The human immune response to respiratory syncytial virus infection

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SUMMARY

Respiratory syncytial virus (RSV) is an important aetiological agent of respiratory infections, particularly in children. Much data 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 in infected humans.

There is an initial strong neutrophil response to RSV infection in humans, positively correlated with disease severity and mediated by 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 re-infection 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. 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.

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 aetiological 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 and the elderly. Much data 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 post mortem 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. First we describe the behaviour of all major immune cell types, encompassing neutrophils, dendritic cells, monocytes, macrophages, eosinophils and T-lymphocytes. Secondly, 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

113 immune molecules have been demonstrated to be involved in the immune response and are
114 reviewed. The global host transcriptional response is also discussed in the context of
115 immune-related pathways. Certain key pathogen-host interactions described herein may
116 represent targets for the development of novel therapeutics. For completeness, we summarise
117 the association between RSV infection and subsequent asthma and also key differences
118 between immune responses in humans and animals used in model systems of infection.
119
120 **METHODS OF SYSTEMATIC LITERATURE REVIEW**
121
122 We conducted a systematic literature review following PRISMA (Preferred Reporting Items
123 for Systematic Reviews and Meta-Analyses) guidelines (PROSPERO registration number
124 CRD42016047320). An electronic literature search of Medline, Embase and Web of Science
125 was performed using the following search terms: ((RSV[Title] OR respiratory syncytial
126 virus[Title])) AND (Immune response OR T-cell OR B-cell OR lymphocyte OR macrophage
127 OR neutrophil OR monocyte OR natural killer cell OR dendritic cell OR immunoglobulin
128 OR IgG OR IgA OR IgE OR cytokine OR chemokine OR interleukin OR interferon) AND
129 (Human OR clinical OR experimental OR neonate OR infant OR children OR adult OR
130 elderly)).
131
132 The last search was conducted on 16th May 2016. The results from the databases were
133 merged and duplicates removed. The combined results of the electronic database search were
134 assessed independently by two authors and discrepancies discussed and agreed upon
135 according to the inclusion and exclusion criteria. Publications in all languages describing
136 primary research in humans were included (clinical, *ex vivo*, post mortem). Editorials,
137 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 2541 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) 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 normalised (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 express RSV proteins F, G, and N proportionately, implying stoichiometric expression thus intact intracellular virions (12). RSV genomic RNA and mRNA is 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 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 up-regulated in nasopharyngeal fluid and NETs are present in BAL 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 (NK) cells

RSV infection results in reduced total systemic NK cell counts albeit with an increase in an activated sub-set 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 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 tract of infants ventilated due to severe RSV-bronchiolitis (BAL and tracheal aspirate), possibly suggesting migration to the lungs (20, 21).

Dendritic Cells (DC)

Conventional (cDC) and plasmacytoid (pDC) DCs 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 pro-inflammatory phenotype (20). Circulating cDCs express the activation marker CD83 and the co-stimulatory molecule CD40. Concentrations of innate immune pro-inflammatory cytokines (IL-6, TNF-α, IL-8) and T-cell derived cytokines (IFN-γ, IL-13, IL-10, IL-2) in BAL correlate with cDC counts. In subsets of infants with severe RSV-bronchiolitis (pre-term infants and infants aged four months or more) pulmonary pDC counts are low compared to 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 from RSV-infected infants and adult transplant recipients co-express 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 TLR8 expression and TNF-α production during acute RSV-infection, which subsequently normalises 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 hospitalised 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 this response may have a role in resolution (30, 38, 40, 41). In contrast to the apparent pro-resolution 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 the section on Th2 responses below.

T-lymphocytes

An initial transient systemic T-cell lymphopenia occurs during RSV-LRTI. Counts of CD8+, CD4+, CD3+ and γδ-T-cells are all reduced, compared to convalescence and non-infected 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 down-regulated 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 sub-sets) 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 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) 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 compared to severe infection (53). In experimental RSV infection of adults, the arrival of CD8+ T-cells to the lungs (in BAL) is associated with a reduction in pulmonary viral load (54). The frequency of pre-existing RSV-specific pulmonary CD8+ T-cells in BAL is inversely associated with pulmonary viral load and symptom severity.

During acute infection, there is up-regulation of Fas and TRAIL receptor expression on circulating CD4+ and CD8+ T-cells compared to 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 (Figure 1a). Furthermore, programmed cell death 1 (PD-1) protein expression is specifically up-regulated on pulmonary CD8+ T-cells during RSV-LRTI (55). PD-1 is a T-cell co-inhibitory 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 (Figure 1b).

RSV infection may also impair differentiation of CD8+ T-cells into memory cells by inducing mammalian target of rapamycin (mTOR) activation (Figure 1c) (56). mTOR mRNA expression is increased in the lungs of infants with RSV-bronchiolitis compared to 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 compared to 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, 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, haematopoietic stem cell transplant recipients are also at increased risk of severe RSV disease and peripheral blood lymphopenia has been identified as a specific risk factor for RSV-LRTI (61)

Cellular Response in Term and Pre-Term Infants

Total cellularity, neutrophil counts, macrophage counts and lymphocyte counts in BAL from infants ventilated due to RSV-bronchiolitis are all higher in term compared to pre-term infants, possibly 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 post-mortem 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 anti-viral B-cell responses and type I IFN-induced proteins (myxovirus resistance protein A, 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, co-localized 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 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 predominantly driven 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 post-infection 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 auto-antibodies was not studied (nor their presence pre-infection) 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 pre-inoculation nasal RSV-IgA and serum anti-RSV neutralizing antibody titres are associated with protection from infection and reduced viral replication (73-77). RSV-specific nasal IgA, serum IgG and serum neutralizing titres in adults are also all associated with protection against natural RSV re-infection (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 natural influenza virus infection, where influenza-IgA producing memory B-cells are detectable (74). Overall, a possible deficit in IgA memory especially in children, 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 non-infected children, circulating RSV-IgG is present at the highest level in those <1 month old, likely derived from trans-placental maternal antibody transfer (82). IgG levels decrease after three months until two years, when levels increase again. The avidity of IgG is significantly lower amongst symptomatic RSV infected infants aged 1-3 months than in age-matched controls. Similarly, in children aged ≥24 months, total IgG affinity was lower for children with RSV-LRTI compared to milder URTI. Serum RSV-IgG and nasal RSV-IgA neutralizing activity is quantitatively higher in children aged 9-21 months compared to those aged 4-8 months (the age group with a higher incidence of RSV infection) (83). In infants there is a reverse correlation between pre-existing serum IgG and the development of nasal IgA following infection, suggesting 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 against more serious lung involvement. Following natural re-infection in adulthood, there is an eightfold increase in serum neutralization titre but this is short-lived, with a fourfold drop by one year in the majority of cases (85).

386 The serum neutralizing antibody response and nasal IgA and IgG response to the G
387 glycoprotein are RSV-group specific (86, 87). In contrast, antibodies to the F glycoprotein
388 are cross-reactive between RSV groups (88).
389
390 **Other Mechanisms of RSV-specific Antibody Activity**
391
392 Maximal cell-bound C3 is present during the convalescent phase and is associated with cell-
393 bound IgG and IgM (89). RSV antigen containing immune complexes are detectable in the
394 upper airways of infected infants from three days after the onset of illness, and detectable up
395 to 36 days after (90). The appearance of such immune complexes coincides with the failure to
396 detect RSV antigen in airway epithelial cells, possibly due to antibody-dependent cell-
397 mediated cytotoxicity (ADCC), which occurs in infants with primary RSV infection (91).
398 ADCC activity correlates with the titre of RSV-IgG in the upper airways and is greater during
399 re-infection than primary infection.
400
401 **Immunoglobulin E**
402
403 An IgE response is mounted against the RSV F and G glycoproteins and may play a
404 deleterious role (92). In infants with RSV-bronchiolitis there is a higher proportion of
405 circulating CD23+ B-cells (CD23 is the low-affinity IgE receptor on mature and activated B-
406 cells) than in non-RSV-bronchiolitis and non-infected infants (93). Nasopharyngeal RSV-
407 IgE, histamine and leukotriene C4 levels are inter-related and associated with bronchiolitis
408 (where peak levels correlate with hypoxia), compared to other manifestations of infection
409 (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 titres 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 characterised by production of IFN-γ, IL-1, IL-2, IL-12, IL-18, TNF-α. IL-12 induces IFN-γ production and favours Th1 cell differentiation. The Th1 response is pro-inflammatory and 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, 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 compared to 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 IFN-γ mRNA expression is lower in

435 hypoxic patients(47). Furthermore, circulating IL-12 levels are lower in severe RSV-LRTI
436 compared to mild infections or controls (48, 98).
437
438 **Respiratory tract**
439
440 IFN-γ levels are also elevated in the nasal mucosa (37, 103-106) and in the lung (20, 106).
441 The respiratory tract IFN-γ response exerts a protective effect, with lower IFN-γ production
442 associated with increased severity scores, hypoxia and need for ventilation (106-110). In
443 RSV-LRTI, the nasopharyngeal IFN-γ:IL-10 ratio increases from presentation to discharge,
444 in parallel with clinical recovery, strengthening the association of IFN-γ with protection (41).
445
446 Other Th1-associated cytokines are also elevated in the nasal mucosa (IL-1, IL-2, IL-12, IL-
447 18, TNF-α) (37, 111, 112) and in the lungs (IL-1, IL-2, TNF-α) (20, 113). TNF-α levels are
448 highest during the acute phase of infection then decline during recovery (37, 105, 113-115).
449 Raised IL-6 mRNA and protein have been observed in BAL and nasopharyngeal fluid from
450 infants with severe RSV infection and a high ratio of IL-6:TNF-α is associated with reduced
451 disease severity (113, 116). In children with only URTI there is reduced nasal production of
452 anti-inflammatory IL-10 and this is inversely related to TNF-α production (117). It has been
453 suggested that a reduction in IL-10 production facilitates a robust TNF-α response, limiting
454 the infection to the upper airway.
455
456 Increased nasal concentrations of IL-1α are associated with the need for ventilation in
457 children with RSV-LRTI (118, 119). There is also an increase in nasal IL-18 concentrations
458 and the number of IL-18 positive cells in children with RSV-bronchiolitis compared to URTI

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

Th2 Responses

The Th2 response, characterised 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 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 of supplemental oxygen (99, 122, 124, 125). In comparison to 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 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 hospitalised due to RSV infection have higher nasal IL-6 concentrations than those not requiring hospitalisation (137). In experimentally infected adults, 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 are 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 ratio is 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 ratios of IL-4:IL-12, IL-10:IL-2, IL-10:IFN-γ, IL-6:IFN-γ, IL-6:IL-2) (37). Overall, these data suggest 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, 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-bronchiolitis has also been described, with 25% of infants only expressing IFN- γ and 50% only expressing 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 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 normalise 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 in children caused by RSV compared to rhinovirus (150). When comparing term and pre-term infants with RSV-LRTI of similar severity, nasal IL-8 and leucocyte counts are higher in the term infants suggesting a more vigorous inflammatory response (151).

Interleukin-17A

Compared to 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 pro-inflammatory IL-17A are higher in patients requiring ventilation (118). When ventilated, tracheal IL-17A concentrations positively correlate with neutrophil counts (152). In infants

559 with mild bronchiolitis, although nasal IL-17A levels are lower initially, they increase during
560 the convalescent phase, hinting at a dual role for IL-17A: deleterious in the acute phase,
561 possibly related to neutrophil recruitment, but potentially involved in the resolution of milder
562 infections (118).
563
564 **CC Chemokines**
565
566 CCL-5 (RANTES), eotaxin and CCL-3 (MIP-1α) production in the nasal mucosa and lung
567 (in BAL) is increased during RSV-LRTI and bronchiolitis (32, 37, 38, 129, 132, 133, 143,
568 149, 153-155). However, nasal and systemic CCL-5 concentrations are lower in patients
569 requiring ventilation (18, 132) inversely correlating with the duration of ventilation and
570 required supplemental oxygen. In RSV-LRTI, the duration of required supplemental oxygen
571 is positively associated with nasal CCL-3 and inversely with CCL-4 (MIP-1β) (107, 108).
572 CCL-3 and eotaxin concentrations in the nasal mucosa are higher in hypoxic bronchiolitis
573 compared to URTI or non-hypoxic bronchiolitis (103, 155, 156). Nasal CCL-3 concentrations
574 are higher in RSV-infected adults who require hospitalisation, compared to those who do not,
575 and are associated with symptom severity in experimentally infected adults (137, 138).
576 However, one study of RSV-LRTI found that increased nasal CCL-2 (MCP-1), CCL-3 and
577 CCL-4 are all positively associated with severity (119).
578
579 **Pattern Recognition Receptors (PRR)**
580
581 PRRs are involved in innate immune recognition of viral pathogens in order to stimulate
582 interferon and cytokine responses. In comparison to healthy controls or infants with
583 rhinovirus or bocavirus infection, in infants with RSV-bronchiolitis there is increased

584 pulmonary expression of TLR-7, TLR-8, RIG-1 and MDA-5 (157). RIG-1 mRNA in the
585 lungs correlated with RSV viral load (157). Furthermore, an individual's TLR4 genotype
586 influences the severity of RSV-bronchiolitis and this is significantly influenced by
587 environmental lipopolysaccharide exposure (139).
588
589 **Innate Interferons**
590
591 IFN- α is produced systemically and in the respiratory tract in response to RSV infection
592 (158). Nasopharyngeal IFN-α titres peak on day 1 of illness and remain elevated for ~6 days,
593 then decrease in parallel with nasopharyngeal RSV antigen levels (158). In peripheral blood,
594 IFN-α levels peak by day 2. Infants aged less than 3 months produce the lowest levels of
595 IFN-α in both the nasopharynx and peripheral blood (158). RSV may be a comparatively
596 weak inducer of type I IFN since nasopharyngeal IFN-α levels are higher in infants with
597 influenza virus, adenovirus and parainfluenza virus infection (158).
598
599 Type III interferons (IFN-λ) are produced in response to viral infection and have type I IFN-
600 like activities. Their receptor complex is primarily expressed on epithelial cells and IFN-λ
601 responsiveness is greatest in organs with high epithelial content such as the lungs. There is a
602 IFN-λ response to RSV-bronchiolitis, with higher nasal levels of IFN-λ 1-3 seen compared to
603 rhinovirus infection (159, 160). IFN-λ mRNA levels correlate with IFN-stimulated gene
604 expression (*MxA* and *ISG56*) (159). Despite their association with antiviral gene expression,
605 higher nasal IFN-λ-1 levels are associated with increased disease severity (159).
606
607 Immunostimulatory defective viral genomes **(**iDVGs) have been detected in the nasal fluid of
608 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 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 non-infected controls (downregulation of miR-34b, miR-34c, miR-125b, miR-29c, mir125a, miR-429 and miR-27b; 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-kappa B signaling and macrophage function (162). miR-26b (thought to target TLR4 based on miRNA target prediction software) has been studied in PBMCs from children with RSV-bronchiolitis where it is up-regulated, negatively correlating with TLR4 expression (163).

GLOBAL HOST TRANSCRIPTIONAL RESPONSE TO RSV INFECTION

Genes and pathways associated with neutrophil function, interferon signalling (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 up-regulated 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 under-expressed (164-166). Under-expression is greater in infants <6 months compared to

those aged 6-24 months (164) and may reflect either low gene expression or migration of peripheral blood immune cells to the infected tissues. In severe disease there is greater up-regulation 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, *ubiquitin D*, *tetraspanin 8*, *mucin 13* and *β-microseminoprotein* are up-regulated and *chemokine ligand 7* is down-regulated compared to 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 (Figure 1). The initial development of cough, wheezing and tachypnoea, usually peaking on days 4-5, 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 respiratory symptoms over a period of 2-3 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 hospitalisation.

We know that pre-existing 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 leucocyte counts and IL-8 levels compared to 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 FEV_1 and FVC measurements on pulmonary function testing (171). Evidence for a causal relationship comes from an intervention trial in premature infants (gestational age 33-35 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 is little 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 VEGF, G-CSF, and IL-10 persists after the RSV episode, all mediators that have been related to asthma and post-virus induced wheeze (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 RESPONSE TO RSV BETWEEN 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 cell counts) but appears to have a less dominant role in mice (15-20% 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 earlier, 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 (Figure 2).

Large quantities of pro-inflammatory 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 pro-inflammatory and T-cell derived cytokine concentrations suggesting 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 co-inhibitory molecule PD-1 and mTOR-mediated suppression of memory CD8+ T-cell differentiation, suggesting T-cell interference is a key viral immune evasion strategy (Figure 1). 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 re-infection 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 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 up-regulation 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, sICAM-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 titres 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 characterised immune processes, especially occurring in the lower airways and lung, require further investigation.

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AUTHOR BIOGRAPHIES

Dr. Clark D. Russell

Clark is a medical trainee in the Regional Infectious Diseases Unit in Edinburgh, Scotland and an honorary clinical fellow of the University of Edinburgh.

Clark's undergraduate medical training was in Edinburgh with an elective in Vancouver in infectious diseases and microbiology. He graduated from the University of Edinburgh with a BMedSci in Infectious Diseases in 2010 then MBChB with honours in 2013 and completed the MRCP (UK) diploma in 2016. He is interested in academic infection medicine and his research experience includes molecular diagnostics, bacterial pathogenesis, host genetics and descriptive clinical studies.

Dr. Stefan Unger

Dr. Stefan Unger is a clinical lecturer at the Department of Child Life and Health at the University of Edinburgh and a pediatrician specialised in respiratory and sleep medicine.

Originally from Germany Dr. Unger qualified in medicine at the University of Edinburgh and trained in paediatrics in Scotland. Dr. Unger conducted an RCT of nutritional supplements in acutely unwell children in rural West Africa during his Medical Research Council (MRC) Career Development Fellowship studying the effect on infectious disease presentations with a focus on respiratory disease. After completion of his PhD with the London School of Hygiene and Tropical Medicine (LSHTM) he specialised in pediatric respiratory medicine with an interest in improving clinical management of bronchiolitis. As a clinical lecturer at the University of Edinburgh his research focuses on the relationship between under-nutrition and immune modulation in lower respiratory tract infections in infancy and subsequent respiratory health in high- and low-income settings.

Mr. Marc Walton

Marc Walton is an undergraduate student at the University of Edinburgh who recently completed the second year of his medical degree (MBChB). He is currently undertaking a one year 'intercalated' degree in neuroscience (Honours) after which he will return to clinical training to complete his medical degree. Marc started working on this systematic review whilst undertaking a period of laboratory work in Professor Jürgen Schwarze's group. His

main research interests lie in neuroscience and paediatrics and he is currently involved in projects relating to the use of outcome measures in intellectual disability; the design of neural implants; and the neuropathology of Alzheimer's disease.

Prof. Jürgen Schwarze

Dr Jürgen Schwarze is the Edward Clark Chair of Child Life and Health at the University of Edinburgh. He is an internationally recognised expert in immune mechanisms of RSV-bronchiolitis and associated airway allergy and a paediatrician specialised in allergy and respiratory medicine.

After qualifying in medicine from Freiburg University, Germany, and training in paediatrics, Dr Schwarze started to work on immune responses in RSV-bronchiolitis and allergic airway disease as a post-doctoral fellow at National Jewish Medical and Research Centre in Denver, Colorado. He then continued his research in this field at Ruhr-University Bochum (Germany) and as a Wellcome Trust Senior Clinical Fellow at Imperial College London. In 2007 he moved to the MRC-Centre for Inflammation Research at the University of Edinburgh. Dr Schwarze's research focuses on the interface between innate (lung epithelial cells, dendritic cells) and adaptive immunity in RSV-infection and subsequent reactive airway disease.

FIGURE LEGENDS

Figure 1: 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 (A) inducing apoptosis (CD4+ and CD8+ T-cells), (B) inducing increased expression of the programmed cell death 1 (PD-1) protein which is inhibitory to activated T-cells (CD8+ T-cells) and (C) promoting activation of the mammalian target of rapamycin (mTOR) pathway, thus preventing memory CD8+ T-cell formation.

Figure 2: Summary of the human immune response to RSV and potential novel therapeutic targets

The role of major cell types (neutrophils, dendritic cells, macrophages, CD8+ T-cells and B-cells) is summarised, 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 protective role of CD8+ T-cell mediated viral clearance is emphasised. Finally, we highlight areas where novel therapeutic interventions could potentially modulate the immune response in favour of the host.

↑ indicates immune cell recruitment to the respiratory tract

*associated with increased disease severity

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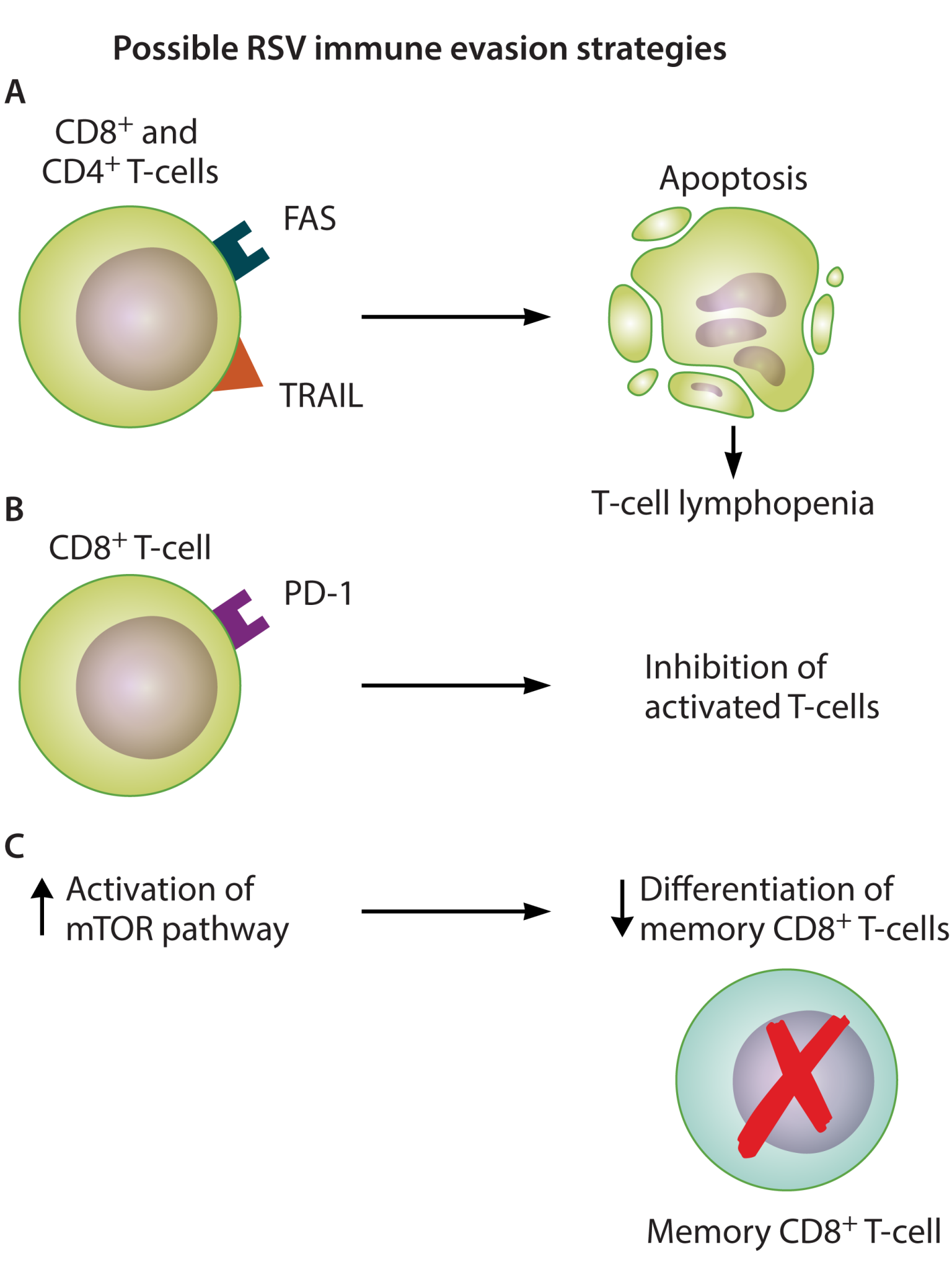
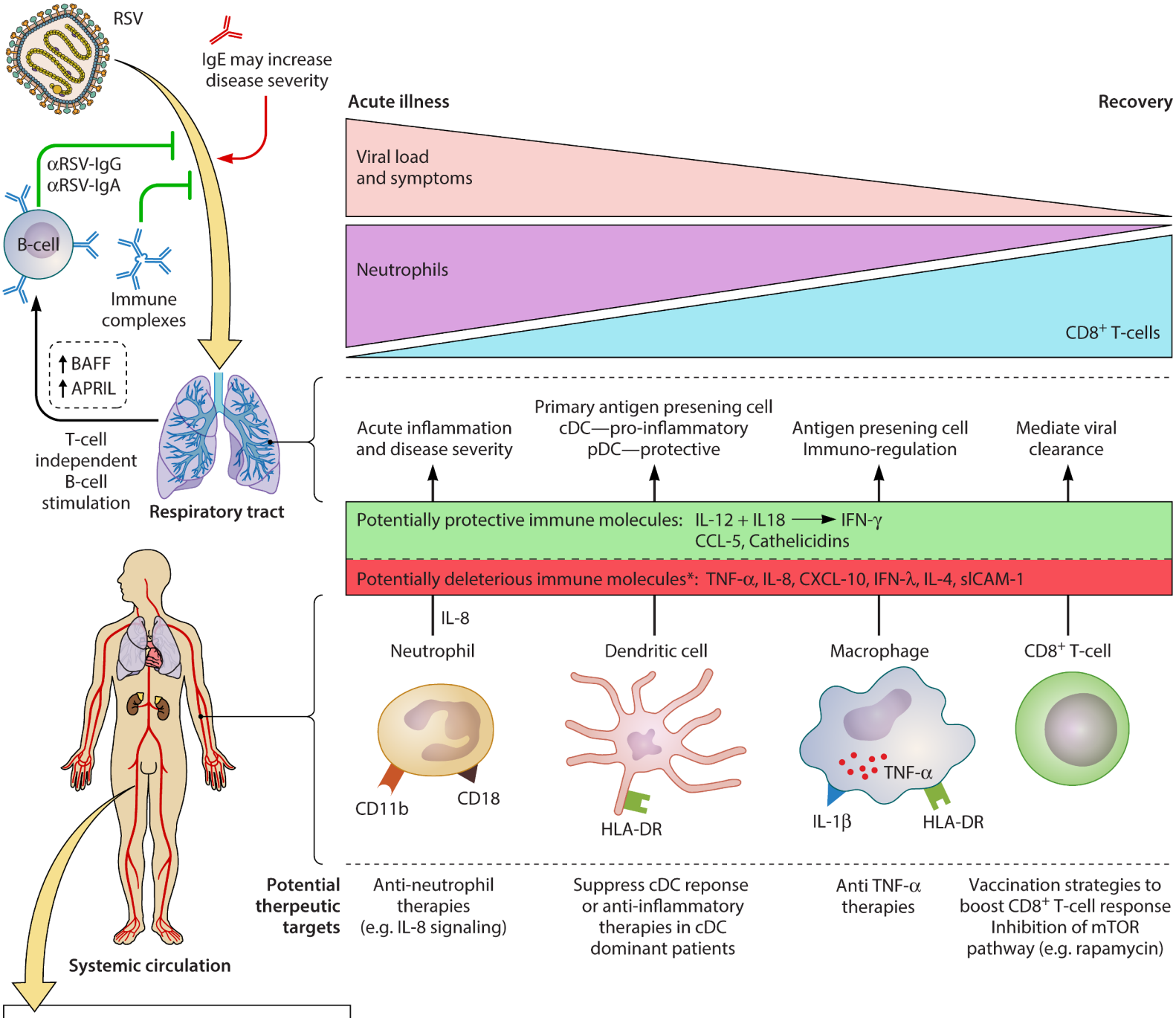
Table 1: Chemokines, cytokines and other immune molecules involved in the human immune response to RSV infection

Immune molecule	Respiratory tract[a]		Systemic	Additional comments	Ref[b]
	Nasal mucosa	Lung			
Th1 cytokines					
IFN-γ	+	+	+	Protective	
IL-12	+	+	+	Protective	
IL-1α & IL-1β	+		+	Deleterious	
IL-2	+	+	+	No association reported 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 association reported with severity	
IL-10	+	+	+	Variable association with severity: see text	
IL-13	+	+	+	No association reported with severity	
Other cytokines					
IL-8	+	+	+	Deleterious: neutrophil chemoattractant	
IL-17A	+	+		Variable association with severity: see text	
IL-33	+			No association reported 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 association reported with severity	158
G-CSF	+		+	Circulating G-CSF levels are higher in infants with RSV-LRTI requiring ventilation	18, 105
Soluble ICAM-1	+		+	sICAM-1 levels in nasal fluid positively correlate with severity	40, 124, 126
Substance P	+	+		Lower concentrations associated with increased severity	106
MBL			–	No association reported with severity	44
Cathelicidin LL-37	+			Protective: in human experimental infection, higher constitutive nasal cathelicidin LL-37 is associated with reduced development of infection	177
Olfactomedin 4			+	Greater PBMC *Olfactomedin 4* gene expression was associated with need for ventilation in RSV-LRTI	19
Surfactant A, B, D		–	+	The pulmonary level of surfactant A and measurable surfactant activity increases 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 KL-6 is greater in infants with RSV-LRTI requiring ventilation	185
sTRAIL		+		No association reported with severity	186

Key. +: increased production; **–**: reduced production

[a]'Nasal mucosa' refers to measurements made in nasal fluid or nasopharyngeal aspirate; 'Lung' refers to measurements made in bronchoalveolar lavage or tracheal aspirate.
[b]References are provided for molecules not discussed in detail in the main text.
Abbreviations used in table. 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: tumour necrosis factor.


Possible RSV immune evasion strategies
A
CD8+ and
CD4+ T-cells
FAS
TRAIL
Apoptosis
T-cell lymphopenia
B
CD8+ T-cell
PD-1
Inhibition of
activated T-cells
C
Activation of
mTOR pathway
Differentiation of
memory CD8+ T-cells
Memory CD8+ T-cell



Global transcriptional response
(peripheral blood)

Regulation	Pathways
↑	Neutrophil functioning Type 1 IFN signalling IFN-stimulated genes Dendritic cell maturation
↓	NK cell functioning B-cell and T-cell responses HLA-I and HLA-II