# The pathogenesis of respiratory syncytial virus disease in childhood

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Respiratory syncytial virus (RSV) is a leading cause of severe respiratory infection in infants and children. RSV is an RNA virus whose genome encodes 10 proteins. The G protein is responsible for viral attachment to cells whilst the F protein promotes syncytia formation. These proteins are also important in the immune response to RSV. Both the innate and adaptive arms of the cellular immune system are involved in the immunological response to RSV. The cytopathic effects of the virus explain many of the pathological findings in RSV disease. However, there is compelling evidence to suggest that the host cell immune response also has a prominent role in disease pathogenesis. Non-immunological factors may also be important.

Respiratory syncytial virus (RSV) is the single most important cause of viral lower respiratory tract infection during infancy and early childhood world-wide.

Despite its discovery in 1955, the burden associated with RSV in the paediatric population is only now becoming fully appreciated. In the US alone, RSV infection is responsible for the hospitalisation of an estimated 50,000–80,000 infants each year[1], the deaths of approximately 500 infants each year[2], and costs $365–$585 million per annum[3]. The majority of admissions to hospital are due to bronchiolitis, the commonest lower respiratory tract manifestation of RSV infection.

RSV is a seasonal virus, with annual outbreaks occurring during winter in temperate climates, and during the rainy season in tropical climates. It is extremely infectious. By 18 months of age, 87% of children have antibodies to RSV and by the age of 3 years, virtually all children have been infected[4]. Re-infection with RSV occurs regularly throughout life although infants are unlikely to get recurrent bronchiolitis.

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The majority of children infected with RSV manifest upper respiratory tract symptoms such as rhinitis, cough and coryza[5]. A third of children infected also develop acute otitis media[5]. Fever, when present, is usually low-grade[5]. Upper respiratory tract symptoms usually precede lower respiratory tract involvement by a few days. Dyspnoea, subcostal recession and feeding difficulties characterise lower respiratory tract

infection. In bronchiolitis, wheeze may be present with a prolonged expiratory phase and crackles. Air trapping results in a rapid respiratory rate, a palpable spleen and liver, and a typical radiographic pattern of hyperinflation with diffuse interstitial markings and peribronchial thickening. Segmental atelectasis is often seen. Bronchiolitis may lead to acute respiratory failure with severe bronchospasm, moderate to severe hypoxia and carbon dioxide retention. Apnoea tends to occur in infants under 2 months of age and often in those born prematurely[4]. Supportive measures are still the mainstay of treatment for bronchiolitis. Therapeutic options for severe disease have not progressed much since Cook and Reynolds[6] commented in 1963 that: 'oxygen is vitally important in bronchiolitis and there is little evidence that any other treatment is useful'.

In industrialised countries, severe bronchiolitis is primarily seen in well-defined high-risk groups such as infants with a history of premature birth, bronchopulmonary dysplasia, congenital heart disease, cystic fibrosis and immunodeficiency[4]. Other risk factors for severe disease include crowded living conditions, maternal smoking, having a low umbilical cord serum RSV antibody and being male[5]. Socio-economic class is also a factor, with infants from low-income families being younger when they first acquire the disease. As a consequence, these children have higher rates of hospitalisation[5]. The presence of maternal IgA antibodies within colostrum gives breast-fed infants some protection against RSV bronchiolitis[7].

The majority of children infected with RSV under 1 year of age develop mild upper respiratory tract symptoms. However, up to 40% develop lower respiratory tract symptoms and 0.5–2% of all infants require admission to hospital[5]. Most RSV-associated bronchiolitis occurs in young children 2–5 months of age. It is unusual in children less than 1 month and in children older than 2 years of age. Of children hospitalised, 1–2% require intensive care and, in these, mortality can reach 10%[5]. Death from bronchiolitis is rare in children without underlying cardiorespiratory or immunological conditions, and the vast majority of children infected with RSV make a full recovery. However, some infants with RSV bronchiolitis subsequently develop recurrent episodes of wheeze and cough, suggestive of asthma[8].

This review will cover recent developments in the biology of respiratory syncytial virus, specifically advances in RSV virology and immunopathology. Non-immunological aspects of disease pathogenesis will also be discussed.

## Properties of the virus

RSV is a medium-sized enveloped RNA paramyxovirus of the genus pneumovirus (Fig. 1). The viral genome is composed of 15,000 nucleotides and encodes 10 viral proteins (Figs 2 & 3). The genome is tightly

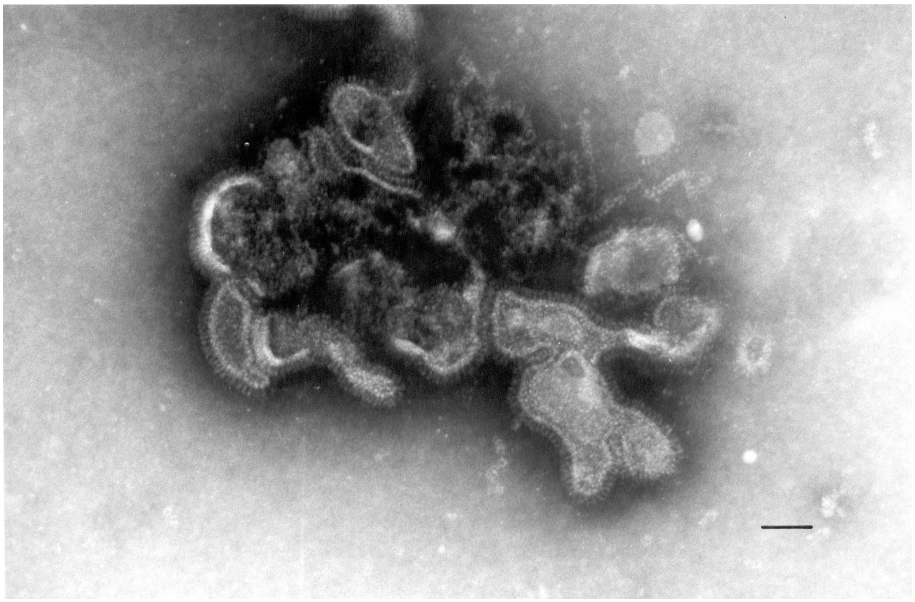

**Fig. 1** Negative stain electron micrograph of respiratory syncytial virus (bar = 100 nm). Kindly provided by the copyright owner, Professor CA Hart.

encapsidated by the nucleocapsid N protein, which, together with the phosphoprotein P and large polymerase subunit L, forms the minimum unit for RNA replication. RNA replication involves the synthesis of a positive-sense, exact-copy, encapsidated, replicative intermediate called the antigenome, which serves, in turn, as the template for progeny genome. As well as the nucleocapsid-associated proteins, transcription requires the M2 open reading frame 1 (ORF1) protein that ensures efficient production of full-length mRNA. The M2 gene also encodes the open reading frame 2 (ORF-2) protein that has a negative regulatory effect and may render nucleocapsids quiescent prior to incorporation into virions. In addition, RSV encodes a matrix protein M, which is thought to mediate interaction between the nucleocapsid and envelope during virion morphogenesis. There are two non-structural proteins, NS1 and NS2, whose functions are unknown, although NS1 appears to be a negative regulatory factor for RNA synthesis. RSV encodes three surface envelope proteins that are components of the virion: the attachment protein G, the fusion protein F, and the small hydrophobic protein SH[9].

The heavily glycosylated RSV G protein comprises 289–299 amino acids and is responsible for viral attachment to cells[10]. Antigenic variability in this protein accounts for the majority of the differences between the two major RSV strains (A and B). The G protein of the A and B strains can differ by up to 47%, whilst G proteins from the same strain can differ by as much as 20%[11]. Despite this variability, all human RSV strains contain an immutable 13 amino-acid region in the G protein that is loop-like in structure[9,11]. This is a possible candidate for the host receptor binding site.

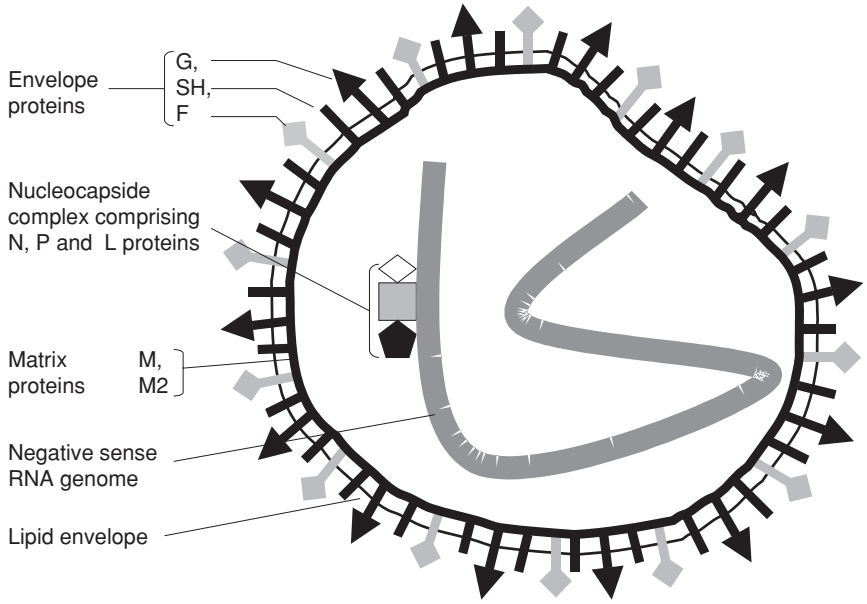


**Fig. 2** A schematic diagram of the RSV virion.

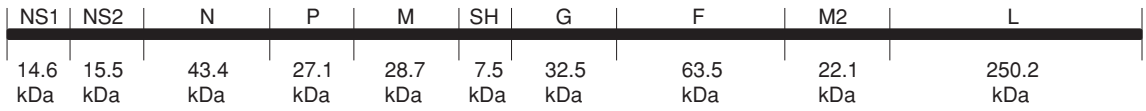


**Fig. 3** The RSV genome depicting the location of the 10 open reading frames and the size of the protein encoded by the individual RNA genes.

Two forms of the G protein are synthesised from alternative initiation codons on the same gene. One form is membrane bound whilst the other, representing 15% of the total, is secreted. The secreted form was thought to be a decoy for antibody, since much of the RSV antibody response is to the G protein, but recent work suggests that membrane and secreted forms provoke differing immune responses[12]. The G protein may not be the only attachment protein, as recombinant RSV lacking G protein can still infect cells[9]. This raises the possibility that the F protein may have additional functions of attachment to a host co-receptor, though no host cell receptor(s) for RSV have been identified.

The RSV F protein in its inactivated form ($F_0$) comprises 574 amino acids and has a trimeric coiled-coil structure, similar to other viral fusion proteins[10]. Activation occurs through cleavage of $F_0$ into two disulphide linked subunits, $F_1$ and $F_2$[10]. The F protein promotes both fusion of viral and cell membranes resulting in the transfer of viral genetic material, and fusion of infected and adjacent cell membranes causing the formation of syncytia[9]. These syncytia are the hallmark of the RSV cytopathic effect and are necessary for cell-to-cell viral transmission. The interaction between the

F protein and a small GTPase, RhoA, facilitates RSV-induced syncytium formation[13]. A possible future therapeutic intervention may involve blocking this interaction[14]. Syncytia formation is also associated with the expression of cytokeratin-17 by RSV-infected respiratory epithelial cells[15]. Cytokeratins are among the major components of the filament networks that make up the cytoskeleton. Cytokeratin-17 expression in RSV infection is neutralised by anti-RSV F protein antibody[15].

The precise role of the third transmembrane protein, SH (comprising 64 amino acids), is unknown at the moment. The SH protein is not required for viral replication or syncytium formation although it does facilitate fusion[9]. Recombinant RSV without the SH gene, when inoculated intranasally into mice, is indistinguishable from wild-type with regard to replication in the lower respiratory tract, but replication is restricted 10-fold in the upper respiratory tract[16]. This site-specific attenuation of viral replication may have implications for future vaccine development.

## The pathogenesis of RSV bronchiolitis

The classic understanding of the pathogenesis of infections assumes that disease manifestations are a direct result of microbial replication and cytotoxicity. While these mechanisms may be important in bronchiolitis, much work has focused on the immunological and non-immunological responses to RSV infection and their role in disease pathogenesis. Several observations suggest that immunological mechanisms may be the key to the severity of RSV bronchiolitis in infancy. First, the disease is most prevalent when the infant is least immunologically mature, despite the possession of maternally derived specific RSV antibody; second, the experience with vaccine-enhanced disease (see later). The immunological response to RSV infection in humans can be divided into innate and adaptive arms.

### *Innate immunity*

This evolutionarily primitive response contributes to the earliest phase of the host defence against foreign organisms. The innate immune response recruits effector molecules and phagocytic cells to the site of the infection through the release of cytokines. It is rapid, does not rely on clonal expansion, and lacks immunological memory.

Pulmonary surfactant is the first line of innate lung defence. It consists of a layer of phospholipids (primarily lecithin and sphingomyelin) together with several surfactant proteins. Broncho-alveolar lavage fluid from RSV-infected ventilated infants shows a decrease in surfactant protein A, B and D concentrations[17]. Surfactant protein A is a member

of the collectin family, a group of structurally related proteins which bind to surface oligosaccharides on a range of pathogens and mediate a number of activities that contribute to the innate immune response, including opsonisation and complement activation. *In vitro* surfactant protein A has been shown to neutralise RSV by binding to the F protein but not the G protein[18].

Recognition of microbial products by host cells is mediated by pattern-recognition receptors. Two important members of this receptor group, CD14 and Toll-like receptor 4 (TLR4), are essential for the innate response to components of Gram-negative and Gram-positive bacteria, mycobacteria, spirochaetes and yeast. Recent work in monocytic cells suggests that the RSV F protein activates the innate immune response via CD14 and TLR4 receptors[19]. Thus a common receptor pathway initiates the immune response to a variety of bacterial, fungal and viral pathogens including RSV.

In RSV infection, the most important cellular components of the innate response are phagocytic cells (neutrophils and macrophages), eosinophils and natural killer cells.

#### Neutrophils

Neutrophils are the predominant airway leukocytes in RSV bronchiolitis[20]. In a study of RSV infection in infants, they were found to represent a median of 93% of cells in the upper airway and 76% in the lower airway[20]. These observations and other studies of neutrophil chemotaxis, adhesion and cytotoxicity, suggest that neutrophils play an important role in the pathological changes that occur in RSV bronchiolitis[21].

In RSV infection, neutrophil chemotaxis is dependent on the production of the potent chemokine, IL-8, by respiratory epithelial cells and macrophages[21]. *In vitro* studies demonstrate a biphasic pattern of IL-8 gene expression in respiratory epithelial cells exposed to RSV[22]. The first peak occurs at 2 h and is independent of viral replication. Thus, an almost immediate inflammatory response can occur before respiratory syncytial virus infection has become established. Late expression (at 24 h) is dependent on viral replication. The importance of IL-8 in human RSV disease is underlined by a recent elegant study demonstrating a genetic polymorphism close to the IL-8 gene, as a determinant of disease severity[23]. Recent work from our group has shown a significant association between nasopharyngeal IL-8 concentrations and disease severity in RSV bronchiolitis[24]. Unsurprisingly, serum and bronchoalveolar lavage samples from infants ventilated with RSV bronchiolitis also show elevated IL-8 levels[25,26].

The process of neutrophil recruitment from the bloodstream into the infected tissue can be divided into four steps: rolling, adhesion, extravasation and migration. As with many infections, the initial step involves the reversible attachment of adhesion molecules (selectins and

integrins) between vascular endothelium and neutrophils[21]. In RSV infection, L-selectin plays a key role in establishing the weak adhesive interaction, which allows the neutrophil to roll along the endothelium. The neutrophil becomes anchored to the endothelium as a result of ICAM-1 induction and the activation of its receptors, the integrins LFA-1 and Mac-1[21]. Both integrin and ICAM-1 expression are increased on peripheral blood neutrophils in RSV-infected infants[21]. Neutrophils then squeeze between endothelial cells and migrate along a chemo-attractant concentration gradient to the site of infection[27]. Once through the vascular endothelium, L-selectin expression is down-regulated in RSV infection, although the physiological relevance of this 'shedding' remains unclear.

Once through the vascular endothelium, the cytotoxic effect of neutrophils is maximised by their retention at the site of infection. In RSV disease, neutrophil–epithelial cell adhesion utilises the same molecular interactions as neutrophil–endothelial cell adhesion. *In vitro* studies on human epithelial cell lines show that RSV infection increases the expression of ICAM-1, VCAM-1 and major histocompatibility complex (MHC) antigens[21]. Neutrophil adhesion can be blocked by antibodies to both integrins and ICAM-1, the latter decreasing neutrophil concentration by up to 70% in rat airways with viral bronchiolitis[28,29]. Other recent studies show that increased neutrophil adherence to respiratory epithelial cells is dose and time-dependent[21,28]. Thus the longer infection lasts, the greater the neutrophil recruitment into the airway. *In vitro* studies show that RSV alone can damage and detach respiratory epithelial cells to a limited extent, and that this effect is augmented by neutrophils[21]. These studies may have clinical implications in RSV-naïve infants, in whom a delayed adaptive immune response with prolonged viral clearance is seen. In these infants, neutrophil recruitment may continue in response to unchecked viral replication with consequent epithelial cell damage by neutrophil disintegration/degranulation. A possible host response to this is the acceleration of neutrophil apoptosis thereby moderating epithelial injury[21].

#### Macrophages

Macrophages are formed when circulating monocytes migrate into tissue. They play an important part in controlling the immune response to viral infection, not only by direct interaction with helper and cytotoxic T-cells but also by the production of cytokines. They can destroy invading pathogens but more commonly act as antigen presenting cells. Macrophages, along with respiratory epithelial cells are the first cells to encounter RSV in the airways[30]. Both cell types respond to infection by secreting cytokines. *In vitro* RSV-infected respiratory epithelial cells secrete RANTES, MIP-1$\alpha$, IL-6, IL-8 and IL-1$\beta$[31], whilst macrophages secrete IL-1$\beta$, IL-6, IL-8, IL-10, IL-12 and TNF-$\alpha$[32,33]. These cytokines further up-regulate the immune response increasing

vascular permeability and causing the activation and recruitment of lymphocytes, neutrophils, NK cells and possibly eosinophils to the site of infection. Comparative studies on mononuclear cells from neonatal cord blood and adults have shown that production of IL-6 and TNF-α in response to RSV infection is less efficient in neonates compared with adults[34]. When apoptosis of RSV-infected neonatal and adult mononuclear cells was compared, decreased cellular apoptosis from both sources was observed although the decrease was more pronounced in neonatal cells[35]. These observations may help to explain the mechanism behind the more severe disease seen in young infants.

A recent study investigated the association between monocyte IL-12 production and disease severity in 30 children ventilated for RSV bronchiolitis[36]. An inverse relationship between duration of ventilation and IL-12 production was observed. The authors postulated that a low monocyte IL-12 response during initial RSV infection might adversely affect the clinical outcome of patients with severe RSV bronchiolitis. Thus monocyte production of IL-12, which is an antiviral cytokine, may be influential in the type and duration of clinical disease seen in RSV infection.

#### Eosinophils

Given the clinical similarities between viral bronchiolitis and asthma, one might anticipate a role for eosinophils in RSV disease. Both eosinophils and eosinophil RNases possess antiviral activity[9], and RSV infected respiratory epithelial cells up-regulate the expression of the eosinophil chemo-attractants, RANTES and MIP-1α[26,31]. Several groups have shown evidence of eosinophil degranulation in both nasopharynx and lung parenchyma during RSV infection[9]. Other studies provide support for the development of a long-standing inflammatory reaction in the airways after RSV bronchiolitis, in which eosinophils play an important role[37–39]. In infants with RSV bronchiolitis, blood eosinophil cationic protein (ECP) levels were found to be higher during convalescence than during acute disease. Eosinophil secretion of ECP is also associated with wheezing during RSV infection[9].

Probably the strongest evidence for the involvement of eosinophils in the immunopathogenesis of RSV bronchiolitis comes from clinical trials of a formalin-inactivated vaccine[5]. Subsequent exposure to RSV resulted in increased mortality and morbidity amongst infants vaccinated. *Post mortem* examination revealed massive pulmonary eosinophil infiltrates.

However, despite these findings, both murine and human studies of primary RSV infection have failed to identify eosinophils in RSV infected lungs[18,40], although they constitute up to 8% of cells in lavage fluid from asthmatic airways[41]. This may reflect an inability by the immune response to activate eosinophil migration fully, or a possible restriction of eosinophil movement to lung and nasopharyngeal parenchyma.

#### Natural killer cells

Comparatively few studies have focused on the role of natural killer (NK) cells in RSV infection. Recent work in mice has shown that NK cells accumulate in the lung in the first few days of RSV infection and are responsible for much of the early production of IFN-γ[42]. NK cells are activated by IFN-β, IL-12 and TNF-α, all cytokines found in the RSV infected lung[29–31]. IL-12 and TNF-α synergistically up-regulate IFN-γ production by NK cells[27]. In children admitted to hospital with RSV bronchiolitis, blood NK cell counts are significantly lower than controls, suggesting that NK cells are recruited from the peripheral circulation into other tissues, probably the lungs[43]. NK cells recognise virus-infected host cells by identifying decreases in MHC class I molecule expression. RSV increases MHC class I expression which, whilst initially protecting infected host cells from NK cell-mediated cytotoxicity, ultimately makes them more susceptible to cytotoxic lymphocyte induced death. The strength of the NK cell response may determine the type of cell-mediated T helper response[11].

### *Adaptive immunity*

The adaptive immune response features immunological memory and is based on the clonal selection of lymphocytes bearing antigen specific receptors. Adaptive immunity may be divided into humoral and cell-mediated responses. In RSV infection, the humoral response is primarily involved in protective immunity whilst the cell-mediated response promotes viral clearance.

#### Humoral

All term, new-born babies have specific RSV neutralising antibodies from the placental transfer of maternal immunoglobulin. Most severe RSV disease occurs between 2–6 months of age, when protection from maternal antibody should be present. This suggests that anti-RSV antibodies may have a role in the immunopathogenesis of RSV bronchiolitis[8]. However the relative sparing of serious RSV disease in infants younger than 6 weeks of age and the correlation between disease severity and low umbilical cord blood RSV antibody titre, argue against the concept that local or systemic antibody production plays a major part in disease pathogenesis[5].

RSV infection provokes the production of serum antibodies in even the youngest children, although the antibody titres produced in infants are low compared to older children and adults[8]. Humans develop antibodies to most RSV proteins, although the F and G proteins stimulate the production of potent neutralising antibodies[7], which are important in protective immunity[8].

During primary infection, serum IgM antibody is present within a few days and remains detectable for 1–2 weeks. IgG antibody appears in the second week, peaks in the fourth week and declines after 1–2 months. The IgA serum antibody response in infants is more variable and may not occur[5]. Serum RSV-neutralising antibodies appear to have a protective effect, as children with high titres (> 1/100) are less likely to develop bronchiolitis than those with lower titres. Furthermore, recent clinical trials of parenteral administration of RSV-neutralising antibodies to high-risk infants show some evidence of protection from severe disease. Re-infection enhances the serum antibody response of all three immunoglobulin classes.

RSV also provokes a secretory antibody response in humans. Both secretory IgA and secretory IgG provide some protection against infection in the upper and lower airways, respectively[1].

The possible role of IgE in RSV bronchiolitis and the subsequent development of wheeze is an area of ongoing debate. Welliver *et al* found RSV IgE in the secretions of infants during the recovery phase of RSV bronchiolitis[8]. Levels of IgE were also high in infants with acute bronchiolitis and correlated with the degree of hypoxia[8]. Recurrent wheezing following bronchiolitis was associated with the initial RSV–IgE response, as well as with a family history of asthma[9]. However, other studies have detected no secretory or serum virus specific anti-RSV IgE in acute or convalescent samples in infants with RSV infection[44].

Evidence from animal models indicates that antibody protects against RSV disease[9]. Once infection is established, however, it is the cell-mediated response that promotes viral clearance.

#### Cell-mediated

The importance of cell-mediated immunity in RSV disease is demonstrated in children with deficient cellular immunity who shed virus for months compared to immunocompetent individuals who clear RSV within weeks[5]. As described elsewhere in this issue, the cell-mediated immune response is classified by lymphocyte surface antigen expression into $CD8^+$ cytotoxic lymphocytes (CTL) and $CD4^+$ T helper cells. Both these cell types have antiviral as well as immunopathogenic capabilities. $CD4^+$ T helper cells are subdivided into Th1 and Th2 lymphocytes on the basis of cytokine secretion. Th1 responses are characterised by IFN-$\gamma$, IL-2 and TNF-$\alpha$ secretion, whilst IL-4, IL-5, IL-10 and IL-13 secretion characterises Th2 responses. Evidence for Th1/Th2 involvement in RSV disease comes primarily from murine studies, where vaccination with a variety of RSV proteins and peptides followed by RSV challenge allows the manipulation of T-cell responses and disease severity.

### *Cytotoxic lymphocytes*

RSV-specific $CD8^+$ lymphocytes are important in both the recovery from, and the pathogenesis of, RSV disease. In the murine model of RSV infection, CTLs clear virus from persistently infected, immunodeficient (irradiated) mice, but may induce an enhanced inflammatory response in the lung[45]. Similarly, CTLs accelerate the clearance of RSV from the lungs of immunocompetent mice but also cause pulmonary injury[9,45]. Other studies have characterised T-cell subset responses and their functional contributions in mice during RSV infection[46]. When both T lymphocyte subsets were depleted, RSV replication was markedly prolonged, yet with no overt evidence of illness, suggesting that host immune response rather than viral cytocidal effect was the primary determinant of disease in mice. Passive transfer of RSV-specific $CD4^+$ and $CD8^+$ lymphocytes to infected mice decreased pulmonary shedding of virus, though some studies demonstrate increased pulmonary damage[9]. Thus, whilst a strong $CD8^+$ response to RSV infection is important for recovery, too vigorous a response may be harmful.

There are few studies evaluating CTL activity in infants and children. In one, the cytotoxic response to respiratory syncytial virus (RSV) was studied in peripheral blood mononuclear cells (PBMCs) from RSV-infected infants compared to controls. CTL activity peaked within 1 week of infection and appeared to be age-dependent, with over 65% of infants 6–24 months of age and 35–38% of infants under 5 months of age exhibiting cellular cytotoxicity to RSV[47]. Other studies show decreased blood $CD8^+$ CTLs in infants admitted for RSV bronchiolitis compared with convalescent samples taken a week later[43]. In a recent study, serum CTL and cytokine responses in a cohort of infants who were followed for three consecutive RSV seasons were measured before their first infection with RSV and in subsequent infections[48]. During the first year of life, nearly 80% of infants infected with RSV developed RSV-specific CTL activity. CTL response to RSV infection was short-lived, but could be preserved and enhanced by a new exposure to the virus. RSV specific CTL levels positively correlated with IFN-$\gamma$ concentrations and inversely with IL-4 concentrations. Infants with detectable CTL activity in the first year of life were less likely to have significant lower respiratory tract disease during subsequent RSV epidemics.

### *T helper cells*

Dramatic evidence supporting an immunopathological mechanism in RSV disease comes from studies of a formalin-inactivated vaccine tested in the 1960s[11]. The intramuscular alum precipitated vaccine did not protect infants from RSV infection. Indeed, as mentioned earlier, children who received the vaccine displayed greater morbidity and mortality with subsequent natural infection compared to unvaccinated control children. Histopathological examination in the children that died revealed florid pulmonary eosinophilia with associated haemorrhagic

necrosis, features not associated with primary RSV infection. Over the past 10 years, animal models have helped to elucidate possible mechanisms behind this vaccine-augmented pathology[5].

In murine models of RSV infection, the induction of different $CD4^+$ T-cell responses may be dependent on the sensitising RSV antigen. Priming mice with recombinant vaccinia virus expressing the F protein induces a Th1 $CD4^+$ T-cell response and a strong CTL response[11]. Immunising mice with RSV G protein promotes activation of a Th2 $CD4^+$ T-cell response and induces eosinophilic infiltrates in lung following subsequent RSV infection[11]. As immunisation with G or formalin-inactivated vaccine results in similar disease profiles upon RSV infection, it has been postulated that G epitopes are responsible for vaccine-enhanced disease. This concept is reinforced by the identification of a G protein epitope associated with eosinophil induction[49]. However, the epitope in question is a component of wild-type RSV that is known to induce Th1 responses[11]. Other recent studies have shown that it is possible to elicit both Th1 and Th2 responses from the same epitope on the G glycoprotein (amino-acids 184–198) implying that MHC–ligand complexes derived from RSV G protein do not direct the differentiation of $CD4^+$ T lymphocytes towards a particular Th1 or Th2 phenotype[50]. Interestingly, there are also differences between immunisation with secreted or membrane-bound proteins. Priming mice with a secreted form of the F protein induces an intermediate Th2-like phenotype with both IL-4 and IL-5 being produced along with RSV F-specific CTLs and high levels of IFN-$\gamma$[51]. The secreted form of G is much more potent than the membrane-bound form in its induction of pulmonary eosinophilia following RSV challenge[11].

The situation is no less complicated in humans. Many groups have tried to identify a dominant Th1 or Th2 cytokine profile in blood, nasopharyngeal aspirates and broncho-alveolar lavage taken from infants with RSV disease. The idea that RSV bronchiolitis might be a Th2-type disease has been studied because it may help to explain the development of post-bronchiolitic recurrent wheeze[8]. However, only limited evidence for the role of Th2 cytokines in the pathogenesis of bronchiolitis has been found to date. Roman *et al* reported that stimulated PBMCs from children with RSV bronchiolitis show an increased IL-4/IFN-$\gamma$ ratio compared to healthy control children although levels of both cytokines were decreased[52]. Renzi *et al* has shown IFN-$\gamma$ expression by PBMCs to be lower in severe compared to mild RSV disease[53]. The same group demonstrated a link between Th2 responses and early wheezing after RSV bronchiolitis[38]. Other recent work examined cytokine profiles in infants with different manifestations of RSV infection. A predominant Th1 cytokine response characterised by IFN-$\gamma$ production was demonstrated regardless of clinical severity[54]. IFN-$\gamma$ protein concentrations are also elevated in nasopharyngeal secretions of infants with RSV infection and correlate with wheezing[55].

Ultimately, multiple factors probably affect the type of T helper cell response elicited in humans during RSV infection. Intrinsic host factors such as atopy, the 'cytokine milieu' present during antigen priming and the presence of immunomodulatory effector cells such as CTLs may all contribute to Th1/Th2 differentiation[11].

### *Non-immunological factors*

The anatomy of the airway predisposes infants to severe RSV disease[5]. In laminar flow, airway resistance is inversely proportional to the fourth power of the radius of the airway. Thus obstruction of the small airways in infants has a greater clinical significance than obstruction in the peripheral airways of an older child or adult. The physiological culmination of the inflammatory response to RSV infection in the lung is the narrowing of the infants airway. Sloughed necrotic epithelium and excessive mucus secretion also add to airway obstruction and the formation of mucus plugs[5]. This results in air trapping and hyperinflation or collapse of distal lung tissue. Several studies have suggested that a proportion of children show evidence of small airways obstruction many years after bronchiolitis[56]. This may be compatible with a pre-existing abnormality, damage sustained by the lung at the time of infection or with mild asthma. It is possible that these infants have relatively smaller airways that predispose them to both RSV bronchiolitis and to recurrent lower respiratory symptoms in childhood. One study suggested that the frequency of lower respiratory symptoms in early life is largely predetermined by lung function in early infancy[57].

Studies on neurogenic-mediated inflammation in the airway may also provide further insight into the pathogenesis of RSV disease. Recently, RSV has been shown to make airways abnormally sensitive to the pro-inflammatory effects of substance P by up-regulating neurokinin-1 gene receptor expression, thereby increasing the density of substance P receptors on some cells[58]. Among these cells are cellular mediators of inflammatory and immune responses such as endothelial cells, lymphocytes, macrophages and mast cells. The potentiation of this inflammatory pathway is a long-term phenomenon, and may predispose to persistent airway inflammation and hyper-reactivity.

## Key points for clinical practice

- Respiratory syncytial virus is the most important cause of lower respiratory tract infection in infants and young children
- RSV causes a spectrum of clinical disease in infants ranging from mild upper respiratory tract symptoms to respiratory failure
- All aspects of the immune system are involved in the host defence against RSV

- There is a delicate balance between the protective and disease-enhancing effects of the host's immune response to RSV
- The end result of these inflammatory changes in the lung is the narrowing of the airway with the consequent clinical symptoms of bronchiolitis
- Clarifying the pathogenic mechanisms that underlie RSV disease may provide an explanation for the observed spectrum of RSV disease and its long-term consequences

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