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Disease Severity in Respiratory Syncytial Virus Infection: Role of Viral and Host Factors

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Abstract

Respiratory syncytial virus (RSV) is not only a major cause of severe lower respiratory tract infection (LRTI) in infancy but is increasingly recognised as an important pathogen in later life. RSV infection is associated with a wide spectrum of disease ranging from asymptomatic infection to life-threatening bronchiolitis and pneumonia. Research has demonstrated that there exists a complex interplay between viral and host factors that determines the severity of disease following RSV infection. Several factors determine RSV virulence including the infective properties of individual strains and viral load (VL). Disease outcome from RSV infection is also impacted considerably by a variety of host factors with the host immune response increasingly recognised as pivotal. This chapter outlines our current understanding of these factors and provides an oversight of their relative importance.

Keywords: respiratory syncytial virus, disease severity, viral load, immunology, genotype

1. Introduction

Respiratory syncytial virus (RSV) has long been recognised as a cause of severe lower respiratory tract infection (LRTI) in early childhood with increasing evidence of its role as an important pathogen in later life [1]. RSV has many intriguing features including its worldwide distribution, its capacity to cause severe disease in early childhood and its extended impact on respiratory health [2]. Consequently, RSV has been the focus of comprehensive study including host and viral determinants of disease severity.

Serologic data has demonstrated that a high proportion of children (between 50 and 70%) will be infected with RSV in the first year of life [3, 4]. Asymptomatic infection is infrequent during infancy with most infants developing clinical features of an upper respiratory tract infection alone [5]. Following an initial prodromal URTI phase, 25–40% of those infected will progress to develop signs and symptoms of bronchiolitis with tachypnoea and chest recession [5]. Bronchiolitis is usually a mild illness in most infants, but a small proportion (2–3%) will develop severe bronchiolitis necessitating hospitalisation [6]. It has been estimated that nearly 33.8 million new cases of RSV-associated LRTI occur worldwide in children

under 5 years of age leading to approximately 3.4 million hospitalizations annually [7]. Mortality from RSV infection in developed countries is rare during infancy although there are an estimated 66,000 and 239,000 yearly deaths in children younger than 5 in the developing world [7, 8].

The major clinical manifestation of RSV in older children and adults is upper respiratory tract illness (rhinitis and acute otitis media) although symptoms tend to last longer and there is an increased incidence of cough and wheeze compared to other respiratory viral infections [9, 10]. Immunity following RSV infection is only effective for a matter of months before the individual is once again susceptible to reinfection [11]. Consequently, reinfection occurs throughout life. LRTI is unusual although RSV accounts for a significant percentage (>4%) of community-acquired pneumonias during epidemics [12]. Elderly adults have an increased risk of lower respiratory tract involvement, with 30–40% of patients having auscultatory findings on examination of the chest [13].

RSV is evidently associated with a wide spectrum of disease which has led to significant interest into those factors that determine the nature of the clinical response to infection. Host, viral and geographical factors interact to dictate the clinical outcome of any viral infection. The viral and host factors that influence the human response to RSV infection are the focus of this chapter.

2. Viral factors

2.1 The virus

Respiratory syncytial virus is a medium-sized (120–300 nm), enveloped, single-stranded RNA virus of the *Paramyxoviridae* family that is a ubiquitous pathogen in all age groups. In 1956, it was first isolated from upper respiratory tract specimens collected from a chimpanzee with coryza [14], being subsequently recovered from two children in Baltimore with lower respiratory tract disease [15]. Its identification as a principal cause of bronchiolitis took a further 4 years [16].

2.2 Viral genome and proteins

RSV has a negative polarity RNA genome composed of 15,000 nucleotides with an estimated weight of 500 kDa. The non-segmented RNA genome encodes 10 units from which 11 proteins are translated. The RSV nucleocapsid core consists of the viral genomic RNA wrapped with N protein (called the N-RNA template), the major viral phosphoprotein, encoded by the P gene and the major subunit of the RNA-dependent RNA polymerase, encoded by the L gene. The genome also encodes for two matrix proteins. The first matrix protein, M, is essential for RSV replication and passaging and plays a central role in virus assembly. The second matrix protein, M2, is localised within the nucleocapsid and has an essential role in the production of complete mRNA during replication. The NS1 and NS2 gene sequences encode for the so-called nonstructural proteins which have been shown to have multiple functions including abrogation of the cellular antiviral response and induction of interferon (IFN) transcription.

Three surface proteins are also encoded within the RSV genome, the fusion (F) protein, the G attachment (G) protein and the small hydrophobic (SH) protein. The F protein plays a central role in virus entry. It mediates fusion between the viral and cellular membrane, thereby allowing the nucleocapsid to enter the cytoplasm of the host cell. The G protein is essential for initial RSV attachment and interaction with the host cell and is important for in vivo replication. The SH protein is a short

integral membrane protein that is not essential for RSV replication in cell culture but is involved to some degree in RSV survival in vivo.

2.3 Viral load

The estimation of viral load (VL) has proved invaluable for predicting disease outcome in viral infections such as hepatitis C [17] and HIV [18]. The relationship between the quantity of RSV in respiratory tract specimens and disease severity has been the focus of multiple studies over the last 30 years. There has been significant variability in study findings to date, with some identifying a positive correlation between viral load and severity [19–21], while others have found no significant correlation [22, 23] or an inverse relationship [24]. These disparate outcomes are likely related to the variable nature of the studies with significant differences in age range, enrolment criteria, quantification techniques (plaque assay vs. PCR) and timing of sample collection. The majority of studies have evaluated VL in hospitalised children only and at a single time point.

A number of recent studies have investigated the dynamics of viral load through the analysis of sequential specimens collected during the course of the illness. Garcia-Maurino et al. studied 150 children <2 years of age (39 outpatients and 111 inpatients) over 2 successive winters [25]. Children who required hospitalisation had significantly lower VLs assessed using quantitative PCR on nasal swabs. Sequential VL evaluation (only evaluated in those hospitalised) demonstrated higher initial VLs and a faster VL decline in those requiring ward care only compared to those requiring PICU care. These findings are consistent with the results from a smaller study of 33 infants hospitalised with RSV bronchiolitis using nasosorption sampling of the upper airway [26]. Faster viral clearance was also associated with milder disease and a shorter length of stay in 219 children in Boston with RSV infection whose upper airway VL was assessed using plaque assay [27]. However, by contrast, higher VLs on day 3 of admission were associated with increased disease severity requiring PICU admission.

Studies to date highlight the complexity of the interaction between VL and disease severity. Further studies are required to clarify this relationship and should ideally analyse sequential specimens for changes in viral load in a tightly defined cohort of children and include both hospitalised and community infants with mild disease.

2.4 Genetic and antigenic variability

Through the application of monoclonal antibody technology, two major antigenic groups of RSV have been identified—the A and B subgroups [28]. Epidemiological studies have revealed that these groups have existed for over 40 years and have a worldwide distribution [28]. Both groups appear to circulate concurrently with geographical and temporal clustering frequently reported [29, 30]. The prevalence of each subgroup follows an irregular, alternating pattern with subgroup A predominating in most analyses [29]. The link between the major antigenic groups of RSV and disease severity is unclear. RSV-A has been associated with a more severe disease course in a number of studies [31–33], while others have identified no difference between the subgroups [34–37] or increased severity due to infection with RSV-B [38, 39]. While these inconsistent findings could result from differences in study design and geography, the presence of varying RSV genotypes is likely to be a significant confounding factor.

The RSV genome demonstrates significantly more variability than previously understood [1].

The G protein has been demonstrated as the greatest source of intragroup variability with nucleotide differences of up to 20% [40]. These differences reside predominantly within two variable regions that flank a relatively conserved central ectodomain of the G protein gene.

The single-stranded, non-segmented nature of the RSV genome precludes the genetic rearrangements that typify the dramatic antigenic shifts of the influenza virus [41]. However, there is a considerable potential for genomic mutation given the inability of RNA polymerase to proof-read during replication of the genome. This provides an opportunity for antigenic drift to occur under the influence of selective pressures from the environment. The current variability of the G protein between RSV strains may therefore be explained by the progressive accumulation of change together with survival and extinction of particular genotypes [42].

Prior to whole-genome sequencing, genetic studies have assessed strain variability within the two major groups primarily through analysis of the G protein gene [42, 43]. Subsequent phylogenetic analyses have identified multiple lineages within both group A and group B viruses with marked similarities observed between strains from different locations and time periods [29, 42, 44]. This has led to the recent subclassification of RSV into 14 RSV-A genotypes and 24 RSV-B genotypes [45]. Several distinct RSV strains appear to cocirculate within an individual community during each annual epidemic with the predominant strain varying year to year [44, 46]. Multiple elements appear to determine prevailing annual strains including herd and maternal immunity, changes in social contacts and migration as well as viral factors [29, 47–49].

RSV's capacity for genomic mutation is exemplified by the emergence of two novel genotypes, RSV-B BA and RSV-A ON1 genotypes. In 1999, the BA genotype was first detected in Buenos Aires, Argentina [50], and has since spread gradually and sequentially throughout the world [51], becoming the predominant group B genotype, and even replacing all previous circulating RSV-B genotypes in some regions [52, 53]. Subsequently, the RSV-A genotype ON1 was identified in Ontario, Canada, in 2010 [54] and has rapidly spread worldwide [55]. Both genotypes demonstrate a 60–72 base pair duplication in the G protein gene which may change the antigenic properties of the protein enabling evasion of the host immune response [50, 56].

The possibility that distinct RSV genotypes may have differing virulence has led to several studies in this area. Much interest has concerned the possible association of the ON1 genotype with less severe disease. Panayiotou et al. first described this association in 99 children <2 years of age hospitalised with a RSV respiratory tract infection over 3 successive Cypriot winters [39]. The ON1 genotype was associated with significantly milder illness (as determined by a clinical severity score) than either GA1 (RSV-A) or BA (RSV-B) genotypes. This finding has also been observed in a number of subsequent studies including a recent study of 329 infants with a clinical diagnosis of bronchiolitis and infected with RSV alone [57, 58]. Infants with the NA1 genotype had a milder clinical course both in terms of clinical severity scores and need for admission to PICU. Conversely, other studies have reported a similar clinical course with different genotypes [36, 59] or that genotype ON1 was associated with more severe disease in a group of Vietnamese children hospitalised with signs of LRTI [60].

Once again, the lack of a consistent association between genotype and disease severity is presumably due to variable study design and geographic factors. However, other factors should be considered. All studies to date have encompassed multiple years to enable analysis of different viral strains as the dominant genotype varies year to year. This inherently introduces a significant confounder with changes between years in staff, clinical practice, herd immunity, etc. In addition, there is increasing evidence of virulence factors encoded in regions of the genome outside

the G protein hypervariable region that has been traditionally used for genotyping. Studies in the mouse model have demonstrated that specific sequences within the conserved central domain of the G protein influence RSV binding to the chemokine receptor CX3CR1 impacting host response [61] and sequences contained in the F protein are associated with significant differences in disease severity [62].

The possibility of different RSV genotypes having distinct infective properties is an attractive explanation for the diverse severity of RSV disease which merits further study. It is apparent that the interaction between RSV strain and disease is complex. Whole-genome sequencing perhaps provides the best future opportunity to further our understanding [1].

3. Host factors

3.1 Predisposing health factors

Pre-existing illness and disease have been long recognised as having a significant impact on the severity of RSV disease. RSV infection in children with congenital immunodeficiency (particularly of cell-mediated immunity) is associated with a more severe clinical course [63], and individuals requiring immunosuppressive therapy (such as haemopoietic cell transplant (HCT) recipients and patients undergoing chemotherapy) also experience more severe disease. Following HCT, RSV infection is associated with significant morbidity, including complications such as late graft dysfunction and bronchiolitis obliterans syndrome. Mortality rates range from 7 to 83% depending on a number of risk factors including lymphopenia and high-dose total body irradiation [64–66]. Adult patients with leukaemia are particularly vulnerable to RSV infection with reported mortality rates between 20 and 85% in those developing pneumonia [67] although children with leukaemia appear to follow a more benign clinical course [68].

Pre-existing cardiopulmonary disease has a significant impact on the clinical response to RSV infection. Underlying congenital heart disease in infants and children has long been recognised to be associated with more severe outcomes from RSV, including more frequent hospitalisation, longer lengths of stay and higher rates of intensive care unit admission [69]. Similarly, children with bronchopulmonary disease have markedly increased morbidity and mortality due to RSV [70]. Adults with underlying cardiac and pulmonary diseases are also prone to severe respiratory illnesses with RSV infection [71]. Adults with chronic obstructive pulmonary disease are particularly vulnerable to RSV infection with a high incidence of lower respiratory tract disease and frequent hospitalisation [71].

The impact of RSV at the extremes of age is significant. Premature birth is a significant risk factor for severe RSV disease; those born at less than 32 weeks of gestational age have approximately double the hospitalisation risk of infants born later [72]. Incomplete immunological and pulmonary maturation and reduced transfer of maternal antibodies are all thought to contribute to this increased risk. The burden in the elderly is also significant with evidence that the impact is similar to that of non-pandemic influenza, both in the community and in nursing homes [73]. RSV outbreaks in nursing homes have been well documented, studies reporting infection rates of over 10% with pneumonia in up to 55% of those affected and mortality rates of up to 20% [73]. A recent prospective, international study detected RSV in over 7% of moderate to severe acute respiratory episodes observed in elderly adults living at home and found that the incidence of RSV infection increased with age [13].

3.2 Predisposing airway geometry

The potential impact of reduced premorbid lung function on the response to a subsequent RSV infection has been of interest for many years. Two initial studies published in 1995 demonstrated that a reduced maximum expiratory flow at functional residual capacity (V_{\max} FRC—believed to reflect the size of intrapulmonary airways) measured in infancy before any lower respiratory tract illness was associated with subsequent virus-associated wheeze [74, 75]. Subsequently, a large prospective study of 2133 infants who had neonatal lung function performed found that those subsequently hospitalised with RSV infection had significantly decreased lung function compared to those with RSV infection managed in the community [76]. These data together would suggest that congenitally smaller airways may predispose infants to more severe RSV disease.

3.3 Host immune factors

The immune response to infection can be divided into two arms:

1. Innate immunity—this refers to nonspecific defence mechanisms that activate immediately or within hours of exposure to an infecting organism. The efficacy of these mechanisms does not rely on clonal expansion.
2. Adaptive immunity—the adaptive immune response relies on the clonal expansion of antigen-specific lymphocytes and can take several days to complete. A principal feature of the adaptive immune response is the generation of immunological memory enabling a more rapid and effective response to pathogens that have been previously encountered.

3.3.1 Innate immunity

The innate immune response to RSV infection consists of a coordinated response incorporating a variety of cell types and their products. The character of this response is a critical determinant of the outcome of infection. A slow, weak or inappropriate response will result in delayed viral clearance enabling the virus to spread to the lower airway producing enhanced pathology. An inappropriate innate response will also have a direct impact on the nature and efficacy of the adaptive immune response.

The importance of innate immunity is highlighted by genetic association studies which have identified the strongest associations with severe disease due to polymorphisms of the innate immune system at both the allele and genotype level [77, 78].

3.3.1.1 Toll-like receptors

Type I interferons (IFN-I, α/β) are an important part of the innate immune response to viral infection. Various cell intrinsic pattern recognition receptors (PRRs) including the Toll-like receptors (TLR) recognise RSV as foreign and potentially dangerous. This leads to the activation of key transcription factors including interferon regulatory factors which regulate the expression of IFN-I and pro-inflammatory cytokines [79]. IFN-I upregulates transcription of multiple interferon-stimulated genes (ISGs) via the IFN-I receptor, which interfere with viral replication, thus facilitating viral clearance.

TLR polymorphisms have been widely studied for their potential association with severe RSV disease. Tal et al. identified an overrepresentation of a

heterozygous genotype in two TLR4 mutations (Asp299Gly and Thr399Ile) among a group of infants necessitating hospitalisation compared to a community-managed group [80]. Similarly, the same mutations were significantly associated with hospitalisation in a group of primarily premature infants with symptomatic RSV infection [81]. However, subsequent studies have failed to confirm these findings [82, 83] although one study has identified significant variability between different RSV epidemics in the genetic risk of severe disease associated with these polymorphisms [83]. This highlights the complex interplay between host genetic factors and the predominant circulating viral strain.

3.3.1.2 Surfactant proteins

The surfactant proteins (SPs) primarily reduce surface tension of the alveoli to prevent lung collapse but also make a significant contribution to innate immunity. In vitro, SP-A enhances uptake of RSV by macrophages, reduces RSV-induced suppression of host cell tumour necrosis factor- α (TNF- α) and augments the production of anti-inflammatory interleukin-10 (IL-10) [84]. SP-D-deficient mice develop severe disease on exposure to RSV and have delayed viral clearance [85]. Studies on ventilated infants with RSV infection have demonstrated low surfactant activity and reduced levels of SP-A, SP-B and SP-D compared to controls [86, 87]. In addition, genetic analyses have identified certain polymorphisms of the surfactant proteins that are associated with severe disease in RSV-infected infants [88, 89].

3.3.1.3 Inflammatory mediators

The initial contact between RSV and the host is typically at the nasal epithelium. The epithelial surfaces form a physical barrier that is impermeable to most infectious agents, acting as the first line of defence against invading organisms. In response to contact with RSV, the epithelium also releases a variety of pro-inflammatory mediators and cytokines that play an important part in the innate immune response. A number of in vitro studies have demonstrated the production of IL-1; IL-6; IL-8; tumour necrosis factor- α ; regulated upon activation, normal T-cell expressed and presumably secreted (RANTES); and CXCL8 by RSV-infected primary airway epithelial cell cultures [90–92]. Macrophages and neutrophils are important phagocytes but also produce a wide range of immunological mediators, including MIP-1 α , RANTES, IL-1, IL-6 and IL-8, when infected with RSV in vitro [93, 94]. Such mediators have a significant impact on both early inflammation as well as subsequent immunological responses. For example, IL-8 is chemotactic for neutrophils as well as being a potent activator. Similarly, MIP-1 α and RANTES activate both monocytes and eosinophils as well as being potent CD4⁺ T-cell chemoattractants.

Respiratory secretions from infants and children infected with RSV have been found to contain many of these mediators [38, 95–97]. Some studies have also identified correlates between certain inflammatory mediator concentrations and disease severity. Higher levels of IL-8 have been identified in severe disease with a strong correlation with oxygen requirement and need for ventilation [96, 98]. Nasal concentrations of MIP-1 α are significantly increased in children with RSV bronchiolitis that require oxygen therapy [99] and in RSV-infected adults that require hospitalisation [100].

There has been much interest in the contribution of RANTES, a potent chemoattractant for eosinophils, T cells and monocytes, to the pathogenesis of RSV disease. RANTES levels in tracheal aspirates have been found to correlate inversely with markers of disease severity in RSV bronchiolitis [95], and the ratio of TNF-receptor to RANTES in nasopharyngeal aspirates is significantly

raised in infants with severe RSV disease [101]. The potential protective impact of RANTES has been further explored through analysis of promoter and intronic polymorphisms in the RANTES gene. Certain polymorphisms (e.g., -403 G/A) have been associated with reduced disease severity and greater production of RANTES [102, 103].

3.3.1.4 Eosinophils

Eosinophils have been traditionally regarded as important in the innate immune response to helminthic infection although several studies have suggested their possible contribution to RSV immunity. Eosinophil cationic protein (ECP), a cytotoxic protein released with eosinophil activation, is found in nasal lavage fluid and serum from children with severe RSV infection [104]. ECP levels in nasopharyngeal secretions have been found to correlate with disease severity [105, 106] suggesting a role for the eosinophil in the pathogenesis of RSV disease.

3.3.2 Adaptive immunity

3.3.2.1 Humoral immunity

The developing foetus acquires maternal IgG antibodies to RSV through the placenta actively. The transfer rate gradually increases from 30 weeks of gestation to a maximum between 38 and 40 weeks. Following birth, levels of neutralising antibody slowly diminish with a mean half-life of 26 days [107]. The quantity of antibody transferred is important in protecting infants against infection; higher specific antibody titres are associated with milder disease following RSV infection [108]. Infants also acquire antibodies from the mother through breast feeding with breast fed infants having significantly reduced oxygen requirement and length of stay following hospitalisation with RSV bronchiolitis [109].

There is considerable variability in the humoral response of infants to primary infection. During the first year of life, there is rapid maturation of the immune system, and this may account for much of this variability. In the first 6 months of life, the serum IgA response is greater than that of IgG [110]. In older children, there is a predominant rise in IgG levels with IgG1 and IgG3 subclasses accounting for the major part of this response [110], whereas IgG1 and IgG2 subclasses form the bulk of the adult response [111]. As well as these qualitative differences in antibody response with age, there is also quantitative variation with generally very low titres of antibody produced in early infancy. The antibody titres against both the F and G surface proteins of RSV in infancy are 10 to 12 times lower than those observed in children over 12 months of age [112]. This blunted response appears to be caused by a combination of factors including the presence of residual maternal antibody and immaturity of the immune system [113].

Murine studies have demonstrated that the humoral response is an important factor in RSV disease severity. B-cell-depleted mice, which consequently have no antibody response to infection, experience greater illness on primary RSV infection [114]. Similarly, higher neutralising antibody titres in children are associated with a lower incidence of severe lower respiratory tract disease once infected with RSV. Clinical severity scores inversely correlate with antibody titres to the F protein in infants with RSV infection [115], and therapeutic trials of polyclonal and monoclonal antibodies have demonstrated reduced disease severity in treated infants [116, 117].

3.3.2.2 Cell-mediated immunity

The cellular arm of the adaptive immune response is orchestrated primarily by cytotoxic T lymphocytes (CTLs) and T-helper (Th) lymphocytes. Cell-mediated immunity is extremely important for the eradication and abrogation of the clinical response to RSV infection as highlighted by the severe impact of infection in individuals with severe congenital/acquired impairment of cellular immunity [63, 118].

3.3.2.2.1 Cytotoxic T lymphocytes ($CD8^+$ T cells)

CTLs are pivotal for the control of many intracellular pathogens and have the capacity to differentiate into long-lived memory CTLs which provide a rapid, robust response to subsequent infection. CTLs have been found to have an important role in clearing RSV during animal studies. Mice that have been rendered athymic are unable to produce a CTL response to RSV infection and become chronically infected. Clearance of the virus can be subsequently achieved by injecting the mice with primed RSV-specific CTLs [119]. There is also some evidence that CTLs can contribute to severe disease if produced in large numbers [120].

Unfortunately, the role of CTLs in human RSV infection has been little studied largely due to the relatively modest RSV-specific T-cell responses observed in the blood and the low numbers of RSV-specific T-cell memory cells present between infections. Using HLA tetrameric staining, RSV-specific $CD8^+$ T cells were analysed in bronchoalveolar lavage fluid and blood specimens from infants requiring ventilatory support for RSV bronchiolitis. RSV-specific T-cell numbers peaked in blood around days 9–12 (at the time of recovery), and there was no correlation between cell numbers and parameters of disease severity [121]. A similar pattern is observed in experimental infection of adult volunteers where antigen-specific $CD8^+$ T-cell numbers peak 10 days after initial infection at approximately the same time as a fall in viral load and resolution of symptoms [122]. This would perhaps suggest an important contribution to viral clearance although the impact of CTLs on disease severity in man remains undefined.

3.3.2.2.2 T-helper lymphocytes ($CD4^+$ T cells)

$CD4^+$ T cells recognise antigens presented on MHC class II molecules, which are found on antigen-presenting cells. They respond by releasing cytokines which play a major role in instigating and shaping adaptive immune responses. The cytokines produced have been used to differentiate these cells into the two major classes of effector T cell—T helper (Th) 1 (producing type 1 cytokines) and Th2 (producing type 2 cytokines) [123]. In general, type 1 cytokines (such as interferon-gamma ($IFN-\gamma$) and IL-12) favour the development of a strong cellular immune response and are an important component of an effective response to intracellular pathogens including viruses. Type 2 cytokines (such as IL-4 and IL-5) favour a strong humoral response to infection by promoting B-cell proliferation and increased production of antibodies. Type 2 cytokines also mediate allergic responses. Cross regulation occurs between the two responses, and responses deviated toward type 2 or away from type 1 are associated with more severe disease in several infectious disease model [124, 125].

Indirect evidence suggests that T-helper lymphocytes have a significant role in RSV disease with $CD4^+$ T cells constituting the largest lymphocyte population in bronchoalveolar lavage fluid obtained from infants ventilated for RSV bronchiolitis [126]. Subsequent studies have focussed on the exact nature of this $CD4^+$ T-cell response.

Murine studies have demonstrated that prior sensitisation to RSV surface proteins followed 3 weeks later by RSV infection can induce polarised cytokine responses which follow broad type 1 and type 2 repertoires [127]. Type 2 responses were associated with enhanced disease characterised by pulmonary haemorrhage and eosinophilia while those with type 1 responses had reduced immunopathology and enhanced viral clearance [128]. A type 2 cytokine response also correlates with quantitated pulmonary pathology following primary RSV infection of BALB/c mice [129].

Multiple human studies have examined type 1 and type 2 immune responses to RSV and have largely identified a similar predominance of type 2 cytokines in those with more severe disease. Type 1 cytokines (including IFN- γ and TNF- α) are increased in the circulation, the nose and lung during an RSV LRTI [96, 130, 131]. Lower IFN- γ levels are associated with increased severity scores, hypoxia and need for ventilation [97, 131–134]. Type 2 cytokines (including IL-4, IL-10 and IL-13) are also increased in the blood, nose and lung during RSV LRTI [95, 132, 135]. However, higher type 2 cytokine levels are generally associated with more severe disease. Systemic IL-10 levels correlate with disease severity in RSV LRTI [136, 137] although respiratory tract IL-10 levels would appear to be associated with milder disease features [95].

Multiple studies have also evaluated the type 1/type 2 immune balance through the quantification of cytokine ratio. The ratio of IL-4 to IFN- γ is raised both systemically and in the respiratory tract in patients with bronchiolitis that require oxygen therapy suggesting a skew towards type 2 responses [138, 139]. Similarly, IL-4:IFN- γ and IL-10:IL-12 ratios are raised in nasal lavage obtained from infants with RSV who develop bronchiolitis compared to those that develop signs of an URTI only [23], and type 2 cytokines predominate in nasopharyngeal specimens from children with hypoxic RSV LRTI [96].

Overall, current evidence would support a bias towards a type 2 T-helper cell response in severe RSV infection. Further studies are, however, required to confirm these findings and would ideally analyse prospectively the cytokine response both systemically and within the airway. Such studies should, also, examine both children and adults with RSV infection using relevant study groups (URTI, LRTI) and strictly control for potential confounding factors such as sampling times (relative to infection onset) and age differences between groups.

3.3.2.2.3 Regulatory T cells

Regulatory T cells (Tregs) are immunoregulating cells that maintain the immunological equilibrium by controlling effector T-cell activation and hence preventing tissue damage during the immune response to infections [140]. Animal studies in Treg-depleted mice have highlighted their importance. RSV infection of Treg-depleted mice results in severe disease with a significantly elevated viral load and an exuberant cytotoxic T-cell response highlighting the importance of Tregs for both viral clearance and control of the RSV-specific T-cell response [141]. A recent study of infants with severe bronchiolitis demonstrated a prolonged reduction in Tregs in these patients compared to a similarly aged, healthy control group [142]. While intriguing, further studies are required to confirm this finding and to clarify the role of Tregs in severity of RSV disease.

4. Conclusion

The broad spectrum of disease due to RSV infection likely represents a complex interplay between multiple viral and host factors. Despite significant research in

this area, our understanding of the relative importance of each factor is limited although recent studies that have examined multiple components of the virus-host interaction have provided some insight. Individual viral strains have differing infective properties which primarily determine RSV virulence alongside other factors such as viral load. The host immune response is increasingly recognised as an important determinant of disease severity. Other host factors including underlying immunological/cardiopulmonary disease and small airway geometry in infancy also appear to have an important impact.

Each year 2–3% of all infants require hospitalisation due to RSV outbreaks [6]. These epidemics result in substantial healthcare costs with direct medical costs for children <5 years old amounting to over \$600 million in the United States alone [143]. Despite the substantial impact of RSV, advances in effective prevention and treatment have been slow. Future studies that consider fully the contribution of both viral and host factors in the pathogenesis of severe disease will undoubtedly facilitate the development of effective therapies.

Conflicts of interest

Dr. Legg has no potential conflicts of interest.

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