REVIEW

The Human Immune Response to Respiratory Syncytial Virus Infection

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SUMMARY Respiratory syncytial virus (RSV) is an important etiological agent of respiratory infections, particularly in children. Much information regarding the immune response to RSV comes from animal models and *in vitro* studies. Here, we provide a comprehensive description of the human immune response to RSV infection, based on a systematic literature review of research on infected humans. There is an initial strong neutrophil response to RSV infection in humans, which is positively correlated

with disease severity and mediated by interleukin-8 (IL-8). Dendritic cells migrate to the lungs as the primary antigen-presenting cell. An initial systemic T-cell lymphopenia is followed by a pulmonary $CD8^+$ T-cell response, mediating viral clearance. Humoral immunity to reinfection is incomplete, but RSV IgG and IgA are protective. B-cell-stimulating factors derived from airway epithelium play a major role in protective antibody generation. Gamma interferon (IFN-γ) has a strongly protective role, and a Th2-biased response may be deleterious. Other cytokines (particularly IL-17A), chemokines (particularly CCL-5 and CCL-3), and local innate immune factors (including cathelicidins and IFN-λ) contribute to pathogenesis. In summary, neutrophilic inflammation is incriminated as a harmful response, whereas $CD8^+$ T cells and IFN-γ have protective roles. These may represent important therapeutic targets to modulate the immunopathogenesis of RSV infection.

**KEYWORDS** immunology, respiratory syncytial virus

INTRODUCTION

Respiratory syncytial virus (RSV) is an enveloped single-stranded RNA virus belonging to the *Pneumoviridae* family of the *Mononegavirales* order. Infections occur worldwide, with outbreaks in temperate climates occurring primarily during the winter months. RSV is an important etiological agent of respiratory infections, particularly in children, causing a spectrum of illness encompassing upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI), including pneumonia and bronchiolitis, which are associated with greater morbidity and mortality. Natural infection results in incomplete immunity, permitting recurrent infection in childhood as well as infections in adults, including the elderly. Much information regarding the immune response to RSV comes from murine and other animal models and *in vitro* human cell culture studies. While important for hypothesis generation, these methodologies may not provide a completely accurate reflection of the immune response during infection in humans. Here, we provide a comprehensive description of the human immune response to RSV infection, based on a systematic literature review exclusively of clinical, *ex vivo*, and postmortem data from naturally and experimentally infected humans.

In this review, we consider the existing data describing the major cellular and humoral components of the immune response to RSV, distinguishing events occurring systemically from those occurring locally within the respiratory tract (Fig. 1). First we describe the behavior of all major immune cell types, encompassing neutrophils, dendritic cells (DCs), monocytes, macrophages, eosinophils, and T lymphocytes. Second, the anti-RSV antibody response and its regulation is discussed. Next, the distinct Th1 and Th2 responses to RSV and the effect of their balance on disease progression are considered. Several chemokines, cytokines, and other immune molecules have been demonstrated to be involved in the immune response and are reviewed. The global host transcriptional response is also discussed in the context of immune-related pathways. Certain key pathogen-host interactions described herein may represent targets for the development of novel therapeutics. For completeness, we summarize the association between RSV infection and subsequent asthma and also key differences between immune responses in humans and those in animals used in model systems of infection.

METHODS OF SYSTEMATIC LITERATURE REVIEW

We conducted a systematic literature review following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (PROSPERO registration number CRD42016047320). An electronic literature search of Medline, Embase, and Web of Science was performed using the following search terms: (RSV[Title] OR respiratory syncytial virus[Title]) AND (immune response OR T cell OR B cell OR lymphocyte OR macrophage OR neutrophil OR monocyte OR natural killer cell OR dendritic cell OR immunoglobulin OR IgG OR IgA OR IgE OR cytokine OR chemokine OR interleukin
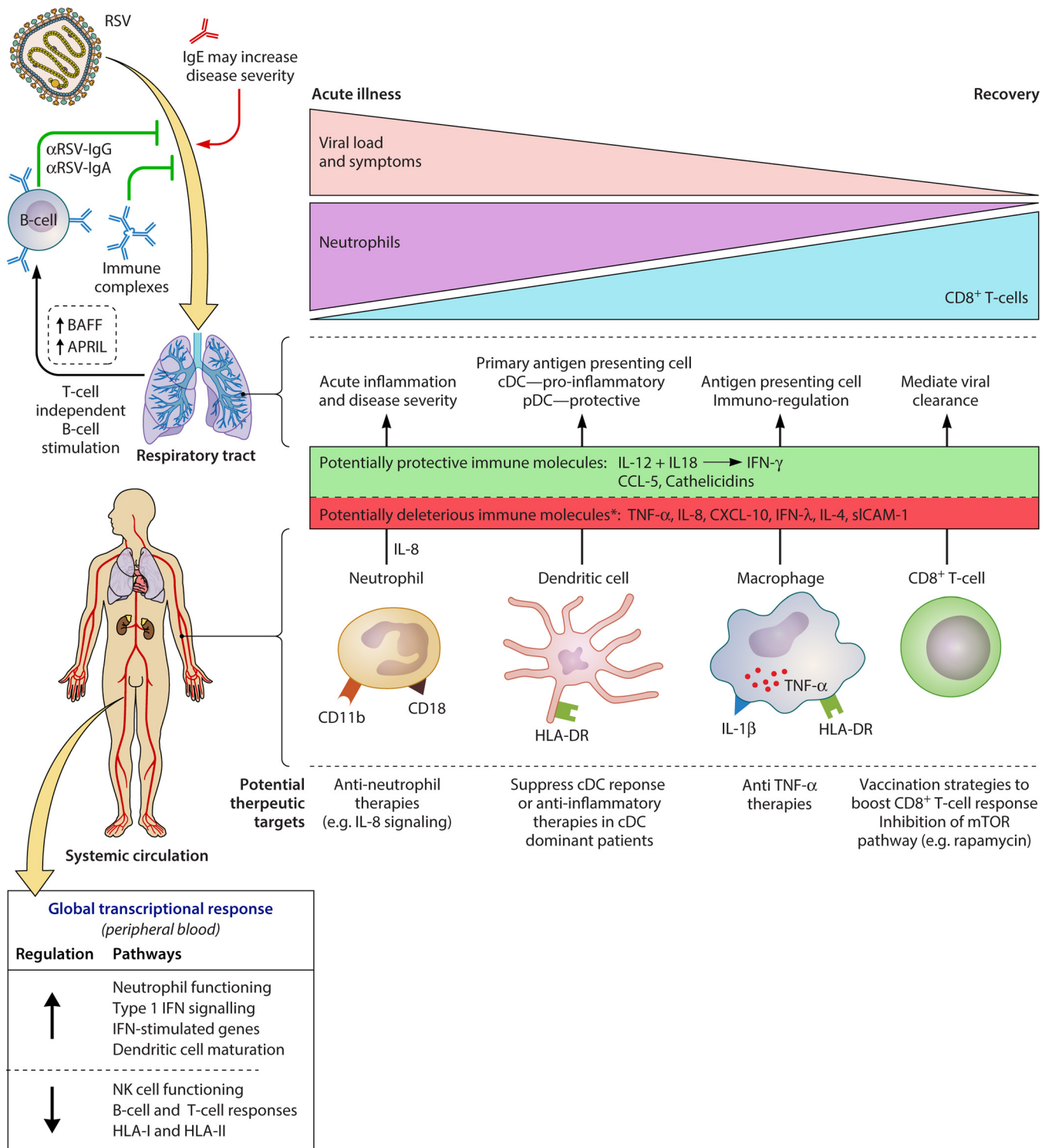
FIG 1 Summary of the human immune response to RSV and potential novel therapeutic targets. The roles of major cell types (neutrophils, dendritic cells, macrophages, CD8^{+} T cells, and B cells) are summarized, in addition to key antibody, cytokine, chemokine, and other immune molecule responses. Major transcriptional changes (in peripheral blood) of immune-related pathways are shown. The deleterious role of neutrophilic inflammation and the protective role of CD8^{+} T-cell-mediated viral clearance are emphasized. Finally, we highlight areas where novel therapeutic interventions could potentially modulate the immune response in favor of the host. ↑, immune cell recruitment to the respiratory tract; *, association with increased disease severity.

OR interferon) AND (human OR clinical OR experimental OR neonate OR infant OR children OR adult OR elderly).

The last search was conducted on 16 May 2016. The results from the databases were merged and duplicates removed. The combined results of the electronic database

search were assessed independently by two authors, and discrepancies were discussed and agreed upon according to the inclusion and exclusion criteria. Publications in all languages describing primary research in humans (clinical, *ex vivo*, or postmortem) were included. Editorials, reviews, commentaries, and opinion pieces were excluded. Articles were limited to those published after 1990. Additional articles of interest were identified from reviewing the bibliographies of relevant articles. The literature search resulted in 2,541 publications after removal of duplicates and pre-1990 publications. Two authors reviewed titles and abstracts and identified 268 records that then underwent full text review. Of these, 166 met the inclusion criteria. A further 9 articles were identified through other sources, including bibliographies of identified articles.

SYSTEMIC AND PULMONARY IMMUNE CELL RESPONSES TO RSV INFECTION

Neutrophils

RSV infection elicits a strong systemic and especially respiratory tract neutrophil response (1–4). Neutrophils are the predominant cell type in bronchoalveolar lavage (BAL) fluid from the lungs of ventilated infants with severe RSV bronchiolitis and those with milder infection (5). These cells are activated during the initial pathogenesis of RSV LRTI, producing neutrophil elastase (6, 7) and expressing activation markers (CD11b, CD18, and CD54 [ICAM-1]) (8, 9). The peak neutrophil response coincides with maximum clinical severity and viral load, and by the time infants with severe infection are discharged from the intensive care unit (ICU) after ventilation, neutrophil counts in peripheral blood have normalized (10). Widespread neutrophil infiltration is seen in lung tissue from fatal cases of RSV LRTI (3, 11).

During severe infection, the virus interacts directly with neutrophils. Cells from peripheral blood and BAL fluid express RSV proteins F, G, and N proportionately, implying stoichiometric expression and thus intact intracellular virions (12). RSV genomic RNA and mRNA are also present intracellularly (12, 13). This could be explained by phagocytosis of virions or replication of RSV within neutrophils. These RSV-containing neutrophils detected in the peripheral blood may have transmigrated from the lungs into the circulation.

Neutrophil apoptosis and neutrophil extracellular trap (NET) formation ("NETosis," a unique form of neutrophil cell death) are active during infection. Proteins involved in apoptosis (annexin V and the Fas death receptor CD95) are upregulated in nasopharyngeal fluid, and NETs are present in BAL fluid from ventilated children (8, 14). NETs may prevent spread of infectious virions and comprise a web-like DNA backbone studded with histones and cytotoxic/antimicrobial proteins.

Natural Killer Cells

RSV infection results in reduced total systemic natural killer (NK) cell counts, albeit with an increase in an activated subset that lacks expression of CD94 (15, 16). Circulating NK cells have higher expression of the inhibitory leukocyte immunoglobulin-like receptor subfamily B member (LILRB1), suggesting that they may contribute to regulation of inflammation during infection (17). Lower systemic total counts correlate with greater severity of infection, and NK cells are sparse in lung tissue from fatal cases (3, 15, 18, 19). In contrast, there is accumulation of granzyme B-expressing NK cells in the respiratory tracts of infants ventilated due to severe RSV bronchiolitis (BAL fluid and tracheal aspirate), possibly suggesting migration to the lungs (20, 21).

Dendritic Cells

Conventional dendritic cells (cDCs) and plasmacytoid DCs (pDCs) are mobilized from the circulation to the nasal mucosa early during infection, with a further increase in DC counts during subsequent convalescence (22, 23). The RSV fusion protein is present within HLA-DR+ DCs in the nasal mucosa, and the selective emigration of DCs, but not monocytes, highlights their likely role as the primary antigen-presenting cell during RSV infection (23). Low numbers of blood pDCs have been associated with the development

of RSV bronchiolitis, suggesting either increased emigration to the respiratory tract or an insufficient pDC response in severe RSV infection (24).

cDCs and pDCs have also been found in the lower airways of infants ventilated due to severe RSV bronchiolitis, where cDCs exhibit an activated proinflammatory phenotype (20). Circulating cDCs express the activation marker CD83 and the costimulatory molecule CD40. Concentrations of innate immune proinflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor alpha [TNF-α], and IL-8) and T-cell-derived cytokines (gamma interferon [IFN-γ], IL-13, IL-10, and IL-2) in BAL fluid correlate with cDC counts. In subsets of infants with severe RSV bronchiolitis (preterm infants and infants aged 4 months or more), pulmonary pDC counts are low compared to those in term-born and younger infants, suggesting an inadequate antiviral response as a factor in severe RSV disease (20).

Macrophages and Monocytes

Alveolar macrophages obtained from BAL fluid from RSV-infected infants and adult transplant recipients coexpress RSV surface glycoproteins, HLA-DR molecules, IL-1β, and cytoplasmic TNF-α, suggesting a local immune-regulatory and antigen-presenting role (25, 26). The cells appear to be infected productively, as viral replication from the cells can be confirmed *ex vivo* (25).

CD69$^+$ monocytes are present in lung tissue from fatal cases of RSV infection (11). In the peripheral blood, monocytes display reduced Toll-like receptor 8 (TLR8) expression and TNF-α production during acute RSV infection, which subsequently normalizes in convalescence (27). In contrast, circulating monocytes increase their expression of TLR4 in RSV infection (28).

Eosinophils

Eosinophils are activated during the acute phase of RSV LRTI and may contribute to recovery. Expression of the myeloid activation marker CD11b on circulating eosinophils from infants with RSV LRTI is increased and inversely correlates with the required duration of supplemental oxygen (29). In comparison to children hospitalized due to influenza virus or adenovirus infection, those with RSV infection have higher systemic eosinophil counts during recovery but not at presentation (30). Despite a lack of data demonstrating significant eosinophil recruitment to the respiratory tract, there is evidence of eosinophil activity during bronchiolitis. Leukotriene C4, eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein (ECP) are elevated in the respiratory tract in RSV bronchiolitis, detectable in nasal fluid (leukotriene C4 and ECP) and lower airway secretions (EDN and ECP) (31–33), while one study did not find increased ECP levels (34). Nasopharyngeal ECP concentrations are also elevated in children with RSV LRTI (not specifically bronchiolitis) and URTI (35–39). Nasal ECP concentrations correlate with nasal concentrations of the neutrophil chemoattractant CCL-3 (MIP-1α) and systemic neutrophil and eosinophil counts (37, 39). Concentrations of CCL-5 (RANTES) (an eosinophil chemoattractant), ECP, and eotaxin all increase during the progression from acute illness to recovery in RSV LRTI and correlate with respiratory tract eosinophil counts, suggesting that this response may have a role in resolution (30, 38, 40, 41). In contrast to the apparent proresolution role of eosinophils themselves during RSV infection, it seems that a Th2-biased response, of which eosinophilia is a component, may be associated with more severe disease, and this is discussed in detail in "Th2 Responses" below.

T Lymphocytes

An initial transient systemic T-cell lymphopenia occurs during RSV LRTI. Counts of CD8$^+$, CD4$^+$, CD3$^+$, and $\gamma\delta$-T cells are all reduced compared to those during convalescence and in noninfected infants (2, 15, 16, 18, 19, 30, 42–44). There is no increased expression of CD11a (LFA-1α) in circulating T cells, suggesting that these cells are not activated, nor is there increased expression of CTLA-4, a marker of downregulated T-cell activation (45, 46). Absolute T-cell counts during RSV infection are inversely associated

with age; thus, T-cell lymphopenia is more pronounced in younger patients (42). Children with more severe illness and those requiring ventilation have reduced circulating T-cell counts (all subsets) compared to those with less severe infection, and in lung tissue from fatal cases, CD4$^+$ and CD8$^+$ T cells are sparse (3, 16, 43, 47, 48). During the course of disease, circulating CD8$^+$ T-cell counts increase (16, 49). In mechanically ventilated infants with severe RSV LRTI, systemic effector CD8$^+$ T-cell counts are low during maximum symptoms and viral load and then peak during convalescence (after the systemic neutrophil response) (10, 49). At the time of ICU discharge, circulating CD8$^+$ T-cell counts are temporarily elevated, whereas neutrophils are normal.

Circulating FOXP3 mRNA and counts of FOXP3$^+$ CD4$^+$ regulatory T cells (comprising suppressive resting Treg cells [CD45RA$^+$ FOXP3^{lo}] and suppressive activated Treg cells [CD45RA$^+$ FOXP3^{hi}]) are reduced in infants hospitalized with RSV bronchiolitis and for at least 3 weeks following acute infection (50, 51). Whether this represents apoptosis or recruitment to the lungs is unknown. Absolute counts of circulating regulatory T cells do not correlate with disease severity (52).

CD4$^+$ and CD8$^+$ T cells are present in BAL fluid obtained from infants with RSV LRTI, with a predominance of CD4$^+$ T cells (4, 5). During the course of infection, the expansion of CD8$^+$ T cells is greater than that of CD4$^+$ T cells, and the CD8$^+$ T cells exhibit an effector phenotype (HLA-DR$^+$ granzyme B$^+$ CD38$^+$). Lower respiratory tract (tracheal aspirate and BAL fluid) granzyme A and B levels are elevated in ventilated patients, and granzyme B is expressed by CD8$^+$ T cells (21). In bronchiolitis specifically, peripheral blood RSV-specific cell-mediated cytotoxic immune responses are more frequent in infants with mild infection than in those with severe infection (53). In experimental RSV infection of adults, the arrival of CD8$^+$ T cells to the lungs (in BAL fluid) is associated with a reduction in pulmonary viral load (54). The frequency of preexisting RSV-specific pulmonary CD8$^+$ T cells in BAL fluid is inversely associated with pulmonary viral load and symptom severity.

During acute infection, there is upregulation of Fas and TRAIL receptor expression on circulating CD4$^+$ and CD8$^+$ T cells compared to that in in convalescence (42). Systemic concentrations of soluble Fas ligand and caspase-1 are elevated. An inverse correlation exists between CD4$^+$ T-cell Fas expression and cell counts. Therefore, one mechanism underlying systemic lymphopenia may be the induction of T-cell apoptosis as a viral immune evasion strategy (Fig. 2A). Furthermore, programmed cell death 1 (PD-1) protein expression is specifically upregulated on pulmonary CD8$^+$ T cells during RSV LRTI (55). PD-1 is a T-cell-coinhibitory receptor that is inhibitory to activated T cells; therefore, PD-1 upregulation could be another immune evasion strategy to blunt the cytotoxic T-cell response (Fig. 2B). RSV infection may also impair differentiation of CD8$^+$ T cells into memory cells by inducing mammalian target of rapamycin (mTOR) activation (Fig. 2C) (56). mTOR mRNA expression is increased in the lungs of infants with RSV bronchiolitis compared to those with human metapneumovirus and rhinovirus infection (and healthy controls), and the RSV cases have a higher proportion of CD8$^+$ mTORser2448$^+$ T cells, indicating activation of the mTOR pathway by phosphorylation on serine 2448 (56). Higher prolactin and lower leptin levels have been associated with lymphopenia in severe RSV infection, suggesting a neuroendocrine component, although these hormonal differences could also be explained by the systemic effects of critical illness (57).

Defective T-cell responses. Deficits in systemic CD4$^+$ and CD8$^+$ T-cell responses may contribute to RSV susceptibility in the elderly, as these subjects have lower levels of RSV-specific CD4$^+$ and CD8$^+$ T cells than younger adults (58, 59). Interestingly, there is no decrease in the level of influenza virus-specific CD8$^+$ T cells with increasing age (59). Furthermore, immunosuppressant drugs prescribed for solid organ transplant recipients (glucocorticoids, calcineurin inhibitors, azathioprine, mycophenolate mofetil, and sirolimus) all have inhibitory activity against T cells, thus impairing the ability of these patients to clear opportunistic RSV infection, resulting in more severe RSV disease (60). Similarly, hematopoietic stem cell transplant recipients are also at increased risk of

Possible RSV immune evasion strategies
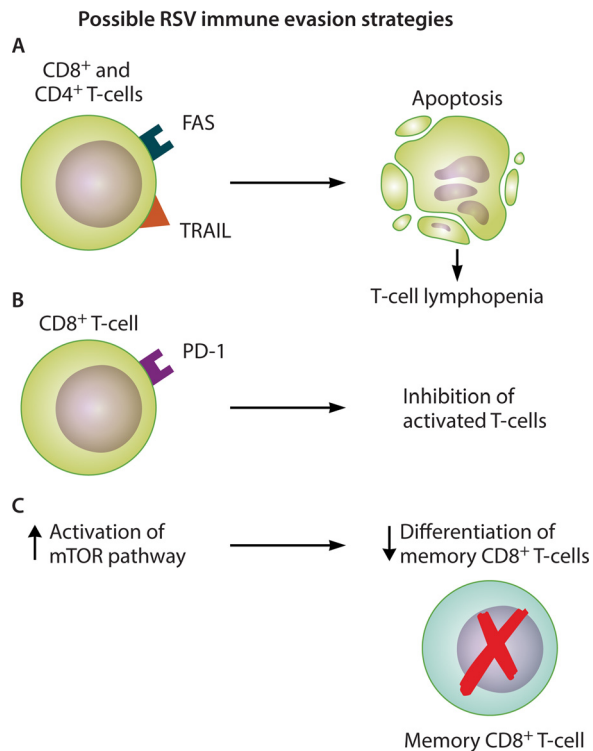
FIG 2 Mechanisms of RSV T-cell interference as a potential immune evasion strategy. RSV infection is associated with an initial systemic T-cell lymphopenia that is quantitatively associated with disease severity. RSV may interfere with T-cell responses by inducing apoptosis (CD4$^+$ and CD8$^+$ T cells) (A), inducing increased expression of the programmed cell death 1 (PD-1) protein, which is inhibitory to activated T cells (CD8$^+$ T cells) (B), and promoting activation of the mammalian target of rapamycin (mTOR) pathway, thus preventing memory CD8$^+$ T-cell formation (C).

severe RSV disease, and peripheral blood lymphopenia has been identified as a specific risk factor for RSV LRTI (61).

Cellular Response in Term and Preterm Infants

Total cellularity, neutrophil counts, macrophage counts, and lymphocyte counts in BAL fluid from infants ventilated due to RSV bronchiolitis are all higher in term than in preterm infants, which possibly is related to immune system maturation (62).

B-LYMPHOCYTE RESPONSES AND ANTIBODY PRODUCTION DURING RSV INFECTION

Antibody Production and B-Lymphocyte Stimulation

There is an increase in circulating B cells, including mature (CD19$^+$ CD5$^+$) and precursor (CD19$^+$ CD10$^+$) cells, in infants with RSV LRTI, and CD20$^+$ B cells and IgM$^+$, IgG$^+$, and IgA$^+$ plasma cells are prominent in postmortem lung tissue from infants with fatal RSV bronchiolitis (43, 63, 64). Antibody responses target the F and G glycoproteins and increase between the acute and convalescent phases of natural primary infection of infants (65). Bronchiolitis may lead to a greater IgG response (66). Type I interferon (IFN) is implicated in early antiviral B-cell responses, and type I IFN-induced proteins (myxovirus resistance protein A and 2',5'-oligoadenylate synthetase 1) are present in high concentrations in bronchiolar and alveolar epithelial cells from RSV-infected infants (63). The B-cell-stimulating factors, a proliferation-inducing ligand (APRIL) and B-cell-activating factor (BAFF), are also present, colocalized to infected epithelial cells. APRIL and BAFF receptors are expressed on a subset of perialveolar plasma cells. In infants ventilated due to severe RSV bronchiolitis, pulmonary BAFF levels are increased (67, 68). BAFF mRNA levels are elevated in bronchial brushings, further suggesting that airway epithelial cells are the source (67). RSV IgA, IgG, and IgM are present in the lungs

of infants with RSV LRTI, together with higher quantities of BAFF and APRIL but lower levels of T-cell-dependent cytokines (IL-2, IL-4, and IL-10) (63, 69). APRIL concentrations correlate positively with RSV IgA and IgM levels and inversely with hypoxia. Thus, the pulmonary antibody response to RSV seems to be driven predominantly by T-cell-independent antibody production via B-cell-stimulating factors (APRIL and BAFF), likely derived from infected pulmonary epithelial cells. In adults with RSV infection, a longer duration of virus shedding is associated with prolonged presence of circulating RSV-specific plasma cells, suggesting that persistent antigenic stimulation in the lung drives B-cell stimulation (70). Similarly, in elderly adults with nosocomial RSV infection, the highest IgG and IgA responses postinfection are seen in patients with more severe illness, perhaps correlating with viral load (71).

In comparison to healthy controls and rotavirus-infected infants, there is a high prevalence of anti HEp-2 (antinuclear) antibodies in infants with RSV LRTI (72). Decay of these autoantibodies was not studied (nor was their presence preinfection), but further investigation of subsequent development of autoimmune disease seems warranted.

Protective Effects of RSV IgG and RSV IgA

In experimental infection of healthy adults, higher preinoculation nasal RSV IgA and serum anti-RSV neutralizing-antibody titers are associated with protection from infection and reduced viral replication (73–77). RSV-specific nasal IgA, serum IgG, and serum neutralizing titers in adults are also all associated with protection against natural RSV reinfection (78, 79). In experimental infections, nasal RSV IgA appears to confer more protection than serum neutralizing antibody, and the response may be more durable (74, 80). Similarly, in infants and children with natural infection, it is the development of the IgA response that appears to correlate with recovery (81). During convalescence, circulating RSV IgG- but not IgA-producing memory B cells are present, in contrast to the case for natural influenza virus infection, where influenza virus IgA-producing memory B cells are detectable (74). Overall, a possible deficit in IgA memory, especially in childhood, when IgA appears to offer important protective immunity, may contribute to recurrent infections (74, 81). In contrast, in elderly patients it is a deficit in circulating serum neutralizing antibodies that appears to predispose to RSV disease (79).

In symptomatic RSV-infected and noninfected children, circulating RSV IgG is present at the highest level in those <1 month old, likely derived from transplacental maternal antibody transfer (82). IgG levels decrease after 3 months until 2 years, when levels increase again. The avidity of IgG is significantly lower among symptomatic RSV-infected infants aged 1 to 3 months than in age-matched controls. Similarly, in children aged ≥24 months, total IgG affinity was lower for children with RSV LRTI than for those with milder URTI. Serum RSV IgG and nasal RSV IgA neutralizing activity is quantitatively higher in children aged 9 to 21 months compared to those aged 4 to 8 months (the age group with a higher incidence of RSV infection) (83). In infants there is a reverse correlation between preexisting serum IgG and the development of nasal IgA following infection, suggesting that maternally derived IgG may suppress the IgA response (84). These observations suggest that good IgG and IgA avidity for RSV contributes to protection against both the development of symptomatic infection and more serious lung involvement. Following natural reinfection in adulthood, there is an 8-fold increase in serum neutralization titer, but this is short-lived, with a 4-fold drop by 1 year in the majority of cases (85).

The serum neutralizing antibody response and nasal IgA and IgG response to the G glycoprotein are RSV group specific (86, 87). In contrast, antibodies to the F glycoprotein are cross-reactive between RSV groups (88).

Other Mechanisms of RSV-Specific Antibody Activity

Maximal cell-bound C3 is present during the convalescent phase and is associated with cell-bound IgG and IgM (89). RSV antigen-containing immune complexes are detectable in the upper airways of infected infants from 3 days after the onset of illness and up to 36 days after (90). The appearance of such immune complexes coincides with

the failure to detect RSV antigen in airway epithelial cells, possibly due to antibody-dependent cell-mediated cytotoxicity (ADCC), which occurs in infants with primary RSV infection (91). ADCC activity correlates with the titer of RSV IgG in the upper airways and is greater during reinfection than during primary infection.

Immunoglobulin E

An IgE response is mounted against the RSV F and G glycoproteins and may play a deleterious role (92). In infants with RSV bronchiolitis, there is a higher proportion of circulating CD23$^+$ B cells (CD23 is the low-affinity IgE receptor on mature and activated B cells) than in those with non-RSV bronchiolitis and noninfected infants (93). Nasopharyngeal RSV IgE, histamine, and leukotriene C4 levels are interrelated and associated with bronchiolitis (where peak levels correlate with hypoxia) compared to other manifestations of infection (URTI or pneumonia) (36, 94). In children with RSV bronchiolitis or pneumonia, higher serum IgE at admission has been associated with prolonged fever and worse symptoms and IgE titers and eosinophil counts with the development of wheeze during RSV LRTI (95–97).

Th1 AND Th2 RESPONSES TO RSV INFECTION

Th1 Responses

Th1 responses are characterized by production of IFN-γ, IL-1, IL-2, IL-12, IL-18, and TNF-α. IL-12 induces IFN-γ production and favors Th1 cell differentiation. The Th1 response is proinflammatory and is important in the generation of cell-mediated immunity required for the control of intracellular pathogens. Therefore, it is an inherently appropriate response to viral infection.

Systemic. Markers of the Th1 response (IFN-γ, soluble tumor necrosis factor receptor II, and soluble interleukin-2 receptor [sCD25]) are elevated in the circulation during RSV LRTI, and systemic IFN-γ exerts a protective effect (97–100). Children ≤6 months with RSV bronchiolitis have a reduced IFN-γ response, possibly contributing to the increased incidence of RSV disease in the younger age group (101). In infants with RSV LRTI, systemic IFN-γ concentrations are lower in those with severe disease (48, 98). Infants with RSV bronchiolitis requiring ventilation have lower IFN-γ concentrations than those with milder disease, and undetectable circulating IFN-γ positively correlates with the need for ventilation (102). Low IFN-γ/IL-10 ratios are associated with hypoxia and wheeze (99). During the acute phase of RSV LRTI, peripheral blood mononuclear cell (PBMC) IFN-γ mRNA expression is lower in hypoxic patients (47). Furthermore, circulating IL-12 levels are lower in severe those with RSV LRTI than in those with mild infections or controls (48, 98).

Respiratory tract. IFN-γ levels are also elevated in the nasal mucosa (37, 103–106) and in the lung (20, 106). The respiratory tract IFN-γ response exerts a protective effect, with lower IFN-γ production associated with increased severity scores, hypoxia, and need for ventilation (106–110). In RSV LRTI, the nasopharyngeal IFN-γ/IL-10 ratio increases from presentation to discharge, in parallel with clinical recovery, strengthening the association of IFN-γ with protection (41).

Other Th1-associated cytokines are also elevated in the nasal mucosa (IL-1, IL-2, IL-12, IL-18, and TNF-α) (37, 111, 112) and in the lungs (IL-1, IL-2, and TNF-α) (20, 113). TNF-α levels are highest during the acute phase of infection and then decline during recovery (37, 105, 113–115). Raised IL-6 mRNA and protein have been observed in BAL fluid and nasopharyngeal fluid from infants with severe RSV infection, and a high IL-6/TNF-α ratio is associated with reduced disease severity (113, 116). In children with only URTI, there is reduced nasal production of anti-inflammatory IL-10, and this is inversely related to TNF-α production (117). It has been suggested that a reduction in IL-10 production facilitates a robust TNF-α response, limiting the infection to the upper airway.

Increased nasal concentrations of IL-1α are associated with the need for ventilation in children with RSV LRTI (118, 119). There is also an increase in nasal IL-18 concentrations and the number of IL-18-positive cells in children with RSV bronchiolitis compared

to those with URTI (117). In bronchiolitis, nasal IL-18 production is associated with nonhypoxic infection, consistent with its role in stimulating IFN-γ production (117, 120).

Th2 Responses

The Th2 response, characterized by IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 production, is involved in the generation of antibody (in particular IgE) and eosinophil responses. This response is associated with atopy and also protection against parasitic infections, and it may counteract and limit Th1-mediated inflammation.

Systemic. Systemic IL-4, IL-6, IL-10, and IL-13 levels are elevated in children with RSV LRTI (37, 97, 101, 121–123). Systemic IL-6 and IL-10 levels correlate with disease severity in RSV LRTI, including the requirement for supplemental oxygen (99, 122, 124, 125). In comparison to those during influenza A virus infection, the systemic concentrations of IL-4, IL-5, and CCL-5 are higher during RSV LRTI (126).

Respiratory tract. Elevated concentrations of IL-4, IL-6, IL-9, IL-10, and IL-13 have been found in nasal washes (37, 109, 127–130) and in the lung (20, 131–133) in children with RSV LRTI. Respiratory tract IL-10 production appears to exert a protective effect in RSV LRTI, with concentrations inversely correlating with the duration of required supplemental oxygen and symptom severity (108, 128, 132). In very young infants (<3 months), this effect appears to be reversed, with IL-10 concentrations correlating with severity (125), and nasal IL-10/CCL-5 ratios are only inversely correlated with duration of mechanical ventilation when infants older than 5 months are considered (134).

IL-6 levels are strongly elevated in BAL fluid from infants ventilated due to severe RSV bronchiolitis (20, 113) and are elevated to a lesser extent in the respiratory tract in infants with milder infection (37, 117, 132). There are inconsistent data associating the nasal IL-6 response with severity. In infants with RSV bronchiolitis, nasal IL-6 concentrations are higher in those requiring ventilation and correlate with the degree of hypoxia (111, 118, 135, 136). Similarly, adults hospitalized due to RSV infection have higher nasal IL-6 concentrations than those not requiring hospitalization (137). In experimentally infected adults, the nasal IL-6 concentration is positively correlated with viral load and symptom severity (138). In contrast, in a cohort of children with RSV bronchiolitis, higher nasal IL-6 concentrations were associated with a shorter requirement for supplemental oxygen (108).

Th1/Th2 Balance

A high nasal and systemic IL-4/IFN-γ ratio, a marker of Th2 bias, is associated with severe (hypoxic) RSV bronchiolitis (103, 123, 139). Independent of the ratio, IFN-γ concentrations are lower and IL-4 concentrations higher in infants with severe bronchiolitis. Also in severe RSV bronchiolitis, circulating CXCR3$^+$ T-cell (Th1) counts are significantly reduced during acute infection compared to convalescence, but CCR4$^+$ T cells (Th2) are not (140). An excessive Th2 or deficient Th1 response may be associated with the development of bronchiolitis compared to milder URTI with RSV: the nasal IL-4/IFN-γ and IL-10/IL-12 ratios are higher in infants with bronchiolitis (141). In a cohort of children with hypoxic RSV LRTI, comparison of systemic and respiratory tract cytokines showed a predominance of Th2 cytokines in nasopharyngeal fluid (higher pulmonary/systemic of IL-4/IL-12, IL-10/IL-2, IL-10/IFN-γ, IL-6/IFN-γ, and IL-6/IL-2 ratios) (37). Overall, these data suggest that a Th2-biased response may be associated with more severe manifestations of RSV infection, consistent with it being either an inappropriate response to acute viral infection or one that is required to limit a potentially detrimental Th-1 response in severe RSV infection.

However, such findings are not entirely consistent throughout the literature, and there are reports of elevated IFN-γ/IL-4 ratios in children with more severe manifestations of RSV infection (bronchiolitis, pneumonia, and any LRTI) compared to controls, albeit not stratified by severity of infection within the groups (98, 142, 143). A heterogeneous polarization of pulmonary Th responses in infants with severe RSV bronchi-

olitis has also been described, with 25% of infants expressing only IFN-γ and 50% expressing only IL-4, although again overall supporting a Th2 bias in severe disease (144). In comparison to infection with human metapneumovirus (hMPV), infants with RSV infection have similar nasopharyngeal IFN-γ levels but higher IL-4 levels, consistent with a Th2-biased response that is distinct from the response to hMPV (34).

There are lower counts of *in vivo* RSV-specific T cells in the elderly, and in *in vitro* experiments, both isolated T cells and peripheral blood mononuclear cells from healthy elderly patients produce less IFN-γ when stimulated with RSV F protein or RSV, respectively (58, 59, 145). Although this finding has not been confirmed by *in vivo* experiments, it does hint at a defective Th1 response in the elderly which may contribute to the higher incidence of severe RSV disease in this population.

CHEMOKINES, CYTOKINES, AND OTHER IMMUNE MOLECULES EXPRESSED DURING RSV INFECTION

Overview

A comprehensive list of immune and lung structural proteins involved in the response to RSV infection is presented in Table 1. Key molecules are discussed here.

Interleukin-8

Systemic and respiratory tract production of IL-8, a neutrophil chemoattractant, is increased during RSV LRTI, and circulating concentrations normalize during convalescence (37, 102, 105, 111, 121, 122, 133, 146–149). Higher circulating and respiratory tract IL-8 levels are associated with hypoxia and need for ventilation in infants (18, 37, 102, 135, 136, 147). IL-8 production in the nasal mucosa is also higher during LRTI caused by RSV in children than with rhinovirus (150). When comparing term and preterm infants with RSV LRTI of similar severity, nasal IL-8 and leukocyte counts are higher in the term infants, suggesting a more vigorous inflammatory response (151).

Interleukin-17A

Compared to those in infants with non-RSV LRTI, circulating Th17 cell counts and IL-17 levels are higher in infants with RSV bronchiolitis (51). In these infants, nasal concentrations of proinflammatory IL-17A are higher in patients requiring ventilation (118). When patients are ventilated, tracheal IL-17A concentrations positively correlate with neutrophil counts (152). In infants with mild bronchiolitis, although nasal IL-17A levels are lower initially, they increase during the convalescent phase, hinting at a dual role for IL-17A: deleterious in the acute phase, which is possibly related to neutrophil recruitment, but potentially involved in the resolution of milder infections (118).

CC Chemokines

CCL-5 (RANTES), eotaxin, and CCL-3 (MIP-1α) production in the nasal mucosa and lung (in BAL fluid) is increased during RSV LRTI and bronchiolitis (32, 37, 38, 129, 132, 133, 143, 149, 153–155). However, nasal and systemic CCL-5 concentrations are lower in patients requiring ventilation (18, 132), inversely correlating with the duration of ventilation and required supplemental oxygen. In RSV LRTI, the duration of required supplemental oxygen is positively associated with nasal CCL-3 and inversely associated with CCL-4 (MIP-1β) (107, 108). CCL-3 and eotaxin concentrations in the nasal mucosa are higher in hypoxic bronchiolitis than in URTI or nonhypoxic bronchiolitis (103, 155, 156). Nasal CCL-3 concentrations are higher in RSV-infected adults who require hospitalization than in those who do not, and they are associated with symptom severity in experimentally infected adults (137, 138). However, one study of RSV LRTI found that increased nasal CCL-2 (MCP-1), CCL-3, and CCL-4 are all positively associated with severity (119).

Pattern Recognition Receptors

Pattern recognition receptors (PRRs) are involved in innate immune recognition

TABLE 1 Chemokines, cytokines, and other immune molecules involved in the human immune response to RSV infection[a]

Immune molecule(s)	Production[b] Respiratory tract[c] Nasal mucosa	Lung	Systemic	Comments	Reference(s)[d]
Th1 cytokines					
IFN-γ	+	+	+	Protective	
IL-12	+	+	+	Protective	
IL-1α and IL-1β	+		+	Deleterious	
IL-2	+	+	+	No reported association with severity	
TNF-α	+	+	+	Deleterious	
IL-18	+			Protective	
sCD25			+	Deleterious	
Th2 cytokines					
IL-4	+	+	+	Deleterious	
IL-6	+	+	+	Variable association with severity (see text)	
IL-9	+	+		No reported association with severity	
IL-10	+	+	+	Variable association with severity (see text)	
IL-13	+	+	+	No reported association with severity	
Other cytokines					
IL-8	+	+	+	Deleterious: neutrophil chemoattractant	
IL-17A	+	+		Variable association with severity (see text)	
IL-33	+			No reported association with severity	130
Chemokines					
CCL-2 (MCP-1)	+	+		Deleterious	
CCL-3 (MIP-1α)	+	+		Deleterious	
CCL-4 (MIP-1β)	+			Variable association with severity (see text)	
CCL-5 (RANTES)	+	+		Protective	
CXCL-10 (IP-10)	+		+	Deleterious	
Eotaxin	+	+		Deleterious	
Other					
IFN-λ	+			Deleterious despite stimulating ISG expression	159, 160
IFN-α	+		+	No reported association with severity	158
G-CSF	+		+	Circulating levels are higher in infants with RSV LRTI requiring ventilation	18, 105
Soluble ICAM-1	+		+	Levels in nasal fluid positively correlate with severity	40, 124, 126
Substance P	+	+		Lower concentrations associated with increased severity	106
MBL			−	No reported association with severity	44
Cathelicidin LL-37	+			Protective: in human exptl infection, higher constitutive nasal levels are associated with reduced development of infection	177
Olfactomedin 4			+	Greater expression in PBMC was associated with need for ventilation in RSV LRTI	19
Surfactants A, B, D		−	+	The pulmonary level of surfactant A and measurable surfactant activity increase during recovery	178–181
MMP-9, MMP-3, PGP		+		Elevated pulmonary levels in ventilated infants are associated with hypoxia and acute lung injury	182–184
KL-6			+	Circulating levels are greater in infants with RSV LRTI requiring ventilation	185
sTRAIL		+		No reported association with severity	186

[a]Abbreviations: CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; G-CSF, granulocyte colony-stimulating factor; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; IP-10, IFN-γ-inducible protein-10; ISG, interferon-stimulated gene; MBL, mannose binding lectin; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; PGP, proline-glycine-proline (the product of MMP hydrolysis of collagen); RANTES, regulated on activation, normal T expressed and secreted; sTRAIL, soluble TNF-related apoptosis-inducing ligand; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor.
[b]+, increased production; −, reduced production.
[c]Nasal mucosa, measurements made in nasal fluid or nasopharyngeal aspirate; lung, measurements made in bronchoalveolar lavage fluid or tracheal aspirate.
[d]References are provided for molecules not discussed in detail in the text.

of viral pathogens in order to stimulate interferon and cytokine responses. In comparison to healthy controls or infants with rhinovirus or bocavirus infection, in infants with RSV bronchiolitis there is increased pulmonary expression of TLR7, TLR8, RIG-1, and MDA-5 (157). RIG-1 mRNA in the lungs correlated with RSV viral

load (157). Furthermore, an individual's TLR4 genotype influences the severity of RSV bronchiolitis, and this is significantly influenced by environmental lipopolysaccharide exposure (139).

Innate Interferons

IFN-α is produced systemically and in the respiratory tract in response to RSV infection (158). Nasopharyngeal IFN-α titers peak on day 1 of illness, remain elevated for ~6 days, and then decrease in parallel with nasopharyngeal RSV antigen levels (158). In peripheral blood, IFN-α levels peak by day 2. Infants less than 3 months of age produce the lowest levels of IFN-α in both the nasopharynx and peripheral blood (158). RSV may be a comparatively weak inducer of type I IFN, since nasopharyngeal IFN-α levels are higher in infants with influenza virus, adenovirus, and parainfluenza virus infection (158).

Type III interferons (IFN-λ) are produced in response to viral infection and have type I IFN-like activities. Their receptor complex is expressed primarily on epithelial cells, and IFN-λ responsiveness is greatest in organs with high epithelial content, such as the lungs. There is a IFN-λ response to RSV bronchiolitis, with higher nasal levels of IFN-λ 1 to 3 seen than in rhinovirus infection (159, 160). IFN-λ mRNA levels correlate with IFN-stimulated gene expression (*MxA* and *ISG56*) (159). Despite their association with antiviral gene expression, higher nasal IFN-λ 1 levels are associated with increased disease severity (159).

Immunostimulatory defective viral genomes (iDVGs) have been detected in the nasal fluid of around half of RSV-infected children in one study (161). These RSV genomes have large deletions rendering them unable to replicate without the presence of helper virus. The presence of iDVGs correlates with mRNA levels of *IFNA4* and the ISGs *IFIT1* and *RSAD2*, suggesting that they are sufficient to stimulate an innate interferon response (161).

microRNA

Viral infection (especially with RNA viruses) can subvert cellular microRNA expression, potentially to the benefit of the virus. A distinct microRNA expression profile is detectable in the nasal mucosa of RSV-infected infants compared to noninfected controls (downregulation of miR-34b, miR-34c, miR-125b, miR-29c, mir125a, miR-429, and miR-27b and upregulation of miR-155, miR-31, miR-203a, miR-16, and let-7d) (162). miR-125a and miR-429 are downregulated in mild but not severe infection; the former has roles in NF-κB signaling and macrophage function (162). miR-26b (which is thought to target TLR4 based on miRNA target prediction software) has been studied in PBMCs from children with RSV bronchiolitis, where it is upregulated, negatively correlating with TLR4 expression (163).

GLOBAL HOST TRANSCRIPTIONAL RESPONSE TO RSV INFECTION

Genes and pathways associated with neutrophil function, interferon signaling (including *STAT1*, *STAT2*, *IFITM1*, *OAS2*, *MX1*, *IFI27*, *IFI35*, and *IFIT3*), interferon-inducible proteins (including *IFI44*, *EIF2AK2*, *IFI44L*, *IFI6*, *OAS3*, and *G1P2*), dendritic cell maturation, and inflammation are upregulated in the circulation of children with RSV infection (164–166). Genes and pathways associated with NK cell, B-cell, and T-cell responses, cytotoxic lymphocyte-mediated apoptosis of target cells, HLA class I and II, and antigen presentation are underexpressed (164–166). Underexpression is greater in infants <6 months than in those aged 6 to 24 months (164), which may reflect either low gene expression or migration of peripheral blood immune cells to the infected tissues. In severe disease there is greater upregulation of neutrophil and inflammatory gene expression and greater suppression of T-cell-, NK cell-, and plasma cell-associated genes (164). In comparison, this dysregulation of genes relating to neutrophil, B-cell, and T-cell function is not seen in children with rhinovirus or influenza virus infection (164).

A different transcriptional response is seen in the upper airways of RSV-infected children. In infants requiring supplemental oxygen or mechanical ventilation, the

ubiquitin D, tetraspanin 8, mucin 13, and β-microseminoprotein genes are upregulated and the chemokine ligand 7 gene is downregulated compared to those in infants with milder RSV infection (167).

RELATIONSHIP BETWEEN MOLECULAR AND CELLULAR IMMUNE RESPONSES TO RSV AND PATHOPHYSIOLOGY

Molecular and cellular events during RSV infection are reflected in changes in host physiology observed during the course of disease (Fig. 2). The initial development of cough, wheezing, and tachypnoea, usually peaking on days 4 to 5, develops in parallel to the maximal neutrophil response and viral load (10). This is followed by a convalescent period with a CD8$^+$ T-cell predominant response involved in viral clearance, which coincides with the reduction in the above-mentioned respiratory symptoms over a period of 2 to 3 weeks.

Many of the different cytokines, chemokines, and other immune molecules that are involved in the immune response to RSV infection have been associated with protective or deleterious effects, as listed in Table 1, depending on the perceived severity of disease in the studied patients. This is usually based on the need for ICU admission, endotracheal intubation, and mechanical ventilation but also on composite scores of clinical parameters, including respiratory rate, oxygen saturations, the need for supplemental oxygen, or the need for hospitalization.

We know that preexisting differences in immune status may modulate molecular and cellular responses during RSV infection. Younger infants have more pronounced lymphopenia and reduced IFN-γ responses, possibly reflecting the immunological immaturity of early life (42, 101). Term infants seem to have a stronger inflammatory response, with higher leukocyte counts and IL-8 levels, than preterm infants (151). On the other hand, preterm babies may have an inadequate antiviral response, with reduced pulmonary pDC counts (20). These observations may provide an explanation for the increased frequency of severe RSV disease in preterm and younger term-born infants.

Furthermore, early life microbiome changes in the gut and respiratory tract may influence the host immune responses during RSV disease (168), similar to the distinct patterns of nasopharyngeal microbiota development that have been reported in young infants with cystic fibrosis (169). Certainly, associations between the respiratory and gut microbiome, host transcriptional immune responses, RSV load, and clinical status are now evident and require further detailed investigation (170).

RSV INFECTION AND SUBSEQUENT RESPIRATORY HEALTH

RSV bronchiolitis during early life has been associated with an increase in susceptibility to subsequent episodic wheeze, physician-diagnosed asthma, and decreased forced expiratory volume (FEV$_1$) and forced vital capacity (FVC) measurements on pulmonary function testing (171). Evidence for a causal relationship comes from an intervention trial in premature infants (gestational age 33 to 35 weeks) who received either palivizumab, a humanized monoclonal anti-RSV IgG used in the prevention of severe RSV disease, or placebo during RSV season (172). Palivizumab treatment almost halved (−46.4%) the proportion of infants with subsequent recurrent wheeze compared to placebo. Possible molecular and cellular explanations for such a relationship have been described. There are few human data on potential immune mechanisms for the long-term effects of RSV bronchiolitis, but levels of the cytokines IL-3 and IL-12p40 during RSV disease have been found to correlate with subsequent development of recurrent wheeze (133). Furthermore, elevation of vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF), and IL-10, all mediators that have been related to asthma and postvirus induced wheeze, persists after the RSV episode (173). Higher proportions of nasal pDCs may reflect a heightened antiviral response in the respiratory tract, potentially due to higher viral load, leading to the development of recurrent wheeze and asthma (174). IL-33, although not reported to be associated

with severity of disease, has also been implicated in a Th2-biased response to RSV and may relate to RSV-mediated asthma in later life (130).

KEY DIFFERENCES IN THE IMMUNE RESPONSES TO RSV OF ANIMAL MODELS AND HUMANS

Contemporary data from animal models of RSV infection have been comprehensively reviewed by Borchers and colleagues recently (175). Although similarities are evident, the critical fact remains that human RSV has no animal reservoir and has evolved to infect humans as its natural host, not the commonly used rodent models, which require infection doses far in excess of those needed for human RSV infection. Neutrophilic inflammation contributes significantly to pathogenesis in humans (where neutrophils can constitute up to 85% of BAL fluid cell counts) but appears to have a less dominant role in mice (15 to 20% of cells) (67). In humans the contribution of Th1 and Th2 immune responses is variable and related to pathogenesis, whereas in mice there is generally a robust and reliable Th1 (IFN-γ) response (175). The evidence for an important contribution of eosinophils in humans has been reviewed above, but there is no evidence that these cells have a major role in the pathogenesis of disease in mice (175, 176).

CONCLUSION

By synthesizing the results of a systematic literature review of data exclusively from infected humans, we propose the following model to describe our current understanding of the immune response to RSV infection in humans (Fig. 1).

Large quantities of proinflammatory cytokines are produced in the respiratory tract, with an initial strong activated pulmonary and systemic neutrophil response which correlates with disease severity and is mediated by the neutrophil chemoattractant IL-8. Eosinophil degranulation occurs in the lungs during RSV bronchiolitis, and there may also be a role for CCL-5-mediated eosinophil recruitment to the lungs during recovery from RSV LRTI. Dendritic cells migrate into the lungs, where they are the primary antigen-presenting cell. Circulating cDCs exhibit an activated phenotype, and pulmonary cDC counts correlate with proinflammatory and T-cell-derived cytokine concentrations, suggesting that they contribute to the inflammatory response in a potentially deleterious manner. Alveolar macrophages have an immune-regulatory and antigen-presenting role.

Initially, there is a systemic CD4$^+$ and CD8$^+$ T-cell lymphopenia, without evidence for pulmonary sequestration of T cells. There is active T-cell apoptosis, upregulation of the T-cell-coinhibitory molecule PD-1, and mTOR-mediated suppression of memory CD8$^+$ T-cell differentiation, suggesting that T-cell interference is a key viral immune evasion strategy (Fig. 2). Following the initial neutrophilic response, there is a pulmonary CD8$^+$ T-cell response coinciding with clearance of RSV from the lungs. CD8$^+$ T cells are protective, likely mediating viral clearance and therefore enabling resolution of infection. Humoral immunity to RSV reinfection is incomplete, but RSV-specific circulating IgG and secretory IgA are protective against infection and possibly modify the severity of infection. T-cell-independent B-cell antibody production via B-cell stimulating factors (BAFF and APRIL) derived from the airway epithelium seems to play a major role in protective antibody generation. On the other hand, RSV IgE production is associated with bronchiolitis, where it may have a deleterious effect. There is strong evidence that IFN-γ (and, related to this, IL-12 and IL-18, which promote IFN-γ production/Th1 differentiation) has a protective role in RSV infection. In contrast, a Th2-biased response may be associated with more severe disease manifestations. Global host transcriptional profiling reveals upregulation of innate inflammatory (e.g., neutrophil-related) genes and suppression of genes associated with the adaptive immune response. This is exaggerated in severe disease and is specific to RSV infection. Other cytokines (particularly IL-17A), chemokines (particularly CCL-5 and CCL-3), and local innate immune factors (cathelicidins, IFN-λ, G-CSF, and soluble ICAM-1) have also been associated with the

course of disease. Elderly patients are at increased risk of severe RSV disease, and this susceptibility may relate to defects in circulating neutralizing antibody titers and RSV-specific CD4$^+$ and CD8$^+$ T cells.

Overall, neutrophilic pulmonary inflammation is incriminated as a damaging process, and protective effects of CD8$^+$ T cells and IFN-γ production are consistently reported. While these processes may be important therapeutic targets to modulate the immunopathogenesis of RSV infection, less well characterized immune processes, especially those occurring in the lower airways and lung, require further investigation.

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