Host response to respiratory syncytial virus infection

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Purpose of review
Respiratory syncytial virus (RSV) infection is the leading cause of bronchiolitis and hospitalization in young infants and causes 100 000–200 000 deaths annually. There is still no licensed vaccine against RSV infection and the therapeutic options are mainly supportive. Despite almost six decades of research, important knowledge gaps remain with respect to the characterization of immune mechanisms responsible for protection and pathogenesis, as well as to the identification of risk factors that predict the severity of infection.

Recent findings
Observations made in mouse models and young children suggest that the early innate immune response plays a major role in the pathogenesis of bronchiolitis due to RSV infection. Recent studies have improved our understanding of the role of the adaptive immune response mediated by TH1, TH2, TH17, regulatory T cells, and $CD8^+$ T cells in the pathogenesis and resolution of RSV infection. Moreover, investigations performed in the last years have made important contributions to our knowledge of the immune response in young children, the principal risk group for severe disease.

Summary
A comprehensive understanding of how the protective and deleterious immune response during the course of RSV infection is induced in young children remains a challenge over the coming years.

Keywords
bronchiolitis, immunity, neonatal, pathogenesis, respiratory syncytial virus

INTRODUCTION

Respiratory syncytial virus (RSV), a member of the Paramyxoviridae family, is the leading cause of lower respiratory tract disease during infancy. Infants are infected by RSV during the first year of life, and virtually all by 2–3 years of age [1,2]. The clinical spectrum and severity of RSV infection can range from a mild upper respiratory illness to a severe infection of the lower respiratory tract, usually bronchiolitis. The majority of children display a mild illness of the upper airways; however, 2–5% will develop a severe bronchiolitis, which requires hospitalization [2–4]. These patients will show later a high risk to suffer recurrent wheeze and asthma [5,6]. Immunity to RSV infection is protective, but it does not result in the induction of long-lasting immunity. RSV does not induce an effective immunological memory, and the titers of virus-specific antibodies rapidly decline after primary natural infection. Hence, recurrent symptomatic infections occur throughout life [7]. Worldwide, RSV is estimated to cause more than 30 million new episodes of lower tract illness in children under 5 years of age, 3–4 million hospitalizations and 100 000–200 000 fatal outcomes, with more than 95% of these deaths occurring in developing countries [8,9].

Epidemiological data reveal that 49–70% of children hospitalized because of RSV infection are younger than 6 months, indicating that very young age is the most important risk factor for severe RSV infection [1,9]. Other important risk factors

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KEY POINTS

- RSV infection is the leading cause of bronchiolitis and hospitalization in young infants.
- There is currently no licensed vaccine against RSV infection, and the therapeutic options are limited.
- Our current understanding of the host response to RSV in humans remains rudimentary; however, recent findings strongly suggest that the early innate immune response plays a major role in the pathogenesis of severe infection.
- New studies have provided important information about the role of the adaptive immune response mediated by TH1, TH2, TH17, Tregs, and $CD8^+$ T cells in the course of RSV infection.

associated with severe RSV disease are premature birth [10▪], low birth weight [11], male sex [12], chronic lung disease [13], congenital heart disease [14], immunodeficiency [15], Down syndrome [16], low socioeconomic status [1], and diet [17▪]. The importance of RSV infections in older adults is increasingly recognized [18–20]. Despite the enormous disease burden associated with RSV infection, there is still no vaccine or effective therapy, and the employment of passive immunoprophylaxis with neutralizing antibodies directed to RSV is limited to high-risk babies.

Severe bronchiolitis induced by RSV infection is associated with sloughing and death of airway epithelial cells (which are the primary targets of RSV infection), edema of the airway wall, increased mucus production, and infiltration of the airway by neutrophils and lymphocytes. The cytophatic effects mediated by RSV might explain many of the pathological findings in RSV disease; however, there is compelling evidence supporting that the host immune response has also an important role [2,21,22].

Our current understanding of the host response to RSV in humans remains rudimentary because most observations have been performed in animal models, which do not adequately reflect the course of human infection. Unfortunately, mainly because of ethical concerns related with studying infection in very young children, we know very little about the immune response against RSV at the site of tissue injury, the airway. This review focuses on recent advances in identifying innate and adaptive immune mechanisms involved in the resolution and pathogenesis of RSV infection in infants. For an update on other important topics, such as the analysis of virus characteristics, the epidemiology of RSV infection, and the current efforts to develop well tolerated and effective vaccines for population at risk, we refer the readers to other recent reviews [1,2,23,24,25▪,26–28].

THE IMMUNE RESPONSE OF HEALTHY INFANTS

Newborns and infants are highly vulnerable to infection and this condition is not restricted to RSV. Early acquisition of HIV, hepatitis B virus, and cytomegalovirus is commonly associated with higher levels of virus replication and more severe disease compared with those infections acquired in later life [29–31]. Infants infected by *Mycobacterium tuberculosis* are at least five times more likely to develop active tuberculosis compared with adults, and also show higher rates of severe disseminated disease [32,33]. Infants are also much vulnerable to malaria compared with adults [34]. Moreover, epidemiological data related to infectious disease in the United States in the period 1998–2006 reveal that infants younger than 1 year show a rate of hospitalization 4 to 10-fold higher compared with patients ranged from 1 to 59 years [35,36].

The heightened susceptibility of newborns and infants to a variety of pathogens could be explained considering some signatures that characterize the innate and adaptive immune system during the first months of life [37,38,39▪]. Pattern recognition receptors (PRRs) are expressed by innate immune cells and play an essential role for detecting invading pathogens and initiating both the innate and adaptive immune response [40,41]. Two families of PRRs have shown to act as key sensors of viral infection by recognizing viral nucleic acids: Toll-like receptors (TLRs) and RIG-I (retinoic acid inducible gene)-like receptors (RLRs) [41,42]. A large body of evidence indicates that TLR-mediated responses are defective in neonates [43–45]. Conventional dendritic cells (DCs) are responsible for the initiation of the adaptive immune response and it has been clearly demonstrated that when stimulated through TLRs they produce low levels of the inflammatory cytokines interleukin-12 and type-I interferons (IFNs) [43,46]. Plasmacytoid dendritic cells (pDCs) are the most important source of type-I IFNs during acute viral infections, and previous studies have reported a reduced production of type-I IFNs by pDCs in response to TLR7 and TLR9 ligands, a defect that persists until 6–12 months of age [47–49]. Natural killer (NK) cells also show a defective function in neonates being their capacity to destroy infected cells and to produce the inflammatory cytokines tumor necrosis factor-α and IFN-γ severely compromised [50,51]. The $CD4^+$ T cell compartment has a particular immunological status in

neonates. They show a defect in developing TH1, and a skewing toward TH2 immunity [39▪,52,53], perhaps reflecting the inability of neonatal DCs and macrophages to produce the TH1-polarizing cytokine interleukin-12 [46,52]. The function of TFH is also defective in young infants [53–55], and together with the delayed maturation of follicular DCs and bone marrow stromal cells appears to explain the compromise in B cell responses [28,56]. The $CD8^+$ T cell compartment also shows a strong defect in young infants, which involves the generation of both effector and memory cells [57▪,58,59▪].

RECOGNITION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION BY PATTERN RECOGNITION RECEPTORS AND ACTIVATION OF THE EARLY INNATE IMMUNE RESPONSE

Two families of PRRs are involved in the recognition of RSV: TLRs and RLRs [26,60,61]. TLRs 3 and 7 are expressed in endosomes and recognize dsRNA and ssRNA, respectively, whereas TLR4 is expressed on the cell surface and recognizes the RSV envelope glycoprotein F [62,63]. The RLR family acts as cytosolic sensors of nucleic acids and recognizes viral RNA [41,61]. Early stimulation of PRRs by RSV activates signaling cascades resulting in a number of responses mediated by different cell types: production of mucus, proteases, antimicrobial peptides, type-I and type-III IFNs and chemokines (including interleukin-8) by epithelial cells, the synthesis and release of type-I IFNs by pDCs, the production of a number of cytokines and chemokines by alveolar macrophages, the phenotypic maturation of DCs, and their migration to lung-draining lymph nodes to prime the adaptive immune response against RSV [26,60,61]. The relative contribution of each PRR in the activation of different cell types and different cell functions in the course of RSV infection remains controversial. RSV has shown to persist longer in the lungs of infected TLR4 or TLR2-deficient mice, suggesting that both TLRs play a protective role [64,65]. Silencing of either TLR3 or TLR7 did not impair in-vivo clearance of RSV, but enhanced the production of TH2-type cytokines, inducing goblet cell hyperplasia and mucus overproduction, supporting the notion that these receptors prevent the development of a more severe disease [66,67]. Interestingly, investigations in immunodeficient patients unresponsive to TLR3, TLR4, TLR7, and TLR3 stimulation do not reveal a predisposition to severe RSV infection, suggesting a redundant role for TLRs in host defense [68]. The contribution of RLRs was analyzed in mouse models, and revealed an important role of these receptors in the early innate immune response against RSV, by stimulating the production of a range of inflammatory mediators such as type-I and type-III IFNs, inflammatory cytokines and chemokines, metalloproteinases, and cathepsins [69,70▪,71,72]. Type-I and type-III IFNs play a critical role in antiviral immunity. A comprehensive review of the actions mediated by IFNs in RSV infection is beyond the scope of this review, and is well covered elsewhere [61,73–75].

CELLS OF THE INNATE IMMUNE SYSTEM IN RESPIRATORY SYNCYTIAL VIRUS INFECTION

In a recent study, Mejias *et al.* analyzed the transcriptional profile from the blood of RSV-infected children who required hospitalization [76▪▪]. They reported that RSV infection is associated with the activation of neutrophils, the upregulation of type-I IFN-related genes, enhanced markers of systemic inflammation, and suppression of T and B-cell-mediated immunity. This suggests that RSV disease could be regarded as a dysregulated innate immune response to infection [76▪▪,77]. Consistent with this view, previous studies have shown that severe bronchiolitis in young infants is associated with a strong influx of neutrophils into the airways [78,79], that appear to promote epithelial cell damage and mucus overproduction [21,80]. Interestingly, a genetic polymorphism of interleukin-8 (CXCL8), which plays a critical role in recruitment of neutrophils in the lung [81], has been associated with an enhanced risk of severe bronchiolitis [82].

There is a general agreement that pDCs play a protective role during RSV infection [26]. They are mobilized to the nasal mucosa and the airways, and promote the clearance of the virus through the release of high amounts of type-I IFNs [83–85]. Mouse models of RSV infection revealed that depletion of pDCs resulted in the enhancement of viral load, pulmonary inflammation, and airway hyper-responsiveness [84,85]. It should be noted, however, that the ability of pDCs to produce type-I IFN is limited during the first months of life [47,49,86,87▪], and hence this could compromise the effectiveness of the immune responses against RSV in early life.

Alveolar macrophages are not only strategically located in the lung to function as a first defense system against respiratory pathogens, but also play a major role in the maintenance of lung homeostasis in the absence of stimuli able to disrupt lung structure [88]. Recently, it was shown that alveolar macrophages are essential for protection against influenza virus-induced morbidity, by virtue of their

ability to remove dead cells and surfactant material from the airway, hence maintaining lung function [89▪▪]. Observations made in lung necropsies from infants who died from RSV infection suggest a similar role for alveolar macrophages in the course of severe infection [90]. Moreover, mouse models of RSV infection revealed that depletion of alveolar macrophages dampened the early innate immune response to RSV, and enhanced the peak viral load and airway occlusion [91,92▪].

NK cells play an important role in the control of viral infections by killing infected cells and by priming DC function and T cell responses, via the early production of IFN-γ [93]. Severe RSV bronchiolitis in young infants appear to be associated with a poor infiltration of NK cells in the airway [94], suggesting that they exert a protective role. Animal models of RSV infection, however, have shown that NK cells may contribute to lung injury during the early steps of RSV infection [95], and also stimulate $CD8^+$ T cell responses, avoiding TH2 responses and subsequent allergic sensitization [96,97]. It should be mentioned that neonatal NK cells express phenotypic and functional deficiencies that may compromise the immune responses against RSV in the first month of life [98].

THE ADAPTIVE IMMUNE RESPONSE AGAINST RESPIRATORY SYNCYTIAL VIRUS INFECTION IN YOUNG INFANTS

Conventional DCs play an essential role in the induction and regulation of adaptive immune response, including the generation of cytotoxic responses mediated by $CD8^+$ T cells, the differentiation of $CD4^+$ T cells into different functional profiles, and the promotion of B-cell responses [99]. As mentioned above, neonatal DCs produce low levels of interleukin-12 and type-I IFNs compromising both the differentiation of $CD4^+$ T cells into TH1 cells and the development of $CD8^+$ T cell memory [46,52]. Moreover, mouse models of RSV infection have revealed that neonatal DCs express low levels of co-stimulatory molecules in the course of infection, and a limited ability to process and transport antigens to draining lymph nodes [59▪]. Consistent with these observations, RSV infection of human DCs has shown to induce only a low increase in the expression of the lymph node homing receptor CCR7, while inhibiting DCs ability to activate $CD4^+$ T cells [100,101].

Antibodies play an essential role in antiviral immunity by directly neutralizing free virus particles and also by inducing the opsonization of extracellular virus or infected cells [102]. The two major surface proteins of RSV, the proteins G and F, are the most important targets of neutralizing antibodies [24]. The effectiveness of the humanized monoclonal antibody palizumab directed to the F protein, in the prophylaxis of high-risk infants, indicated that neutralizing antibodies can effectively confer protection against RSV infection [103]. Passive transfer of maternal IgG antibodies through the placenta and colostrums confer protection to neonates, but the levels of maternal antibodies decline rapidly, showing a half-life of 2–3 months [104–107]. Infection by RSV in young infants results in the induction of an antibody response, but fails to establish long-lasting immunity because the antibody titers rapidly decay [108]. Even in the adult, the titers of IgG-neutralizing antibodies rapidly wane after natural RSV infection [109,110], being lower titers of nasal IgA and serum IgG-neutralizing antibodies associated with increased rates of infection [109,111,112].

Children with a defective T-cell response show an increased RSV-mediated disease severity and high viral titers, indicating that T cells are involved in the resolution of the infection [113,114]. Mouse models of RSV infection have clearly shown that $CD8^+$ T cells are not only required for viral clearance, but are also involved in the induction of lung injury [115,116]. On the contrary, observations made during the course of severe infection in young infants suggest that T $CD8^+$ cells are involved in the clearance of RSV, but not in the induction of lung disease. Infants are able to mount a virus-specific $CD8^+$ T cell response; however, accumulation of activated T $CD8^+$ cells was almost undetectable in broncheoalveolar lavage samples at the time of hospitalization, in the peak of illness, but reach high levels 9–12 days after the onset of primary symptoms, that is, at convalescence [117,118]. This suggests that $CD8^+$ T cells do not contribute to pathogenesis associated with RSV infection [119,120].

The relevance of TH2 responses in the pathogenesis of RSV infection in young infants is unclear [22,121▪,122]. Observations made in infants during the course of severe bronchiolitis have shown contradictory results. Although some studies reported a correlation between the levels of TH2 cytokines in broncheoalveolar lavage and the severity of RSV infection [123–125], other studies failed to detect this polarization toward a TH2 profile [126–128]. More recently, the participation of TH17 cells in the pathogenesis of RSV infection has been proposed. TH17 cells play an important role in the immune response against bacteria and fungi, but also are involved in the induction of tissue injury by virtue of their ability to induce the rapid recruitment and activation of granulocytes and macrophages, and

the stimulation of epithelial cells [129,130]. TH17 cells were found to be increased in the tracheal aspirate and the peripheral blood of infants during the course of severe RSV infection [131,132▪▪]. Moreover, experiments in mice revealed that cytokines associated to the TH17 profile, such as interleukin-6, interleukin-23, and interleukin-17, are detected in the airways following RSV infection [133–135]. TH17-derived cytokines appear to promote three major deleterious responses: infiltration of the lung by neutrophils, stimulation of interleukin-13 synthesis and mucus over-production, and inhibition of cytotoxicity mediated by T $CD8^+$ cells [26,136].

Forkhead box $P3^+$ regulatory T cells (Tregs) play a critical role in the control of autoimmune responses. They do not only prevent autoimmunity, but also modulate immune response during infection to minimize tissue injury [137,138]. Mouse models of RSV infection have shown that Tregs play an important role in controlling lung inflammation in the course of RSV infection [139–141]. These studies revealed that infection by RSV induces Treg recruitment in the lung and mediastinal lymph nodes, and also that depletion of Tregs enhances disease severity [139–141]. We have recently reported that RSV infection in young infants who required hospitalization induces a dramatic and prolonged reduction in the frequency of peripheral blood Tregs [142]. Scarce information is available about the function of Tregs during the course of human acute viral infections; however, increased frequencies of Tregs in patients infected by dengue or influenza A virus (H1N1) have been reported [143,144]. Whether the depletion of circulating Tregs during RSV infection is because of the recruitment of Tregs to the lung or the lung-draining lymph nodes, the death of Tregs, or the acquisition of an effect-like phenotype, under the pressure of inflammatory conditions [145], remains to be clarified.

CONCLUSION

Infection by RSV is the common most single cause of hospitalization in young infants, and results in 100 000–200 000 deaths each year. It is also a leading cause of respiratory disease in the elderly and in immune compromised patients. There is currently no licensed vaccine against RSV infection and the therapeutic options are limited. There are still many important gaps in our understanding of the pathogenesis of RSV infection. Why do some children develop severe bronchiolitis, whereas most suffer a mild illness? What is the role of the innate and adaptive immune mechanisms in the protection and pathogenesis of RSV infection? Why do RSV infection fails to induce long-lasting immunity, even in the adult? Answers to these questions are crucial to the development of effective vaccines and new therapeutic tools for the prevention and treatment of RSV infection.

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Conflicts of interest

There are no conflicts of interest.

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