In either case, disease severity (as measured by weight loss or lung pathology) is enhanced by vaccination and the induction of specific T cell immunity. The development of eosinophilia can be inhibited by strong $CD8^+$ T cell responses (reviewed in 12, 18).

$CD8^+$ T Cells

Once activated, $CD8^+$ T cells recognize and kill virus-infected epithelial cells; as the infection resolves, the population contracts to form a pool of local and circulating memory cells that can respond more quickly on subsequent infection. These include the recently described subset of resident memory T (Trm) cells that have innate-like functions, such as early sensing of infection and modulation of the inflammatory environment in sites of pathogen entry (70).

Infection of BALB/c mice with rVV-M2 induces almost exclusively $CD8^+$ T cell responses and lung neutrophilia after RSV challenge, reminiscent of the effects of $CD8^+$ T cell transfer (60). The fusion protein F (the protein usually selected for vaccine development) induces antibody and $CD4^+$ and $CD8^+$ T cell responses. All of these responses are only partially protective against secondary infection and can be associated with enhanced disease as measured by weight loss (reviewed in 12).

It is important to note that the rVV is given peripherally by cutaneous scarification and that the first point of contact between primed T cells and RSV itself is in the lungs.

Murine $CD8^+$ T cells recognize a hierarchy of dominant and subdominant epitopes in RSV, and this is also apparent in humans (70). Although highly immunodominant epitopes that induce large epitope-specific $CD8^+$ T cell responses may be observed in certain inbred mice, it has been reported that those recognizing subdominant epitopes are most protective and less pathogenic (71), but the relevance of these findings to human infection has not been confirmed.

Investigation of T cells against human RSV has been limited by the relatively modest RSV-specific T cell responses that are seen in blood and the very low frequency of RSV-specific memory T cells between episodes of acute infection. In both natural and experimental infection, RSV-specific $CD8^+$ T cells are generally found at much lower frequencies than influenza virus–specific cells (70, 72). Using MHC-peptide tetramers to label and track antigen-specific $CD8^+$ T cells in experimentally infected adults, $CD8^+$ T cells are most numerous approximately 10 days after infection. Their proliferation is associated with the fall in viral load and resolution of symptoms, adding weight to the thought that they may be involved in viral clearance (70). In peripheral blood, epitope-specific $CD8^+$ T cells then rapidly contract, and by 6 months after infection they invariably have returned to low baseline frequencies.

In animals of acute disease, the anatomical location of T cells in relation to infected cells is important in determining phenotype and function, and peripheral T cells are a poor guide to what is happening at the site of infection (73). In experimentally infected adult human volunteers, RSV-specific $CD8^+$ T cells were abundant in the lower respiratory tract, with up to 20% of $CD8^+$ T cells recognizing a single epitope of RSV in some cases. RSV-specific $CD8^+$ T cells in the respiratory tract invariably displayed the hallmarks of Trm cells, with high expression of CD69 and CD103 (70).

Trm cells are formed during acute infection, with precursors migrating from the lymph nodes, where they first encounter antigen, to the site of infection. There, local signals promote tissue-retention molecules and Trm cells remain at high frequencies after infection, acting as innate-like cells that immediately detect a re-encounter with the same antigen. On recognition of antigen, they express IFN-γ and other cytokines that recruit activated $CD8^+$ T cells even of other specificities, thus promoting an antiviral but proinflammatory environment. These cells do not recirculate via the blood; though they can be extremely long-lived in other tissues (such as skin), they have a finite lifespan in lung, perhaps limiting immunopathological responses to respiratory viruses (74). In addition, the frequency of Trm cells in the airways prior to infection negatively correlates with disease severity on subsequent infection, suggesting that these cells play a role in the initial protection against reinfection with RSV.

In healthy volunteers challenged with RSV, Trm cells not only expanded during the acute infection but also continued to be present in enriched numbers into convalescence and were associated with patchy inflammatory changes visible on bronchoscopy up to 28 days after infection (70). This finding is reminiscent of the pathogenic effects of antiviral $CD8^+$ T cells seen in mice.

Regulatory T Cells and IL-10

Tregs are essential modulators of the adaptive immune response, making up 5–10% of $CD4^+$ T cells in the mouse and often (but not invariably) characterized by expression of the transcription factor FoxP3. Absence of $CD4^+$ $FoxP3^+$ Tregs in both mice and humans leads to autoimmunity, and defective or suboptimal Treg function during RSV infection may cause immunopathology.

In RSV-infected mice, Tregs proliferate and accumulate in the lungs, upregulating activation markers and CTLA-4 (75, 76). Depletion of Tregs leads to enhanced viral clearance but also to

disease exacerbation and increased numbers of antigen-specific IFN-γ- and TNF-α-producing $CD8^+$ T cells (77–79). Mice with enhanced disease caused by formalin-inactivated vaccine have a remarkable deficit of Tregs, and selective recruitment of Tregs into the RSV-infected airway by inhalational administration of CCL17/22 attenuates vaccine-enhanced disease (80–82). In addition, increasing Tregs by administration of preformed IL-2/anti-IL-2 immune complexes reduces pulmonary inflammation without inhibition of viral clearance (78). Recent evidence has also implicated Tregs in maintaining neonatal immune tolerance, which can be broken by RSV infection, thus predisposing toward allergic airway disease (83).

Granzyme B production by lung Tregs is important to RSV-specific T effector cell responses (78), and IL-10 production dampens T cell inflammation in the lung (84–87). Tregs may also regulate RSV disease by promoting the production of protective anti-F-specific antibodies (88). They have also been shown to promote early $CD8^+$ T cell responses and viral clearance, which in turn lead to reduced pathology (75, 76).

Therefore, while T cell responses drive the immunopathology during severe RSV infection, immunoregulation of these cells is crucial in order to maintain tissue integrity and function. Murine studies show that the induction of Tregs during RSV infection is crucial to keep the lung T cell responses under control and prevent pathology (77–79, 89), and that bronchiolitis can be viewed as a disease of defective immunoregulation (12).

B Cells and Antibodies

RSV-specific serum antibody is present in virtually every child and adult, reflecting the universality of RSV infection in early life. The only exception is children who are not infected before maternal antibody wanes. Nevertheless, these antibodies are insufficient to prevent reinfection with RSV, which induces local and systemic antibody responses that are only partially protective and limited in duration (68). Serum neutralizing antibody remains a commonly accepted measure of protective immunity and a surrogate of protection in vaccine trials.

It is clear from studies of the effect of passive transfer of immunoglobulin (especially palivizumab) that systemic administration of antibody can protect against RSV infection. Passive antibody affects mainly the lower respiratory tract, reducing the risk of RSV-associated severe disease and hospitalization. However, levels of antibody equivalent to those achieved by palivizumab are rarely achieved by natural infection, and passive antibody administration has no benefit when administered during acute RSV infection. It is only effective as a prophylactic treatment.

However, serum neutralizing antibody titers were only a loose correlate of protection from infection in adult volunteers, in whom RSV-specific nasal IgA correlated better with reduction in risk of PCR-confirmed infection on experimental challenge. This suggests that nasal IgA mediates immune exclusion whereas serum IgG is an indirect correlate of protection in this setting. Notably, the levels of serum and mucosal antibody achieved after experimental challenge are poorly maintained, and decline to preinfection levels within a few months of infection (90).

Signals provided by specialized T follicular helper (Tfh) cells are necessary for B cell functions, especially for optimal affinity maturation and differentiation to long-lived memory and plasma cells (91). There is as yet no literature on Tfh cells in human RSV infection, but it is possible that impaired antigen presentation to $CD4^+$ T cells affects their commitment to the Tfh cell lineage or alters their functional capacity to help B cells. In addition, inhibition of type I interferons or the altered inflammatory milieu may have direct effects on B cell maturation. Whatever the mechanism, the result is that RSV-specific antibodies persist poorly; whereas repeated infections lead to a gradual increase in antibody titer, individual RSV infections induce only transient boosts in serum or mucosal antibody (90).

The recent finding that IgA^+ memory B cell generation is impaired following experimental RSV infection of adults supports the hypothesis that immunomodulation by RSV blocks the generation of long-lived B cells that normally develop after antigen reencounter and confer long-lived, high levels of antibody that can protect against reinfection (90).

IMMUNE MODULATION AND EVASION BY RSV

As noted in the introductory section, one of the most intriguing aspects of RSV's immunobiology is its ability to cause symptomatic reinfection throughout life even in those with healthy and mature immune systems. Among viruses, this is highly unusual. Most acute viral infections, including respiratory infections such as those caused by influenza virus (92) and rhinovirus (93), induce robust homotypic immunity following natural infection that confers almost complete protection for many years.

By contrast, RSV causes repeated infections during childhood (94) and recurrent colds in adults, on average reinfecting every two to three years. Although there is partial protection against the exactly homologous strain of RSV, consistent and durable protection is never achieved. This ability to reinfect is evident both in natural infection and in experimental human challenge, where reinfection with the same strain of RSV can occur as soon as two months after the previous infection (95). However, reinfections are generally milder than primary infections and viral loads are several orders of magnitude lower; secondary infections are generally limited to the upper respiratory tract, except in debilitated older persons and those with immunosuppression, for whom RSV infections of the lung may be insidious and severe.

Partial resistance to reinfection is characterized by induction of immune memory. Until recently, this was believed to be an exclusive feature of adaptive immunity (i.e., B cells and T cells). However, there is increasing evidence that medium- and even long-term alterations in mucosal innate responses to infection can also confer protective innate memory (96), and inflammatory and innate signals are essential for full differentiation of adaptive immunity (97).

Therefore, modulation by RSV of either innate or adaptive responses could be responsible for its ability to reinfect, allowing subsequent RSV infection without extensive viral evolution. While type I interferon inhibition by nonstructural proteins best-studied immunomodulatory mechanism, other viral proteins disrupt the normal inflammatory and immune response. RSV expresses two major surface glycoproteins, F and G proteins, both of which have apparent immunomodulatory properties. The mechanisms by which RSV modulates or evades host responses are summarized in **Figure 5**.

Interferon Blockade by NS1/2

Type I interferon responses are inhibited by RSV's nonstructural proteins (NS1/2), which block interferon production via the inhibition of type I interferons or signaling in infected cells (98). Deletion of NS1, NS2, or both in recombinant viruses additively leads to greater expression of IFN-β in vitro (99). NS2 binds the N-terminal CARD of RIG-I, inhibiting its ability to interact with MAVS (100). In vitro models suggest that NS1 and NS2 reduce STAT2 levels by enhancing proteasome-mediated degradation via formation of a ubiquitin ligase complex that contains the two proteins (101, 102). Furthermore, NS1 disrupts IRF3 binding to the IFN-β promoter by directly binding to it and disrupting its association with CBP (103), leading to inhibition of the production of and downstream responses to type I interferons. This effect is seen especially in human cells. However, it should be noted that susceptibility to reinfection cannot simply be due to such inhibition, since the NS proteins of influenza virus also have interferon-inhibiting functions

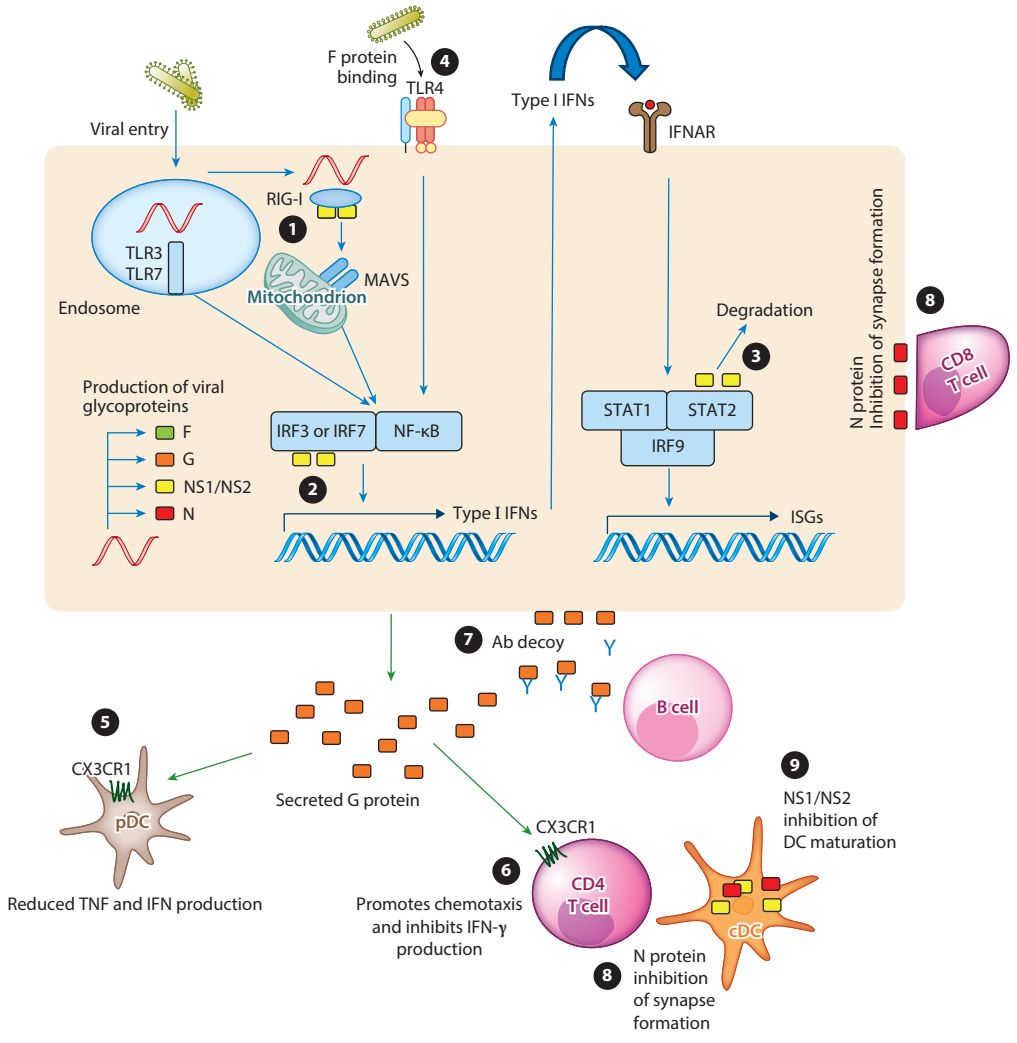

Figure 5

RSV prevents an effective host immune response. RSV interferes with host immunity by diverse actions. ❶ RSV NS2 protein binds RIG-I and impairs innate signaling via MAVS; ❷ NS1 disrupts IRF3 binding to the IFN-β promoter; RSV G and N proteins can also inhibit type I interferon production. ❸ NS1 and NS2 enhance degradation of STAT2; ❹ RSV F protein binds to TLR4 and may cause desensitization of TLR signaling pathways. Secreted viral G protein can bind to CX3CR1 on ❺ pDCs and ❻ some lymphocytes, leading to altered chemotaxis and reduced function. ❼ Secreted RSV G protein can act as a decoy, binding specific neutralizing antibody. ❽ RSV N protein can disrupt the immunological synapse formed by $CD4^+$ T helper cells and $CD8^+$ cytotoxic lymphocytes. ❾ NS1/NS2 reduce maturation of cDCs, attenuating their efficacy as antigen-presenting cells. Abbreviations: cDC, conventional DC; DC, dendritic cell; ISG, interferon-stimulated gene; pDC, plasmacytoid DC; TLR, Toll-like receptor.

but homologous reinfection does not occur (104). However, inhibition of type I interferons does appear to be a major determinant of susceptibility to reinfection with RSV with its far-reaching effects on both innate and adaptive immunity (105).

While monocyte-derived DCs (moDCs) infected with RSV can somewhat upregulate markers such as MHC class I and class II, CD38 and mediators of signal 2, CD80, and CD86, the deletion

of NS1 and NS2 leads to enhanced DC maturation, indicating that these virus-encoded proteins may be inhibiting DC maturation via the inhibition of type I interferons (106). Type I interferons assist T cell expansion and effector differentiation via epigenetic modification, so reductions in type I interferons is likely to have a profound impact on the T cell response (107). In combination, these effects may therefore explain the observation that in vitro moDCs possess limited capacity to induce $CD4^+$ T cell proliferation and cytokine secretion after RSV infection (108).

Immune Modulation by (Fusion) Glycoprotein F

While F's primary function is to fuse the viral envelope with the host cell membrane, in vitro studies show that it may also induce cellular activation via TLR4 (109). The significance of this effect is not clear, but some studies have shown an association of TLR4 polymorphisms and RSV disease, and interaction of RSV proteins with an array of TLRs is likely to have immunomodulatory effects (110).

Immune Modulation by Surface (Attachment) Glycoprotein G

RSV's attachment protein G is known to bind to heparan sulfate moieties on certain cells, but not on ciliated epithelial cells. However, it has remarkable similarities to fractalkine (CX3CL1), a chemokine that is chemoattractive for lymphocytes and monocytes and normally expressed on activated endothelial cells. CX3CL1 and G both have a mucin-like (*O*-glycosylated) extended serine-threonine-rich stalk that ends in a cysteine-rich chemokine domain, which in either case binds the human fractalkine receptor CX3CR1. Both G and CX3CL1 have soluble and membrane-bound forms, but G differs in having a second distal mucin-like domain beyond the chemokine-like motif. CX3CR1 has recently been shown to be present on cultured ciliated airway epithelial cells and to mediate viral binding (31, 111, 112). CX3CR1 is especially expressed on cells with high cytotoxic potential, such as NK cells, cytotoxic T cells, and $\gamma\delta$ T cells. The interaction between RSV and CX3CR1 promotes chemotaxis of such cells, but the benefit to the virus of such an effect is not yet clear.

G protein may also inhibit TLR-induced type I interferon host responses to RSV (110, 113), and the CX3C motif has been associated upon in vitro RSV infection with reduced type I interferon production by human epithelial cell lines, reduced type I interferon and TNF production by pDCs, and reduced IFN-γ by T cells (114). Indeed, treatment of mice with monoclonal antibody directed against the central conserved region of G reduces the pathology caused by RSV, although it is unclear whether this is due to direct reduction of viral load or cytotoxic effectors (115). Neutralizing antibody binds various regions of G, and the soluble form has been suggested to act as a decoy for antibody, preventing its ability to neutralize virus.

Other Possible Immunomodulatory Effects

Several mechanisms by which RSV might interfere with antigen presentation have been proposed. In vitro, RSV-infected DCs have been shown to exhibit impaired immunological synapse assembly, possibly mediated by viral N protein expression, which occurs on the surface of both DCs and epithelial cells and is associated with decreased MHC-peptide clustering (116).

The result of the various immunomodulatory mechanisms in humans is short-lived RSV-specific T cell responses of relatively low magnitude, with some evidence for impaired functionality that is hypothesized to be responsible for the symptomatic reinfection seen throughout life.

INTERACTION OF RSV WITH THE MICROBIOTA AND OTHER INFECTIONS

The mouth, nose, and upper respiratory tract are not sterile, and the viral and bacterial communities of the respiratory tract continuously interact and influence one another and the immune system (117). In infants, acquisition of the microbiota (primarily from the mother) is influenced by route of delivery and breast-feeding and external factors, such as the use of antibiotics and environment, and it drives maturation of the infant immune system (66, 118–121).

The respiratory microbiome may influence susceptibility to and the severity of RSV infection, perhaps by altering innate "tone" in the airways. In turn, RSV infection can itself alter the respiratory microbiome. If these changes persist, this could possibly account for the delayed effects of severe RSV disease on subsequent respiratory health, including the development of wheeze and asthma. The presence of certain bacterial species in the airway and in the profile of fecal microbiota in infants has been associated with an increased risk of subsequent severe RSV infection and with risk of asthma (66, 122–124).

In a recent study, the presence of certain bacterial species during RSV infection in infants was associated with changes to the immune response (including expression of proinflammatory genes, and neutrophil and macrophage activation) and more severe disease (125), and the ability of microbiota to influence host immunity may depend on the host genotype (66). Severe RSV infection may increase susceptibility to bacterial infections for months after recovery from the initial viral insult (126), an effect also seen in mouse models of disease. Viral infections are reported to cause transient desensitization of innate immunity in the lung (127–129).

IMMUNE RESPONSES TO RSV IN THE VERY YOUNG AND THE VERY OLD

The predilection of RSV for the very young and very old (**Figure 1**) reflects the limited physiological reserve of the lung during infancy and old age, combined with age-dependent differences in immune responses to the virus.

IMMUNE RESPONSES IN INFANCY

Although both gestational age and host genetic variation contribute to RSV disease severity, the severity of disease is difficult to predict. This may be because the genetics are complex and polygenic; variation in RSV itself also contributes to disease, and each individual has a unique infection history and baseline respiratory microbiome. Add to this the fact that the pathogenesis may be different in term versus preterm infants, or those with concurrent disease, and there is a very complex set of circumstances that may or may not lead to severe RSV disease.

INNATE RESPONSES IN INFANCY

Despite this complexity, genetic studies have identified numerous genes associated with severe bronchiolitis (38), generally highlighting the importance of innate immune response and airway remodeling genes (39, 40). Innate responses are generally delayed and attenuated in neonates; some studies of severe bronchiolitis have highlighted impaired cytokine production (130, 131) and variations in *Tlr4* in association with severe RSV disease (132–136). For example, a recent study demonstrated that environmental LPS exposure and *Tlr4* genotype combine to cause variations in the severity of RSV disease (66). However, another study did not show an association of *Tlr4* variants with disease severity; instead, the minor T allele of the vitamin D receptor gene was identified as a risk factor (137).

Adult mice lacking TLRs or STAT1 exhibit some features of the impaired innate immune responses, and more severe disease seen in human neonates, with poor viral clearance, exacerbated inflammation and skewed T cell responses (66, 138, 139), although mice lacking all signaling via PRRs (TLRs and RLRs) are still able to mount RSV-specific T cell responses (140). A recent multicenter prospective study of whole blood transcriptomic signatures in infants with RSV infection found strongly differential expression of innate immune genes, including an interferon-related signature that became more marked in convalescence, perhaps indicating inhibition of interferon-related genes at the peak of viral replication (141).

pDCs produce an impaired RIG-I-dependent type I interferon response to RSV in vitro (142), and immature DCs have been shown to promote Th2 cell priming in neonatal mice (143–145). Further, microbial exposure may influence the neonatal response, and treatment of neonatal mice with the TLR ligand CpG can diminish the extent and polarization of the type 2 response upon reexposure to the virus (146).

Together, these studies support the concept that failure to generate an effective or appropriate antiviral innate response may underlie the development of severe RSV disease in infants, at least in some cases.

ANTIBODIES DURING INFANCY

Maternal antibodies seem the most likely explanation of the relative resistance of infants to bronchiolitis in the first few weeks of life. RSV-specific antibodies are transferred to infants from their mothers via the placenta during late pregnancy, and in breast milk (147); this appears to protect infants from RSV infection and to reduce viral load (148). Levels of neonatal serum IgG correlate with protection against infection, severity of infection, and risk of hospitalization (147, 149–153). However, maternal antibodies decline with a half-life of about 38 days and fall below the protective threshold when the infant reaches three to five months of age (147, 154, 155). Preterm infants may also be especially vulnerable to RSV infection because they lack placentally derived antibodies (156), as do those in whom the relative abundance of placentally transferred RSV antibodies is reduced, for example, by infection-associated hypergammaglobulinemia (157, 158).

Natural RSV infection in infants can lead to the generation of a primary IgG and IgA antibody response (159, 160), but the neonatal antibody response is relatively weak, poorly functional, and short lived, declining to preinfection levels within three to four months (161). IgA appears in nasal secretions only around the fifth or sixth day of hospitalization (159, 160), and neutralizing antibody titers peak during convalescence rather than the acute stage of infection (162), so infantile antibody responses are unlikely to modify disease during primary encounter. However, secondary antibody responses may be brisk during reinfection, building to near-adult levels in later childhood (160–165). In addition, there is evidence that preexisting maternal antibodies may interfere with generation of infant antibody responses (162, 166).

Why infection with RSV does not induce very high levels of fully protective antibodies is unknown. Local production of B cell survival factors, such as BAFF and APRIL, is induced in the respiratory epithelium in infants with severe RSV infection by IFN-β and may be a key determinant for optimal local antibody production (45, 167, 168), whereas IFN-γ production in infants may impede it (169).

T CELL RESPONSES, CHEMOKINES, AND CYTOKINES IN INFANCY

Severe RSV infection is associated with both high viral load and pronounced inflammation, production of inflammatory chemokines, and cellular recruitment to the airways (15, 16, 170).

Bronchiolitis has been associated with a dysregulated type 2 polarized immune response, and in general infants produce poor type 1 responses and are biased to promoting Th2 and Th17 responses (171).

The nature of inflammation seen in severe bronchiolitis suggests that enhanced disease in infants is associated with an imbalanced or dysregulated immune response to viral infection, and some reports suggest that this may allow development of inappropriately polarized responses. During acute RSV infection, Th2 polarization is evident in peripheral blood mononuclear cells (PBMCs) in some studies (172, 173). Furthermore, nasopharyngeal samples from infants with RSV infection, particularly younger infants, contain a higher ratio of IL-4 to IFN-γ and *Gata3* expression to *Tbet* expression, and eosinophil cationic protein is sometimes detectable (66, 172, 174, 175). Lower levels of IFN-γ in nasopharyngeal aspirates of infants are associated with more severe disease (176, 177). *IL4* gene polymorphisms are also associated with the development of bronchiolitis (178–180), suggesting that Th2 polarization may be associated with more severe infection in some instances; however, it is important to recognize that bronchiolitis does not result in lung eosinophilia and does not respond to treatments that are effective in asthma.

Cytokines and chemokines produced in experimental primary culture of differentiated pediatric airway epithelial cells largely mirror those present in the airway secretions of infants with severe RSV infection (181, 182). Infected epithelial cells can also produce Th2-promoting cytokines IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) (63, 145, 183, 184), and IL-33 has been reported in the airways of infants with acute RSV infection (185).

In neonatal mice RSV infection leads to the priming of a Th2-biased response to reinfection (186). IL-33 mediates the induction of Th2-biased immunopathology (187), and in humans, polymorphisms of the IL-33 receptor component encoded by *Il1rl1* are associated with increased RSV disease severity (188). In addition, innate lymphoid cells (ILCs) have been shown to increase in neonatal mice after infection (187) and to produce IL-13 in adult mice in a TSLP-dependent fashion (183). More pathogenic strains of RSV are reported to induce greater IL-13 and TSLP-mediated ILC2 proliferation and activation (183), and thus they contribute to a Th2-rich environment in the lung.

The role of Th17 cells in human RSV remains controversial. Neutrophils are typically abundant in the lungs of children with bronchiolitis and RSV pneumonia (170, 189). While some studies have shown elevated Th17 responses in the airways and PBMCs of infants during infection, and some evidence suggests that IL-17 may potentiate neutrophil recruitment, others have shown Th17 responses to be most marked during convalescence (14, 190–192).

IL-9 has been shown to be produced by neutrophils in children with bronchiolitis (193), and polymorphisms in *Il9* have been associated with severe disease in boys (194). IL-9 has also been shown to regulate pathology during RSV infection of mice (195).

In addition, the monocyte chemoattractant CCL2 is found in high levels in bronchoalveolar lavage specimens from children with bronchiolitis (15). Regulatory T cells are found at lower levels in the peripheral blood of children with severe RSV infection (196) and *Il10* polymorphisms have been associated with severe bronchiolitis (197), suggesting that lack of regulation may also allow development of exacerbated inflammation in certain infants.

Impaired type 1 immunity is also apparent in the infant $CD8^+$ T cell response to infection. In infants with severe RSV infection, activated $CD8^+$ T cells are found in the airways and in peripheral blood, but they peak in number during convalescence (9–14 days after onset of symptoms) after the viral load has declined. So in the primary response they probably do not contribute to prevention of infection or substantially contribute to disease severity (10, 192). In contrast, lung $CD8^+$ T cell responses are abundant in adults. Neonatal mice mount a $CD8^+$ T cell response to RSV infection,

but this is a weaker response with an epitope dominance different from that seen in adults (198, 199).

Some infants may be predisposed toward developing type 2 responses to RSV. Most neonates and infants typically develop poor type 1 immunity and $CD4^+$ T cell responses skewed in favor of Th2 and Th17 (200). In vitro, naive cord blood PBMCs produce lower levels of Th1 cytokines and higher levels of Th2- and Th17-associated cytokines in response to stimulation with RSV (200), and low IFN-γ production in naive infant PBMCs is associated with a greater risk of subsequent RSV infection and hospitalization (201).

Murine models also support the concept that Th2 and Th17 responses to RSV can emerge in the absence of Th1-inducing signals or regulation (63, 77, 202). In the neonatal murine model of RSV infection, primary infection in pups leads to eosinophilic inflammation upon reinfection, driven by polarized T cells but also amplified by activation of macrophages and NK cells (186, 203–206). In this model, age is the primary determinant of the nature of the T cell response to RSV infection. Boosting IFN-γ during primary infection in neonatal mice promotes viral clearance and inhibits the development of eosinophilic airway inflammation during adult reinfection (207–209).

Together, these findings suggest that the predisposition or capacity of infants to produce a Th1-polarized IFN-γ response to RSV may protect against viral infection, inappropriate T cell responses, and severe disease.

LONG-TERM EFFECTS OF SEVERE INFANTILE RSV INFECTION

Although there is a remarkable association between RSV infection and later childhood wheeze (noted in the introductory section), these observations do not resolve the issue of causality. In a double-blind, placebo-controlled trial, treatment with the monoclonal antibody palivizumab caused a substantial reduction in wheeze in healthy one-year-olds who were born preterm (210), hinting that RSV infection does have long-term effects on the lung, with airway hyperresponsiveness and asthma diagnosis. However, a high-potency derivative of palivizumab, motavizumab, showed no such effect in term babies despite a substantial reduction in RSV-related hospital admissions (211). The reasons for these apparently contradictory results are not yet clear, but they could involve differences in study populations and endpoints.

PBMCs of infants who have recovered from bronchiolitis are more likely to produce lower levels of IFN-γ or more Th2-polarized T cell responses to stimulation with RSV even years after infection (212–214). Such a polarized response is associated with a predisposition to early wheeze following bronchiolitis (215), infants with low IFN-γ responses to polyclonal stimulation of their PBMCs having a significantly higher risk of wheeze (216).

Following bronchiolitis, increased IL-10 levels in nasopharyngeal aspirates of infants with severe RSV infection are associated with an increased risk of subsequent wheeze (217). IL-10 production by monocytes during convalescence was higher in infants who went on to develop wheeze early after RSV infection, whereas no association was found with IFN-γ and IL-4 responses in this study (176). Polymorphisms in IL-10 family member genes have been associated with recurrent wheeze in postbronchiolitic infants (218), whereas late wheezing, developed at six years, was associated with polymorphism of *IL13* (180). Thus, early wheeze, later development of wheeze, and asthma may be distinct clinical entities with different pathogeneses following RSV infection. Furthermore, it is likely that both genetic predisposition and environmental exposure influence the development of postbronchiolitic wheeze.

Finally, repeated RSV infection of young mice can break tolerance to ovalbumin delivered as an alloantigen in the mother's milk. This effect is dependent on RSV promoting a Th2 phenotype in

Tregs (83), demonstrating that RSV can switch the responses to unrelated antigens in the airways away from tolerance toward a proinflammatory phenotype.

In summary, severe infantile RSV infection may reflect a failure of innate immunity to control the virus, leading to a higher viral load. Combined with unfavorable host genetics and deficient generation of a protective Th1 response, an immune disequilibrium results in inflammation skewed toward harmful immune responses (219), with potential long-term effects on respiratory health.

RSV IN OLDER ADULTS

RSV is increasingly recognized as an important pathogen in older adults, especially those in poor health. Indeed, 78% of RSV-associated deaths occur among persons aged 65 years or more (220), and RSV has been said to cause a disease burden at least comparable to that of influenza in elderly persons (221–223).

Given the aging global population structure, adult RSV-associated disease now poses a progressively increasing burden. For example, a recent North American study examined the impact of RSV in a cohort of 608 healthy elderly patients, 540 high-risk adults, and 1,388 patients hospitalized with acute respiratory illness (222). The risk of severe RSV disease in people over 65 years old is increased by the presence of underlying chronic pulmonary disease, circulatory conditions, and functional disability and is associated with higher viral loads (222–227).

The underlying causes for the susceptibility to severe RSV disease in the elderly are likely complex and multifactorial. As the lung ages, changes in elasticity, cellular composition, barrier integrity, and microbiome, in addition to immunological changes, may contribute to enhanced susceptibility to respiratory infections (228). Innate immunity in the elderly exhibits both diminished antipathogen responses and chronic, low-level activation ("inflammageing") and dysregulation (229, 230), and innate antiviral immunity may be impaired (231); but it is unclear what impact alteration in innate immunity has on RSV infection in the elderly.

Adaptive immunity wanes in the elderly as well (232). A lower frequency of peripheral IFN-γ-producing, RSV-specific T cells, with a shift toward greater IL-10 and IL-13 production, has been reported in elderly persons (233).This may be due in part to a lower frequency of RSV-specific $CD8^+$ T cells (72, 234, 235). Higher viral titers and a diminished cytotoxic lymphocyte response have also been reported in elderly rodent models (236–238).

Owing to a lifetime of exposure to RSV infection, all elderly people have antibodies to RSV, but low neutralizing antibody titers are associated with increased risk of RSV infection and severe disease (odds ratio 5.89) (224, 239, 240). Most studies report a higher baseline and vastly higher induction of serum neutralizing antibody after infection in elderly persons, perhaps resulting from higher viral burden and prolonged and more severe inflammation (239, 241–243). This suggests the ability to mount an antibody response to RSV is not impaired in the elderly. However, elderly persons with higher levels of antibody tend to be resistant to complications of RSV infection, suggesting that induction of a robust antibody response by vaccination might protect this vulnerable age group.

VACCINATION AGAINST RSV

Age-groups that are especially affected by RSV disease are in general those that are poorly responsive to vaccination (244, 245). Infants often respond poorly or inappropriately to vaccines, owing to the immaturity of the infant immune system and interference by maternal antibody (244–246).

In the 1960s, trials of formalin-inactivated alum-adjuvanted RSV vaccines (FI-RSV) proved disastrous, inducing non-neutralizing antibody and cell-mediated responses that enhanced disease

during subsequent natural RSV infection in children younger than two years who were previously seronegative for RSV. Properties of FI-RSV appear to have compounded with differences in the immature and inexperienced infant immune system to create a pathogenic immune response. The enhanced lung inflammation observed in children immunized with FI-RSV can be replicated in many animal models, including mice, cotton rats, cattle, and primates. The immunological causes of this effect are several, including the possible formation of immune complexes in conjunction with inappropriate Th2-polarized and deficient T cell regulatory responses (80–82, 247).

Maternal immunization offers a possible means of extending the duration of postnatal protection beyond the most susceptible period of infancy without the need to directly vaccinate the neonate (248–250). Clinical trials of maternal vaccination led to increased levels of RSV-specific antibody in infants (251), an effect recapitulated in animal models (252, 253). However, maternally derived antibody has a half-life of approximately 38 days, so even if maternally derived antibody is at very high levels, maternal vaccination is unlikely to protect throughout the (most vulnerable) first six months of life let alone until it is possible to achieve a good vaccine response in the second year of life. There is even a hypothetical risk that maternal antibodies could prevent or skew the development of immunity by the neonate, although the likelihood and long-term impact of such an effect is difficult to predict.

Developing an effective vaccine for the elderly should be a priority; however, the elderly respond poorly to vaccination, so any future vaccines will need to prove their efficacy in this age group (246, 254, 255). Challenges of vaccination in the elderly include immunosenescence and preexisting immunity (244, 246, 254, 256). They may be afforded protection through mass vaccination programs of younger adults. In particular, vaccination of health care workers to reduce nosocomial infection may be an effective strategy. Alternatively, vaccination of younger adults could induce lasting, lifelong protective immune memory (256, 257).

The majority of vaccine candidates currently in clinical trials are designed to induce systemic IgG in order to replicate a palivizumab-like effect. Whether this is sufficient to protect populations such as older children and elderly adults remains to be demonstrated, and without high levels of mucosal antibody, the potential for controlling transmission may be limited. In addition, the continued lack of a well-validated correlate of protection retards the development and licensing of these vaccines, which are currently reliant on demonstration of efficacy in large-scale clinical trials. Further understanding of the role of specific antibody subclasses, antigen specificities, and location, and the contribution of local T cell immunity, may help to resolve this important issue.

CONCLUSIONS

RSV employs various immunomodulatory mechanisms that lead to poor immune memory and susceptibility to reinfection, acting at every level of host defense. The result is an immune response that is relatively short-lived, with protective antibodies and T cells declining within weeks or months to levels where protection is no longer achieved. However, the individual mechanisms that contribute to impaired protection are poorly characterized, in part because of the difficulty of studying local mucosal immunity in human subjects. However, with the advent of so many putative RSV vaccines based on different technologies, it may now be possible to develop tools to probe protective immunity against RSV in revealing detail.

As vaccination against RSV disease becomes a possibility, the wider effects of delaying or eliminating RSV infection will take time to become evident. Removing RSV from the respiratory ecosystem may have unanticipated consequences, and delaying first infection until later life may not inevitably be beneficial. It will not be possible to judge all the general effects of vaccines until they are in widespread use.

Understanding the mechanisms and factors that govern maturation of the neonatal response to RSV is crucial to making progress with additional vaccines for infants. Complex interactions between the virus, the microbiome, maternal health, and the infant genome will influence subsequent innate and adaptive immunity and short- and long-term outcomes of infection. Anatomically relevant sampling of immunity in relation to the time course of infection may help elucidate the true heterogeneity of clinical disease caused by RSV infection, taking into account differing etiologies and sequelae.

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