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Cellular and Molecular Characteristics of RSV-Induced Disease in Humans

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1. Introduction

Respiratory syncytial virus is associated with a large spectrum of illnesses ranging from mild upper respiratory tract (URT) disease to severe lower respiratory tract (LRT) disease. All infections start initially in the upper respiratory tract (URTI), where the virus replicates primarily in the nasopharynx (Brandenburg, 2000; Collins and Graham, 2008). The incubation period is thought to be 4 to 5 days before the onset of symptoms, which are characterised by a runny nose/nasal congestion, a non-productive cough, ear pain, sinus pain and sometimes a low-grade fever (Brandenburg, 2000; Freymuth *et al*, 2001; Rietveld, 2003). In most cases the infection induces mild illnesses of the upper airways, such as rhinitis, pharyngitis or otitis media. However, in some cases (mainly children < 2 years of age) the infection progresses within 1 to 3 days to the lower respiratory tract (LRTI) (Brandenburg, 2000; Graham *et al*, 2002; Tregoning and Schwarze, 2010) and may lead to mild or severe LRT diseases (tracheobronchitis or croup, bronchitis, bronchiolitis or pneumonia). The mechanisms by which RSV spreads to the LRT remain unknown but aspiration of nasopharyngeal secretions loaded with progeny virus is a plausible explanation (Brandenburg, 2000; Freymuth *et al*, 2001; Rietveld, 2003).

In two surveillance studies collating clinical data on RSV over a 20-year period, RSV-LRTI was identified in 37.8% children (2 weeks to 5 years-old, Fischer *et al*, 1997) and 26% healthy adults (18 to 60 years, Hall *et al*, 2001) were diagnosed with a RSV symptomatic infection. Moreover, Fischer *et al* (1997) observed that between 0 and 12 months of age almost half of the children diagnosed with RSV infection developed LRT disease, with a peak at 60% for infants aged between 3 to 6 months. For yet unknown reasons, several factors pre-dispose to severe forms of RSV-LRTI, including young age (< 3 weeks to 3 months), old age (> 65 years), premature birth (<34 weeks of gestation) and underlying medical conditions (bronchopulmonary dysplasia, congenital heart disease and congenital or acquired immunodeficiencies) (Brandenburg, 2000; Collins and Graham, 2008).

There are currently three types of severe disease associated with a RSV-LRTI: bronchitis, which is a disease limited to the large airways (bronchus), bronchiolitis, which involves the small conducting airways (bronchioles), and pneumonia, which implicates the involvement of the alveolar compartment (Tregoning and Schwarze, 2010). These illnesses are often characterised by dyspnea (breathing discomfort/difficulties), tachypnea (rapid breathing, >20 breaths/min), chest retractions, polypnea (increase of breathing frequency, bronchial

obstruction), wheezing (bronchiolar involvement) and rales/ronchi (crepitations, alveolar involvement) (Freymuth *et al*, 2001; Rietveld, 2003). Thus, in a 13-year prospective study in infants and children in the United States, RSV was detected in 43%, 25%, 11% and 10% of paediatric hospitalisations for bronchiolitis, pneumonia, bronchitis and croup, respectively (Collins and Graham, 2008). RSV-LRTI can vary from mild to severe, the latter requiring admission to the intensive care unit (ICU) since the patients often need respiration support. Furthermore, Wang *et al* (1995) observed that among children hospitalised with RSV-LRTIs, 0.9% were associated with fatal cases. However, the mechanisms leading to either an URTI or a LRTI upon RSV infection are still not well understood and require further investigation.

In order to facilitate a better understanding of severe RSV-induced diseases, this chapter will review the histopathology of RSV fatal cases, the cellular responses to infection and the factors associated with the severity of the illness in humans.

2. Histopathology of fatal RSV cases

Since its isolation in 1956 several reports have investigated the histopathology associated with fatal cases of RSV in children (Aherne *et al*, 1970; Downham *et al*, 1975; Ebbert *et al*, 2005; Johnson *et al*, 2007; Kurlandsky *et al*, 1988; Neilson *et al*, 1990; Padman *et al*, 1985; Welliver *et al*, 2007) and adults (Levenson *et al*, 1987), starting with the autopsies of the two unfortunate recipients of the first-ever RSV vaccine candidate. Undertaken in the late sixties, this first human RSV vaccine field trial was based on a formalin-inactivated alum-precipitated whole virus (FI-RSV). However, instead of inducing protective immunity, FI-RSV primed the vast majority of vaccinees for exacerbated disease upon exposure to natural RSV infection, leading to the deaths of two of the vaccinated infants. The autopsies of these infants revealed a prominent neutrophilic and lymphocytic pulmonary infiltration, with some evidence of eosinophilia (Graham *et al*, 2002; Prince *et al*, 2001). These observations were further perpetuated by similar findings in animal models of RSV infection (cotton rats, BALB/c mice, monkeys), which led to the general belief of an immunopathogenic nature of RSV disease.

In this section we will comprehensively review the histopathological observations in children and adults in 3 parts: the damage and cytopathic effects caused to the lungs, the site of RSV infection and the type and site of cell infiltrates.

2.1 Damage and cytopathic effects to the lungs

Bronchiolitis and pneumonia are most commonly associated with severe forms of RSV-LRTI and, not surprisingly, are also the most frequent diseases linked with a fatal outcome. As stated above, they involve different areas of the lower respiratory tract and, therefore, their histopathological characteristics are distinct.

2.1.1 Bronchiolitis

In a study involving 22 children, Aherne *et al* (1970) described the hallmarks of each disease when associated with RSV. The earliest lesion of acute bronchiolitis is the necrosis of the bronchiolar epithelium, principally the destruction of ciliated cells, followed by the peri-bronchiolar infiltration of lymphocytes, macrophages and some plasma cells. Markers of apoptosis, such as caspase 3 (Welliver *et al*, 2007) and Fas (Reed *et al*, 2008), were clearly

evident in bronchiolar epithelium cells of children who died from fatal RSV LRTI compared with uninfected paediatric lung tissue. However, alveoli are usually not involved in the bronchiolitis inflammatory process.

The submucosa and adventitial tissues become oedematous and congested but typically there is no damage to elastic fibres or muscle. The secretion of mucus is enhanced and a thick plug forms in the bronchiolar lumina composed of epithelial cell debris, fibrin and inflammatory cells (Fig. 1) (Downham *et al*, 1975). The occlusion of the bronchioles may lead to various degrees of mechanical lesions, the most severe being the complete collapse of the alveoli that the affected bronchiole supplies. At later stages, two types of cells can be seen that are thought to be involved in the regeneration of the bronchiolar epithelium: undifferentiated pleomorphic cells lining small bronchioles, varying in thickness and showing occasional mitotic activity; and more occasionally, elongated basophilic cells spreading over recently denuded lamina propria. In a comparable study of fatal RSV-LRTI in children (1 to 14 months old) Neilson *et al* (1990) similarly reported the occasional uneven proliferation of epithelial cells with protrusion into the bronchiolar lumen, creating a polypoid appearance.

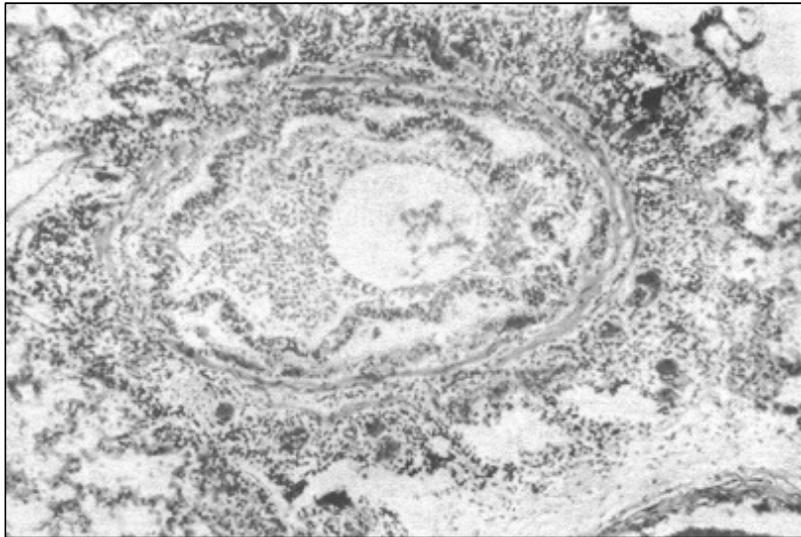


Fig. 1. Histological appearance of lungs of children deceased from a severe RSV bronchiolitis. Varying degrees of peribronchiolar lymphocytic infiltration can be observed alongside a necrosis of the bronchiolar epithelium. The small bronchioles are often plugged with mucus and cell debris. Magnification x 112. Reproduced from [Role of respiratory viruses in childhood mortality, Downham *et al*, 1 (5952), 235-239, 1975] with permission from BMJ Publishing Group Ltd.

2.1.2 Pneumonia

The most thorough description of fatal pneumonia associated with RSV was published in 2007 by Johnson *et al* (2007). In their histopathology report of autopsy tissues from 4 children, they noted extensive damage to the lungs, from medium and small bronchioles to the alveoli. As outlined in an earlier study of fatal pneumonia in infants (Aherne *et al*, 1970), Johnson *et al* (2007) observed widespread destruction of bronchial and bronchiolar epithelium resulting in occlusion of the airways by cell debris (sloughed epithelial cells), fibrin, a minor component of mucin and inflammatory cells (mostly macrophages).

Conversely, Welliver *et al* (2007) were unable to stain for mucus in their autopsy slides. In addition, the small bronchioles were surrounded by fibrotic tissue (excess connective tissue) and the mucosa was often hyperplastic (Kurlandsky *et al*, 1988).

Similar to bronchiolitis, occasional marked uneven proliferation and metaplasia of the bronchiolar epithelium could be detected. These resulted in papillary protusions, which are likely to contribute to airway narrowing. This feature was seen in other comparable studies of infant cases (Aherne *et al*, 1970; Neilson *et al*, 1990) and also in an elderly woman (Levenson *et al*, 1987).

One patient in the Johnson *et al* (2007) study showed multiple syncytia lining the bronchiolar epithelium, an observation that had been previously reported in children (Neilson *et al*, 1990). However, if occasional intra-bronchiolar and intra-alveolar syncytia can be seen in fatal RSV pneumonitis in children (Welliver *et al*, 2007), multiple giant nucleated cells are not a common occurrence associated with RSV pneumonitis. Giant cell pneumonia is the typical disease syndrome of immunosuppressed patients, such as subjects with immunodeficiency diseases or recipients of organ transplant (bone-marrow, lung) (Graham *et al*, 2002). Indeed, several patients in the study by Neilson *et al* (1990) had underlying conditions associated with various degrees of immunodeficiency, which may explain their observations. However, there were no reported underlying conditions that could justify this pattern in the study by Johnson *et al* (2007).

As for bronchiolitis, pneumonia is characterised by a massive bronchiolar inflammation and a peri-bronchiolar infiltration of inflammatory cells, deep to the muscularis layer. The pattern of inflammatory infiltrate around an airway appears to be determined by the distribution of arterioles adjacent to the airway. Sometimes, the infiltrates around the bronchovascular bundle can be so dense that they resemble hyperplastic node follicles, also called bronchiolar-associated lymphoid tissue (BALT), which in turn are responsible for the congestion of the arterioles. Occasionally, BALT nodules projected into the bronchiolar lumens, thereby participating in the narrowing of the airways (Johnson *et al*, 2007).

One of the hallmarks of pneumonitis is the diffuse alveolar damage caused by RSV. This is characterised in children and adults by the proliferation of type II pneumocytes, in order to regenerate destroyed type I pneumocytes, but also interstitial and intra-alveolar fibrosis (Aherne *et al*, 1970; Kurlansky *et al*, 1988; Levenson *et al*, 1987). In addition, an extensive interstitial infiltration occurs, leading to a marked capillary congestion, and is often accompanied by intra-alveolar leakage of fibrin and inflammatory cells (macrophages and occasional neutrophils). Due to a lack of aeration, alveolar parenchyma necrosis and intra-alveolar haemorrhage can be observed in severe cases of pneumonitis, along with oedema and the apparition of a thick hyaline membrane lining the alveoli (Aherne *et al*, 1970; Johnson *et al*, 2007). This latter feature was also observed in elderly patients (Levenson *et al*, 1987).

The observations described above are further supported by Downham *et al* (1975), who corroborated the histopathology described for either bronchiolitis or pneumonia. In this study, they assessed the histological changes in the lungs of 13 children without knowledge of the virus findings. The 2 categories of pathological changes in patients that were later diagnosed with a RSV infection were: 1/ varying degrees of peribronchiolar lymphocytic infiltration, necrosis of bronchiolar epithelium, plugging of the small bronchioles by mucus and cell debris; 2/ lymphocytic infiltration of alveolar walls and interstitial lung tissue,

sometimes associated with a minor degree of peribronchiolar lymphocytic infiltration (Fig. 2). Indeed, the type 1 and 2 patterns showed striking similarities with the histopathology of fatal RSV bronchiolitis and pneumonia cases, respectively.

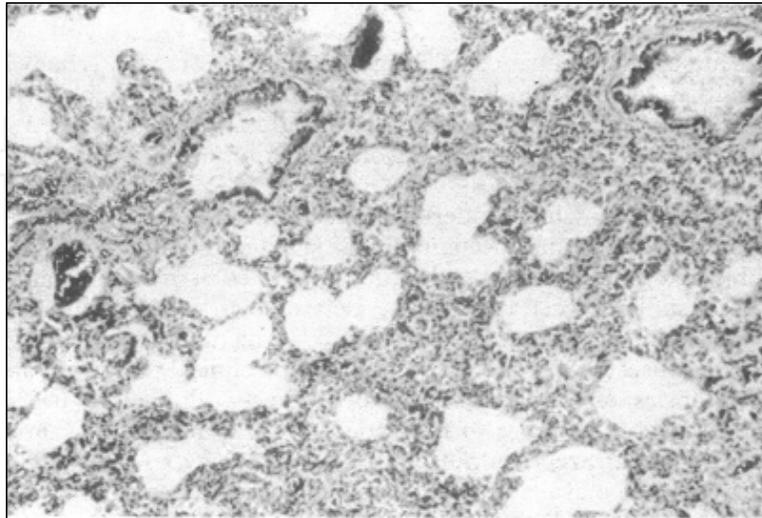


Fig. 2. Histological appearance of lungs of a child that died following severe RSV pneumonia. Cell infiltration of alveolar walls and interstitial lung tissue is clearly identifiable and is associated with a peribronchiolar infiltration. Magnification $\times 84$. Reproduced from [Role of respiratory viruses in childhood mortality, Downham *et al*, 1 (5952), 235-239, 1975] with permission from BMJ Publishing Group Ltd.

2.2 Site of RSV infection in the lungs

In fatal cases of RSV disease, infection was highly restricted to the superficial cells of the respiratory epithelium (Johnson *et al*, 2007; Neilson *et al*, 1990; Welliver *et al*, 2007). In the URT RSV initially replicates in the ciliated cells of the nasopharynx, while in the LRT ciliated cells of the large and small airways and type I pneumocytes in the alveoli are the major targets (Collins and Graham, 2008; Graham *et al*, 2002). This is consistent with the early loss of cilia/ciliated cells observed by Aherne *et al* (1970) in both bronchiolitis and pneumonitis illnesses. This was elegantly demonstrated in 2 recent studies of young children (1 to 15 month-old) who died of “atypical pneumonia” (Johnson *et al*, 2007) or of a severe LRTI (Welliver *et al*, 2007) due to RSV. Although Welliver *et al* (2007) did not mention the type of disease, the histopathological aspects pointed towards a severe form of pneumonitis. In these publications Johnson *et al* (2007) and Welliver *et al* (2007) detected extensive RSV-positive staining in the bronchial and bronchiolar epithelium of all cases (Fig 3a and b), but also in the alveolar epithelium of most patients (Fig. 3a). As expected, Johnson *et al* (2007) observed RSV-positive staining in ciliated cells but also, surprisingly, in non-ciliated cells. As suggested previously (Aherne *et al*, 1970), this may be due to a loss of cilia following RSV infection.

They also noted that the infection was restricted to the superficial epithelium, as basal cells routinely stained negative. Evidence from the infection of well differentiated human airway epithelial cells (WD-HAE) by RSV indicates that progeny virus release is polarised, forcing the virus to the apical surface (Villanave *et al*, unpublished observations; Zhang *et al*, 2002). Coupled with this, the tight junctions are likely to restrict access of the released virus to the basal layers. Interestingly, when Zhang *et al* (2002) damaged their WD-HAE cultures so that

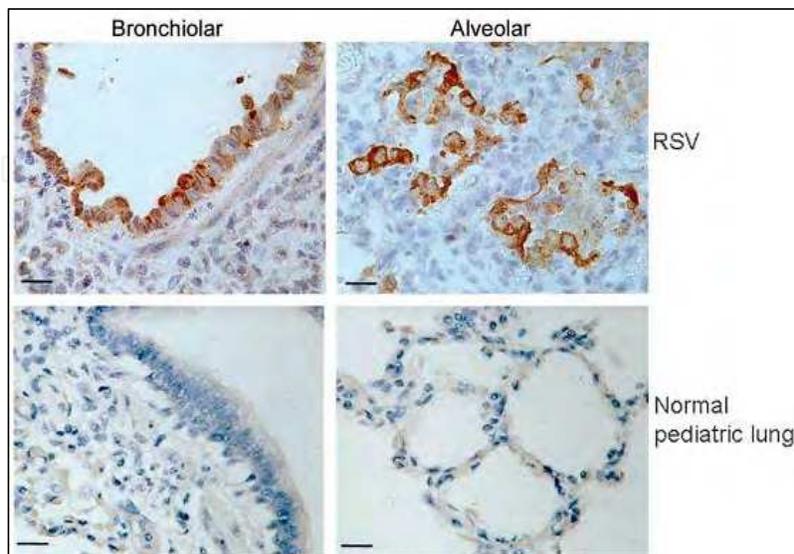
some basal cells were exposed, there was still very little RSV infection of these cells, implying that they somehow have an innate protection from RSV. In addition, Johnson *et al* (2007) further identified the infected alveolar cells as being predominantly type I pneumocytes but also some cuboidal cells thought to be reparative type II pneumocytes. At this level, RSV antigens were detected in a continuous linear fashion along the alveolar surfaces. Similarly, they found that the smallest airways often had circumferential staining, while the larger airways showed a patchy RSV immunostaining, often localised in clusters of 3 to 5 cells.

Several histopathology reports described RSV antigens in giant multinucleated cells (Johnson *et al*, 2007; Kurlansky *et al*, 1988; Neilson *et al*, 1990; Welliver *et al*, 2007) when these syncytia were observable, and also within the material obstructing the lumen of the bronchioli and alveoli (Johnson *et al*, 2007). More specifically, this material was composed of cell debris, infiltrating alveolar macrophages (Johnson *et al*, 2007) and sloughed bronchiolar and alveolar cells (Neilson *et al*, 1990). The positive staining of macrophages for RSV antigens may have resulted from phagocytosed antigen or the infection of these cells by RSV. Indeed, there is evidence suggesting that RSV can infect and replicate in a human macrophage-like cell line (Zhao *et al*, 2007) and in human monocyte-derived macrophages (Spann *et al*, 2004).

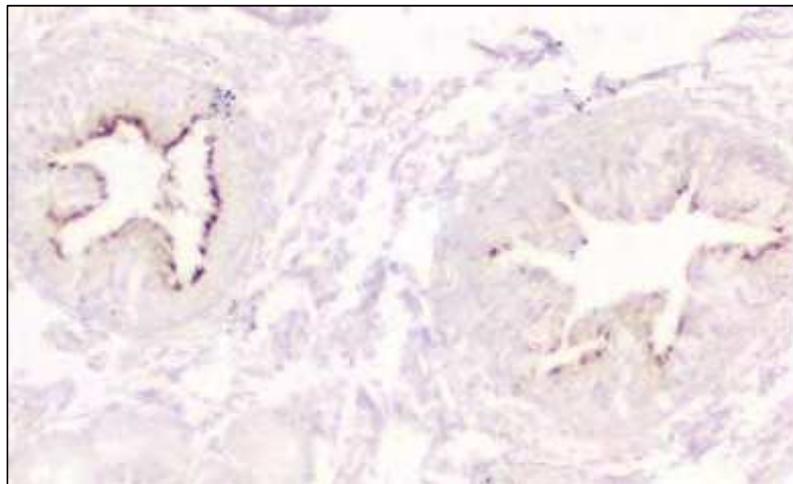
Finally, cytoplasmic inclusions have also been observed, both in children (Neilson *et al*, 1990; Aherne *et al*, 1970) and adults (Levenson *et al*, 1987), although this is not a systematic feature in RSV subjects (Johnson *et al*, 2007). These inclusions can be granular, basophilic and peripherally located (Neilson *et al*, 1990) or homogenous eosinophilic and paranuclear in location (Levenson *et al*, 1987; Neilson *et al*, 1990). They can vary markedly in size and are occasionally surrounded by halos. These inclusions are found in the cytoplasm of bronchial, bronchiolar and alveolar epithelial cells, as well as within syncytia and sloughed intra-bronchiolar and intra-alveolar cells (Aherne *et al*, 1970; Levenson *et al*, 1987; Neilson *et al*, 1990). However, it is still unclear what these inclusions are or to what extent they are involved in the pathology.

Interestingly, Welliver *et al* (2007) compared histopathology findings between RSV and influenza fatal cases and showed that viral antigen was more extensively present in RSV subjects and that damage to the bronchiolar epithelium was also greater. This might suggest an association between viral load and disease severity, as was hypothesised previously (DeVincenzo *et al*, 2005). On the other hand, a closer examination of images from various reports shows vast areas of intact virus-infected cells, especially in the bronchiolar epithelium, implying that the infection might not be the only phenomenon involved in the cytopathic effects (Welliver *et al*, 2007). The uneven distribution of the damage across the lungs, another characteristic associated with RSV fatal cases, further reinforces this impression. For instance, Johnson *et al* (2007) described diffuse pulmonary oedema in areas uninvolved with pneumonitis and Bronchiolitis and Aherne *et al* (1970) found that the severity of the pathological changes varies widely from one region to another. It is also important to note that while the infection can be patchy, the damage to the bronchiolar epithelium and alveoli is often widespread, as is the infiltration of immune cells, such as macrophages and neutrophils. This theory of an immune-mediated disease is supported, at least partly, by work on *ex vivo* models of human WD-PBECS. These models showed limited gross cytopathic effects following RSV infection (mainly restricted to the ciliated cells) (Henderson *et al*, 1978; Zhang *et al*, 2002; Villenave *et al*, - unpublished observations), although increased epithelial cell sloughing was observed compared to uninfected controls.

However, the extent of sloughing and damage *in vivo* is unlikely to explain the widespread sloughing of epithelial and type I/II pneumocytes observed in autopsy cases (Aherne *et al*, 1970; Johnson *et al*, 2007; Welliver *al*, 2007).



(a)



(b)

Fig. 3. Immunohistochemical staining for respiratory syncytial virus (RSV) antigen in bronchiolar and alveolar tissues from infants with lower respiratory tract infection (LRTI). (a) Autopsy tissues were obtained from human infants with fatal cases of LRTI caused by RSV. Normal infant lung tissue (from an infant that died of asphyxia) is stained as a control. Brown stain indicates the presence of viral antigen. Original magnification $\times 40$. (Welliver *et al*, Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses, *The Journal of Infectious Diseases*, 2007, 195, 8, 1126-36, by permission of Oxford University Press) (b) Medium-sized muscular bronchioles demonstrate RSV antigen circumferentially and restricted to the apical epithelium. Dark purple stain indicates RSV antigen. Original magnification $\times 25$. Reprinted by permission from Macmillan Publishers Ltd: [Modern Pathology] (Johnson *et al*, *The histopathology of fatal untreated human respiratory syncytial virus infection*, 20 (1), 108-119), copyright (2007).

2.3 Inflammation and cell infiltration

2.3.1 Bronchiolitis versus pneumonia

One of the common aspects of fatal RSV bronchiolitis and pneumonia is the massive infiltration of multiple inflammatory cell types into the airways. The main cells composing the infiltrates included macrophages, lymphocytes, neutrophils and, to a lesser extent, eosinophils, or subsets of these populations. However, their proportions among the infiltrate and, more importantly, the areas of infiltration differ depending on the form of the disease and the strength of the immune system at the time of death.

The inflammatory cells are centred on the bronchial arteries and arterioles, consistent with their migration out of the bloodstream. They are also massively detected in the sub-muscularis layer of the peri-bronchiolar tissue (Fig. 4), indicating their migration from the arteries/arterioles towards the airways (Johnson *et al*, 2007). Therefore, due to the distribution of the pulmonary arteries, which are surrounding large and medium-sized bronchiolar branches, the inflammatory infiltrate appears symmetrical and circumferential around medium bronchioles and larger airways. On the other hand, because of the diminution of blood vessels converging in the smallest airways, such as terminal bronchioles, the inflammation is often asymmetrical and densely localised between arterioles and the most proximal bronchiole. Overall, the pattern of the inflammatory infiltrate around an airway appears to be determined by the distribution of the blood vessels (arteries and arterioles) adjacent to the airway (Johnson *et al*, 2007).

In the case of pneumonitis, the inflammation is equally important at the alveolar level. In numerous reports of fatal pneumonitis, infiltrating cells are extensively seen in the alveolar interstitium (interstitial pneumonitis) (Fig. 2) and/or within the intra-alveolar exudate (intra-alveolar pneumonia). These observations were consistent in children (Aherne *et al*, 1970; Downham *et al*, 1975; Johnson *et al*, 2007; Kurlansky, 1988; Neilson *et al*, 1990; Welliver *et al*, 2007), adults (Levenson *et al*, 1987) and immunocompromised hosts (Hertz *et al*, 1989; Padman *et al*, 1985)

Irrespective of whether there is a bronchiolar or alveolar inflammation, the main cells migrating from the blood stream towards the airways are mononuclear cells, with a predominance of macrophages followed by lymphocytes. Their distribution is diffuse around the bronchiolar lumen (submuscular area) and within the alveolar parenchyma, and they are a component of the airway plug.

2.3.2 Mononuclear cells

In fatal cases of RSV, macrophages (CD68⁺ cells) were detected in great abundance around the bronchioles and in the bronchiolar exudate, the alveolar interstitium (interstitial pneumonia) and/or within the alveolar lumen (intra-alveolar pneumonia) (Johnson *et al*, 2007, Welliver *et al*, 2007). Thus, despite their submuscular localisation in the case of an acute bronchiolitis (Fig. 5a), macrophages are able to migrate through the smooth muscle towards the bronchiolar lumen. This ability to migrate is illustrated by Johnson *et al* (2007) who observed inflammatory cells moving through gaps across the muscularis (Fig. 5b) and detected the presence of macrophages within the bronchiolar epithelium. On the other hand, at the alveolar level, infiltrated macrophages represent either alveolar macrophages that migrated inside the alveolar space or macrophages that were recruited from the

surrounding arterioles/capillaries. Finally, macrophages were also found to be an important component of the follicle-like expanded BALT (Johnson *et al*, 2007).

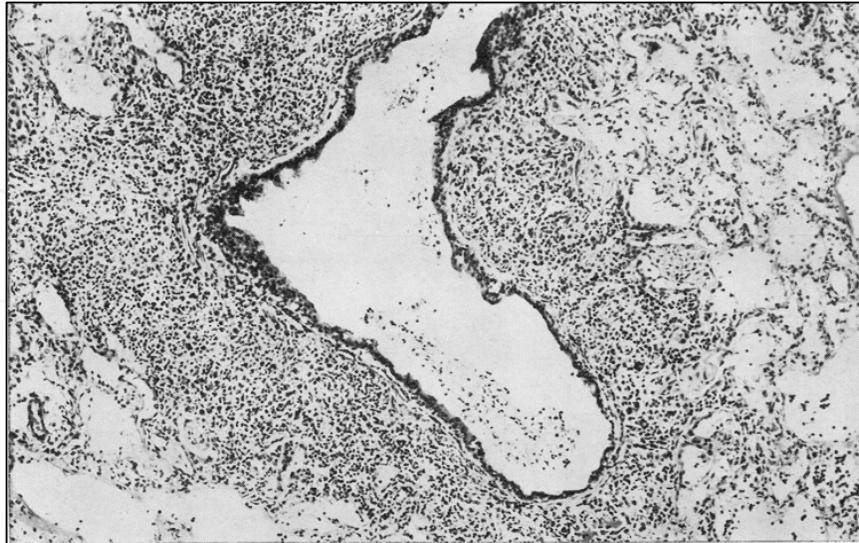


Fig. 4. The peribronchiolar lymphoid infiltration in acute bronchiolitis. Note also the epithelial irregularity. H.E. x 60. Reproduced from [Pathological changes in virus infections of the lower respiratory tract in children, Aherne *et al*, 23 (1), 7-18, 1970] with permission from BMJ Publishing Group Ltd.

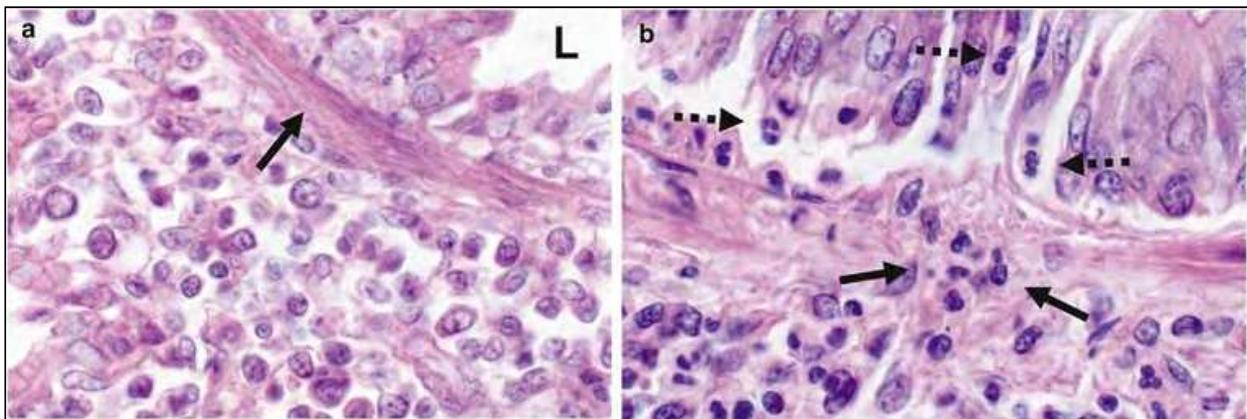


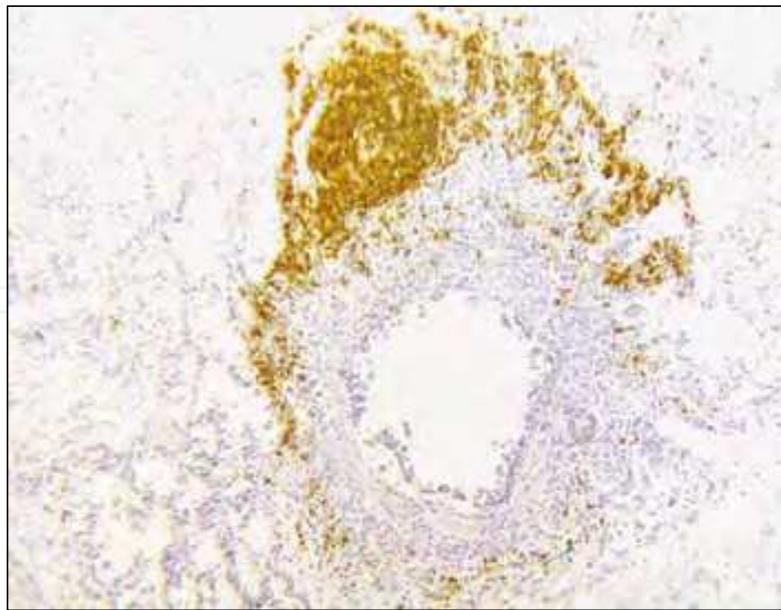
Fig. 5. Relative segregation of mononuclear cells and polymorphonuclear leukocytes in airway. (a) Peribronchiolar mononuclear inflammatory infiltrate is primarily submuscular, and is peripheral to the smooth muscle (arrow). L indicates the airway lumen. (b) While neutrophils are commonly found in BAL, the predominant cells in the peribronchiolar tissues are mononuclear. Neutrophils can sometimes be found migrating across gaps in the muscularis (arrow) towards the airway lumen and interspersed within the epithelium (dashed arrow). Original magnification x 250 (a and b). Reprinted by permission from Macmillan Publishers Ltd: [Modern Pathology] (Johnson *et al*, The histopathology of fatal untreated human respiratory syncytial virus infection, 20 (1), 108-119), copyright (2007).

T Lymphocytes (CD3⁺) are also among the mononuclear cells involved in the inflammation process. Their distribution within the lung tissue is similar to macrophages - diffuse, peribronchiolar and in the alveolar interstitium (Welliver *et al*, 2007; Johnson *et al*, 2007). Lymphocytes have also been found in the basal part of the bronchiolar epithelium (Johnson

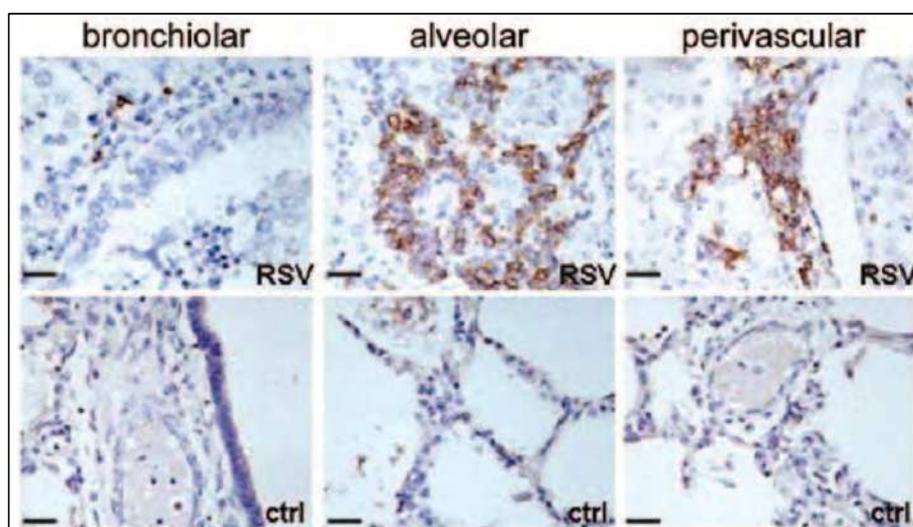
et al, 2007) suggesting that, like macrophages, they can probably migrate through gaps of the muscularis. However, the extent of lymphocytic peri-bronchial and interstitial infiltration varies between studies, and there are discrepancies in the literature about their presence within the bronchiolar or alveolar lumens (Welliver *et al*, 2007; Johnson *et al*, 2007 Padman *et al*, 1985; Aherne *et al*, 1970). It is not clear whether these discrepancies reflect technical variations or actual pathological differences in different individuals, for example due to underlying medical conditions, or the extent of disease at the time of death.

Independent of the extent of lymphocyte infiltration, detailed investigation of lymphocyte subsets indicated that CD3⁺CD8⁺ T cells were predominant over CD3⁺CD4⁺ T cells within the lung tissue. Furthermore, Johnson *et al* (2007) specifically observed that the presence of CD8⁺ T cells was often associated with virus-infected areas, which is consistent with a putative role in virus clearance. However, Welliver *et al* (2007) failed to detect granzyme in lung tissue suggesting that these CD8⁺ T cells might not be fully activated or functional (granzyme being an effector molecule secreted by cytotoxic T cells to kill infected cells). They also failed to detect NK cells (CD56⁺), another cell type involved with cytotoxic responses to viruses. Interestingly, Johnson *et al* (2007) observed that many of the CD3⁺ T cells were actually CD4 and CD8 negative (double negative, DN), a population suspected to be T-regulatory cells (DN Tregs) or NK T cells, but whose role remains to be elucidated. Overall, these findings suggest that T lymphocyte activation and cytotoxic activity may be absent or compromised in the lungs at the time when infants are experiencing their most severe symptoms.

In 1970, Aherne *et al* observed that plasma cells were a component of the peri-bronchiolar and peri-alveolar inflammatory response in fatal cases of bronchiolitis and pneumonitis in children. This observation was recently corroborated by Welliver *et al* (2007) and Johnson *et al* (2007), using infant autopsy tissues. More specifically, large populations of CD20⁺ B cells were detected within the BALT follicles and nodules partially obstructing the airways (Johnson *et al*, 2007) (Fig. 6a), the alveolar interstitium and the perivascular areas, compared to a control group (infants that died of hypoxia) (Reed *et al*, 2009) (Fig 6b). Indeed, this was concomitant with strong IgA-, IgG- and, to a lesser extent, IgM-positive staining within the cytoplasm of cells localised in the alveolar, bronchiolar and perivascular spaces (Fig. 7), identifying these cells as plasmocytes (Reed *et al*, 2009). IgA and IgG staining was particularly strong in the alveolar interstitium and exudate, and in the sub-muscularis layer of the peribronchiolar area. IgA also appeared to be strongly deposited on bronchiolar epithelium, which correlates with its essential involvement in mucosal immunity. Because a vigorous B cell response was observed, while in the same study CD4⁺ T cells were rarely detected, Reed *et al* suggested (2009) the possibility of a T-independent B lymphocyte antibody production. This hypothesis was supported by the perivascular, bronchiolar and alveolar expression of factors known to be involved in T cell-independent B cell activation, Ig production and class-switch recombination. These factors were identified as vasoactive intestinal peptide (VIP), B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL). The up-regulation of the APRIL and BAFF receptors on lymphocytes having plasmocyte morphological characteristics, compared to an age-matched control group, further supported this concept (BAFF-R, transmembrane activator calcium modulator and cyclophilin ligand interactor [TACI] and B cells maturation antigen [BCMA]).



(a)



(b)

Fig. 6. B cell distribution within the lungs of children who died of severe LRTI. (a) Peribronchial lymphoid aggregates are primarily composed of CD20⁺ B lymphocytes (in brown), presumably part of the BALT. During the response to RSV infection, some areas appear to develop the appearance of a hyperplastic lymph node follicle. Original magnification $\times 25$. Reprinted by permission from Macmillan Publishers Ltd: [Modern Pathology] (Johnson *et al*, The histopathology of fatal untreated human respiratory syncytial virus infection, 20 (1), 108–119), copyright (2007). (b) CD20⁺ lymphocytes were detected at autopsy from infants who died of acute RSV LRI (RSV) or asphyxia (ctrl). Original magnification, $\times 40$. Representative fields from bronchiolar (left), alveolar (middle), and perivascular (right) spaces are shown. CD20⁺ cells were detected by immunohistochemistry analysis in formalin-fixed, paraffin-embedded lung tissue. (Reed *et al*, Innate immune signals modulate antiviral and polyreactive antibody responses during severe respiratory syncytial virus infection, The Journal of Infectious Diseases, 2007, 199, 8, 1128–38, by permission of Oxford University Press)

It is important to keep in mind that most of these conclusions regarding lymphocyte subsets associated with RSV LRTI are drawn from only 3 reports and a relatively small number of infants (Johnson *et al*, 2007; Reed *et al*, 2009; Welliver *et al*, 2007). Also, several of the children included in these studies had underlying conditions that are often associated with an immunodeficient status (Down's syndrome, heart disease) (Tregoning and Schwarze, 2010). Therefore, while these publications are of huge relevance for the understanding of RSV pathology, the particular background of these children suggests that generalising these observations to entire populations may be premature and that further studies are warranted.

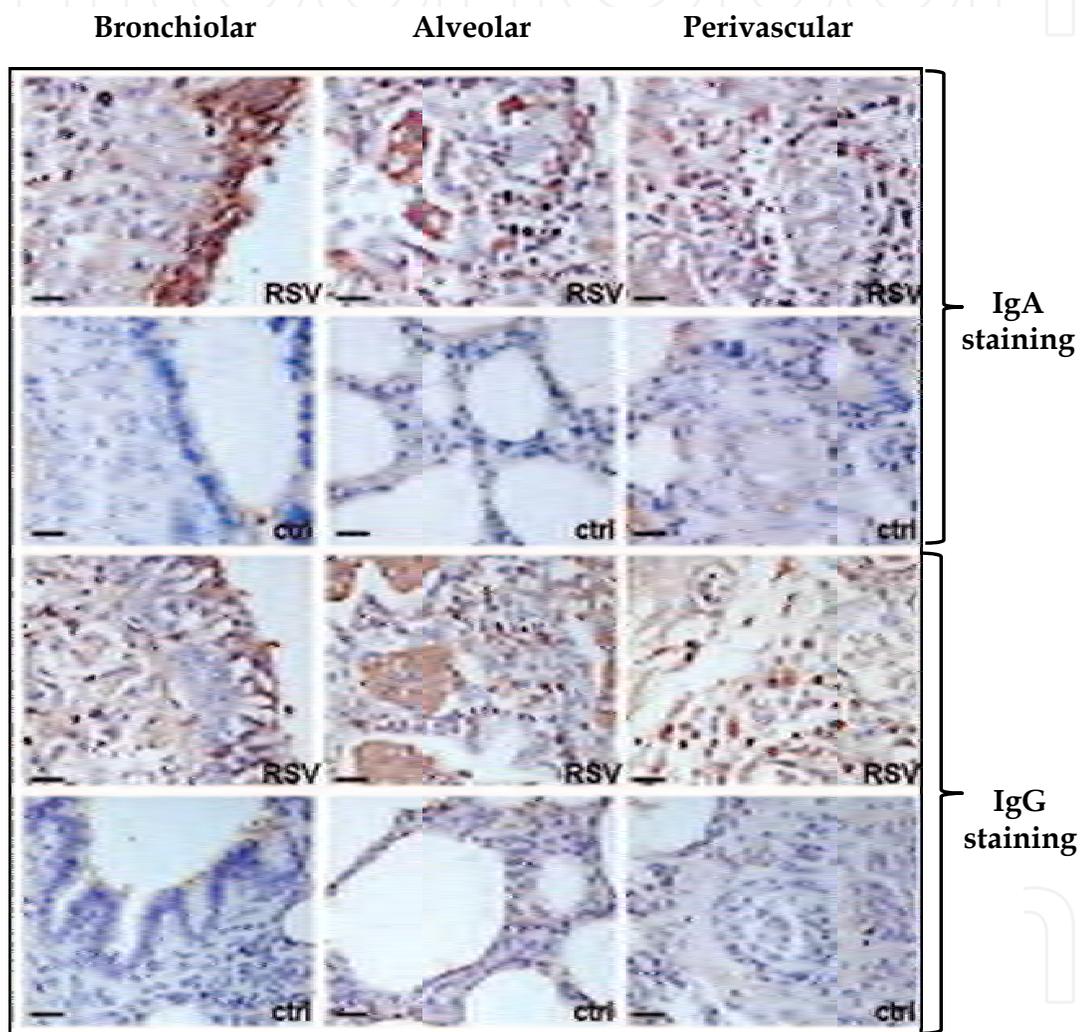


Fig. 7. Detection of immunoglobulin isotypes in samples from individuals with respiratory syncytial virus lower respiratory tract infection (RSV LRTI). IgA (upper panels) and IgG (lower panels) were detected by immunohistochemistry analysis in formalin-fixed, paraffin-embedded lung tissue obtained at autopsy from infants who died of acute RSV LRTI (RSV) or asphyxia (ctrl). Representative fields from bronchiolar (left), alveolar (middle) and perivascular (right) spaces are shown. (original magnification, x40). (Reed *et al*, Innate immune signals modulate antiviral and polyreactive antibody responses during severe respiratory syncytial virus infection, *The Journal of Infectious Diseases*, 2007, 199, 8, 1128-38, by permission of Oxford University Press)

Dendritic cells (DC) are professional antigen presenting cells and essential for inducing adaptive immune response against pathogens. They are found in the peripheral blood or patrolling within tissues in contact with the external environment, such as the skin or the inner lining of the nose/lungs. However, little is known about the extent of their involvement during natural RSV infection and only one publication investigated their presence in fatal RSV cases (Johnson *et al*, 2007). Very few DCs were detected in the lungs and were mainly localised within bronchiolar epithelium and the peribronchiolar connective tissue. It is possible that their low number is a consequence of their migration from the lungs to the lymph nodes after detection and capture of the RSV antigens during the early inflammatory process.

2.3.3 Neutrophils and eosinophils

According to Johnson *et al* (2007), neutrophils were also a component of the inflammatory infiltrate, albeit to a lesser extent than macrophages or lymphocytes. In contrast to the diffuse distribution of mononuclear cells, neutrophils are more densely focalised between the pulmonary or bronchiolar arteries and the airway lumen. This suggests that these cells are responding to chemottractant molecules secreted by the bronchiolar epithelium only, whereas factors attracting mononuclear cells are produced by epithelial cells and cells within the lung parenchyma (Johnson *et al*, 2007). As mentioned above, neutrophils are principally found in the connective tissue of the peribronchiolar area (submuscularis), in the bronchiolar and alveolar lumens but have not been observed within the alveolar interstitium (Johnson *et al*, 2007; Welliver *et al*, 2007). They can also be detected crossing through gaps of the muscular layer and in the basal part of the bronchiolar epithelium, confirming their ability to migrate through tissues towards the airway lumen (Johnson *et al*, 2007).

Noticeably, the histopathology observations regarding the intra-luminal infiltration of macrophages, lymphocytes, neutrophils and eosinophils concur with the cell composition of nasopharyngeal (NPL) and broncho-alveolar lavages (BAL) collected from infants hospitalised with severe bronchiolitis. However, the ratios of cell types are significantly different, with neutrophils consistently being the largest cell population within nasal and pulmonary secretions (70 to 80%), followed by mononuclear cells (Brandenburg *et al*, 2000; Everard *et al*, 1994; Heidema *et al*, 2007; O'Donnell *et al*, 2002; Smith *et al*, 2001). This is further supported by the appearance of neutrophil precursors (myelocytes, metamyelocytes and banded neutrophils), normally residing in the bone marrow, in the peripheral blood of hospitalised RSV patients at a time when disease severity and viral load are declining (Lukens *et al*, 2010). It is likely that the migration of neutrophil progenitors from the bone marrow is a consequence of neutrophil exhaustion (and depletion of the banded-neutrophil pool in peripheral blood) due to a massive recruitment of these cells into the airways. In contrast, in autopsy cases neutrophils were often detected in the minority among the intra-luminal infiltrate compared to macrophages and lymphocytes (Aherne *et al*, 1970; Johnson *et al*, 2007; Welliver *et al*, 2007). The reason for this striking contrast is unclear and warrants further investigation.

Eosinophils can be detected as part of the peribronchiolar cell infiltrate but they are not a dominant component of the inflammatory process (Aherne *et al*, 1970; Johnson *et al*, 2007). For many years these cells were thought to be major players of the inflammatory response against RSV since a significant eosinophilia was one of the hallmarks described of the fatal

enhanced disease associated with FI RSV vaccination (Graham *et al*, 2002). However, re-analysis of the FI-RSV autopsies challenged this dogma, as eosinophils were found to be a minor population among the pulmonary infiltrates with an uneven distribution across lung tissues, while neutrophilia was the principal characteristic of the inflammation (Graham *et al*, 2002; Prince *et al*, 2001). Therefore, it remains to be elucidated whether FI-RSV enhanced disease is representative of a natural primary RSV infection and to what extent eosinophils (and neutrophils) are involved in RSV pathology.

It is noteworthy that the histopathology of the FI-RSV autopsy cases displayed both similarities and discrepancies compared to recent histopathology studies of fatal RSV cases (Johnson *et al*, 2007; Welliver *et al*, 2007). For instance, in the former cases, Prince *et al* (2001) observed a peribronchiolar inflammation composed of 10% neutrophils, 10% macrophages and 80% lymphocytes, while the bronchial exudate contained 50% neutrophils, 30% macrophages and 20% lymphocytes. Eosinophils represented 1-2% of the inflammatory cells of the sections (Prince *et al*, 2001), whereas Graham *et al* (2002) observed many eosinophils. Also, according to the original publication describing the autopsies of the FI-RSV recipients, the alveoli contained scattered infiltrates of neutrophils and macrophages, or of neutrophils and mononuclear cells, depending on the pulmonary lobe examined (Chin *et al*, 1969). While the infiltration of macrophages and lymphocytes in the peribronchiolar and alveolar areas are consistent between reports, discrepancies regarding the amount of infiltrating eosinophils and neutrophils remain. However, comparable disease enhancement does not occur with community-acquired natural RSV infections and re-infections, and, therefore, the relevance of FI-RSV-associated pathogenesis to natural infection is unclear (Collins and Graham, 2008). It is interesting to note that the histopathological hallmarks of fatal RSV bronchiolitis or pneumonia from normal infants were also identifiable in fatal RSV cases of immunocompromised children and adults (Ebbert *et al*, 2002; Hertz *et al*, 1989; Padman *et al*, 1985). However, the mortality and morbidity is a lot higher in the latter groups, reaching 50% of infected patients (Graham *et al*, 2002).

3. Factors associated with severe RSV infection

RSV epidemics usually occur over the winter months in temperate climates and in the rainy season in hotter climates. In the Northern hemisphere peak epidemic months are commonly November to January (Brandenburg, 2000; Garcia *et al*, 2010; Houben *et al*, 2011). Approximately 70% of children encounter their first infection before the age of one, while almost all children are infected at least once before the age of 2 years (Brandenburg, 2000; Rietveld, 2003;) and over 50% will have been infected twice (Brandenburg, 2000). About 40% of young children infected for the 1st time show signs of lower respiratory tract involvement, with some developing severe complications necessitating hospital admission (Brandenburg, 2000). Overall, 0.5 - 2.5% of infants are hospitalised with RSV infection during their first year of life, and 7 - 21% of these children require ventilation support due to respiratory failure (Brandenburg, 2000; Rietveld, 2003). RSV is the most common cause of bronchiolitis hospitalisation, with one study showing that RSV accounted for 60 - 70% of all hospitalisations with bronchiolitis (Garcia *et al*, 2010).

Deciphering the factors associated with severe RSV disease is complicated by the fact that many published studies define disease severity, and patient inclusion criteria in severe

cohorts, differently. Factors such as requirement for mechanical ventilation, admission to paediatric ICU, days in hospital and oxygen saturation levels are commonly used, either alone or together. There are clearly defined groups of infants that are at high risk of severe RSV. These include infants with congenital heart disease, bronchopulmonary dysplasia, congenital or acquired immunodeficiencies or prematurity (<32 weeks gestation). However, the majority of infants hospitalised with RSV have no known high risk factors. The mechanisms leading to severe LRT disease, and possibly death, are still poorly understood. It is likely that a multitude of factors influence the course of disease during an infection. We postulate that the interrelation of various elements associated with the virus, the host and the environment will shape the outcome of RSV disease.

3.1 Viral factors

3.1.1 RSV subgroup

RSV has a single serotype with two major antigenic subgroups (A and B). Both subgroups have a worldwide geographical distribution and circulate independently, while their seasonal distribution is similar each year (Brandenburg, 2000; Collins and Graham, 2008; Rietveld, 2003). In several epidemiology studies, 3 patterns of subgroup A and B circulations within communities could be observed: a strong predominance of RSV A strains, relatively equal proportions of RSV A and B strains and, occasionally, a strong predominance of RSV B strains (Hall *et al*, 1990; Imaz *et al*, 2000; Mufson *et al*, 1988; Taylor *et al*, 1989). Numerous studies have investigated the relationship between disease severity and RSV subgroups but the data are inconclusive. In some studies infections with RSV A strains were associated with increased severity in children (Hall, *et al*, 1990; Imaz *et al*, 2000; Mufson *et al*, 1988; Taylor *et al*, 1989; Walsh *et al*, 1997), while others failed to detect any significant correlation with the subgroup (DeVincenzo *et al*, 2004; Fodha *et al*, 2007; Martinello *et al*, 2002). It is possible that the simultaneous circulation of multiple strains of each subgroup during an epidemic, the year-to-year variation in virus population, the alternated subgroup predominance and the geographical location of the study may explain discrepancies between reports (Collins and Graham, 2008; Fletcher *et al*, 1997; Hall *et al*, 1990; Martinello *et al*, 2002). Interestingly, in several studies of hospitalised children RSV A subgroup infections led to higher mean viral loads compared to RSV B (Devincenzo *et al*, 2004; Fodha *et al*, 2007; Perkins *et al*, 2005). However, in view of the discrepancies, the biological significance of strain variation in relation to disease severity remains unclear.

3.1.2 RSV strain/genetic variation

The antigenic homology between RSV subgroups is approximately 25%. The fusion protein (RSV F) is the most conserved with 90% amino acid sequence identity and 50% antigenic homology between RSV A and B subgroups. The G protein (RSV G) is the most divergent, showing only 53% amino acid relatedness and 1 to 7% antigenic homology (Brandenburg, 2000; Collins et Graham, 2008; Rietveld, 2003). Thus, phylogenetic studies have targeted the G gene to differentiate between RSV strains. For instance, Martinello *et al* (2002) investigated the association between RSV G genotypes and disease severity in children during two RSV seasons. They found that clade RSV A GA3 isolates correlated significantly with increased

clinical severity scores compared to other subgroup A clades (GA2, GA4) and B isolates. Previous reports also addressed this issue, although with a different classification of RSV strains. Specifically, Hall *et al* (1990) grouped RSV A and B strains on the basis of their reaction with various monoclonal antibodies to RSV G protein. Their data suggested an association between one RSV strain (A2) and higher rates of intensive care admission. Furthermore, two studies used restriction length polymorphism (RFLP), either of N and F genes (Savon *et al*, 2006) or N and G genes (Fletcher *et al*, 1997), to define RSV strains. Both studies concluded that RSV genotypes F1N4 (RSV F and N clades) and G were associated either with longer hospital stay (Savon *et al*, 2006) or various degrees of disease severity, according to their severity criteria (Fletcher *et al*, 1997). However, in our view these observations warrant further investigation. Overall, from our current understanding it is reasonable to assume that among the multiple strains simultaneously circulating during an RSV season some could be associated with increased severity.

3.1.3 Viral load

It is evident from the few published studies available that the importance of viral load in relation to RSV disease severity remains controversial. Studies addressing this issue determined viral loads in nasal aspirates from naturally infected infants following hospitalisation or within the community (Buckingham *et al*, 2000; DeVincenzo, 2004; DeVincenzo *et al*, 2005; Fodha *et al*, 2007; Houben *et al*, 2010; Wright *et al*, 2002). With the exception of the study by Wright *et al* (2002), there is a growing consensus that mean viral titres correlate significantly with disease severity. That RSV disease might be driven mainly by the viral load is a seductive idea and would correlate with other virus-associated illnesses, such as HIV, cytomegalovirus and influenza virus (DeVincenzo, 2005).

However, analyses of mean viral titres mask the fact that some individuals in non-severe cohorts may have considerably higher virus titres in their nasal aspirates than some individuals in the severe cohorts. This is clearly exemplified by Buckingham *et al* (2000), who presented the virus load data from individuals in the severe and non-severe disease cohorts. Their data demonstrated that some individuals in the non-severe cohort had virus loads $\geq 2 \log_{10}$ pfu/ml higher than some severe cohort counterparts. Such data argue strongly against virus load as a predictor of disease severity. On the other hand, in contrast to the severe cohort, some individuals in the non-severe cohort had virus loads $\leq 2 \log_{10}$ pfu/ml, which undoubtedly skewed mean data towards reduced values. The cumulative data suggest the possibility that a certain minimum virus replication is required to cause severe illness, but the degree of replication itself may not be the defining characteristic. Interestingly, King *et al* (1993) reported that in infants co-infected with human immunodeficiency virus type 1, prolonged clinical shedding of RSV occurred without substantial disease compared to healthy controls, thereby implying a substantial role of the host immune system in RSV pathology rather than viral cytopathic effects. In our opinion, re-analyses of the published data, in which individual virus titre data are stratified into the severe and non-severe cohorts, will greatly help our understanding of this important issue.

Convincing evidence of a correlation between virus load and disease severity was provided recently in an elegant RSV challenge study in healthy adult volunteers (DeVincenzo *et al*,

2010). While symptoms were mild and restricted to the URT, virus growth and disease severity kinetics paralleled one another, suggesting that the efficiency of virus replication was driving symptom severity. A similar temporal association of virus shedding and respiratory symptom scores and mucus weight in RSV-challenged healthy adults was described by Lee *et al.* (2004). Moreover, the secretion of specific pro-inflammatory chemokines paralleled the viral load and the severity of the disease, hence acting as potentially interesting biomarkers (IL-6, IL-8/CXCL8, RANTES/CCL5, TNF- α , MIP-1 α /CCL3). This is consistent with Noah & Becker (2000), who reported that IL-8/CXCL8, RANTES/CCL5, MIP-1 α /CCL3 and MCP-1/CCL2 concentrations increased in nasal lavages recovered during virus shedding from infected adult volunteers. Surprisingly, infectivity, virus growth kinetics and disease severity appeared to be independent of the initial virus inoculum titres (10^3 to $10^{5.38}$ pfu) under the experimental conditions described (DeVincenzo *et al.*, 2010). This contrasted with the work by Hall *et al.* (1981), in which infectivity and the onset of virus shedding was greatly influenced by the initial inoculum ($10^{5.2} \gg 10^{3.2}$ TCID₅₀) of the adult volunteers. It is possible, however, that these discrepancies might be explained, in part at least, by the use of different RSV strains and/or the fact that volunteers were pre-screened for relatively low virus neutralising antibody titres before inclusion in the study by DeVincenzo *et al.* (2010). The latter hypothesis is consistent with Lee *et al.* (2004), who found a significant correlation between pre-inoculation RSV neutralisation antibody titres and infection rates, irrespective of the infecting doses tested.

As the RSV challenge studies cited above were undertaken in healthy adults, who were undoubtedly repeatedly exposed to RSV infections prior to inclusion, great care must be taken in extrapolating these data and conclusions to infants undergoing primary infection. First, in contrast to the adult challenge experiments, in which symptoms were invariably restricted to the URT, a large proportion of infants undergoing their first RSV infection have symptomatic LRT involvement. Second, such infants are, by definition, immunologically naive relative to RSV and do not possess the plethora of anti-RSV memory T and B cell/antibody responses evident in adults. Third, young infants in particular, in whom the peak of severe RSV disease occurs, are thought to be immunologically immature and are therefore likely to respond quite differently to infection compared with adults.

3.2 Host factors

3.2.1 High-risk profiles in infants

As indicated above, underlying heart and lung conditions are factors predisposing to severe RSV disease. Patients with diseases such as congenital heart disease (CHD) or bronchopulmonary dysplasia (BPD) are often associated with RSV infections of greater severity. These conditions are associated with more frequent admission to ICU, prolonged hospitalisation, increased requirement and duration for supplemental oxygen, the need for mechanical ventilation, increased severity scores and death (Brandenburg, 2000; DeVincenzo *et al.*, 2005; Garcia *et al.*, 2011; Kaneko *et al.*, 2001; Rietveld, 2003; Thorburn K, 2009; Wang *et al.*, 1995; Wright *et al.*, 2002). The precise reasons for aggravated illness in these infants are unclear but pre-existing lung diseases will significantly reduce an infant's functional pulmonary reserve capacity, rendering RSV more likely to overwhelm

quickly and easily the infant's defences. Prematurity (Brandenburg, 2000; Fodha *et al*, 2007; Garcia *et al*, 2011; Rietweld, 2003; Wang *et al*, 1995) and acquired or congenital immunodeficiency status (Brandenburg, 2000; Ebbert *et al*, 2002; Hertz *et al*, 1989; Padman *et al*, 1985; Rietweld, 2003) are also important predisposing factors for severity.

It is thought that the reduced transplacental acquisition of maternal antibodies in pre-term compared to full-term infants may partially explain the aggravated disease associated with RSV infection of premature infants (DeVincenzo *et al*, 2005). Indeed, in a longitudinal study, it was shown that infants with higher maternal neutralizing antibodies had a lower risk of RSV hospitalisation and that once infected they developed significantly reduced severity of disease (Glezen *et al*, 1981). Importantly, although prematurity, congenital heart and lung conditions (Wang *et al* 1995; Rietweld, 2003), and immunodeficiencies (Graham *et al*, 2002; Hall *et al*, 1986; Wang *et al* 1995) have been associated with higher mortality rates in infants, the majority of children admitted to hospital with a RSV infection do not have these high risk factors (Brandenburg, 2000; Wang *et al*, 1995).

3.2.2 Cytokine and chemokine responses

A considerable body of information supports the idea that RSV pathogenesis is, at least in part, immune mediated. In essence, an over-exuberant immune response to infection is thought to cause considerable "collateral" damage in the lungs. The drivers for much of these immune responses to infection are cytokines and chemokines. These are small secreted proteins that are induced following interaction of pathogen-associated molecular patterns with pathogen recognition receptors, such as Toll-like receptors, on host cells. They include an expanding family of proteins, whose interaction with specific cellular receptors stimulates a range of signal transduction cascades resulting in a plethora of immunological functions. Functions of cytokines and chemokines include chemoattraction and activation of immune cells, such as neutrophils, lymphocytes, monocytes/macrophages and dendritic cells, i.e., hallmarks of RSV pathogenesis. Therefore, an over-exuberant cytokine/chemokine response is likely to be associated with RSV immunopathogenesis.

In this regard, regulated upon activation, normal T cell expressed and secreted (RANTES/CCL5), macrophage inflammatory protein 1 α (MIP-1 α /CCL3), monocytes chemotactic protein 1 (MCP-1/CCL2), IL-1 β , IL-6, IL-8/CXCL8, IL-10, IL-18, tumours necrosis factor α (TNF α) and interferon γ -induced protein 10 (IP-10/CXCL10) are among a range of cytokines/chemokines demonstrating increased expression in nasal aspirates, BALs or blood from RSV infected infants (Barmejo-Martin *et al*, 2007; Garofalo *et al*, 2001; McNamara *et al*, 2004; McNamara *et al*, 2005; Murai *et al*, 2007; Noah *et al*, 2002; Roe *et al*, 2011; Van Benten *et al*, 2003). Furthermore, the levels of some of these molecules correlated with disease severity. For example, Garofalo *et al* (2001) reported that MIP-1 α /CCL3 was markedly increased in nasopharyngeal secretions from infants with severe RSV bronchiolitis compared to those with RSV-URTI only or mild bronchiolitis. Similarly, Van Benten *et al* (2003) found that increased numbers of IL-18 positive cells in nasal brushes from RSV-infected infants was specific for bronchiolitis, as similar increases were not detected in infants with only RSV-URTI.

Despite the strong evidence of correlations between cytokine and chemokine expression levels and RSV infections, it is still unclear whether these relationships are temporal or

causal in relation to RSV-induced disease and disease severity. The functions of some of these molecules are consistent with a causal role, but formal proof in humans will be very difficult to achieve. In support of a causal role, an elegant study by Juntti *et al* (2009) indicated that IL-6 and IL-8 responses following LPS stimulation of cord blood samples were predictive of disease severity following later infection. In essence, cord blood from infants subsequently hospitalised with RSV had higher combined IL-6 and IL-8 responses to LPS stimulation than cord blood from RSV-infected infants treated as outpatients. However, another limitation of these studies is the fact that only RSV infection was confirmed in the infants – co-infections with other respiratory pathogens were not considered. Therefore, the possibility remains that some of the data on cytokine/chemokine expression level correlations with RSV disease severity might be compromised by co-infections at the time of sampling.

3.2.3 Genetic polymorphisms

As outlined above, the majority of infants hospitalised with RSV have no underlying high-risk profiles. Furthermore, only a small proportion of infants from any given birth cohort end up with severe RSV disease. This suggests the possibility that genetic polymorphisms may be implicated in predisposing infants to severe disease. A number of groups have attempted to address this question using genetic association studies. Not surprisingly, in view of their correlations with severe RSV disease, much of the initial work focussed on specific cytokine/chemokine genes and their receptors. A comprehensive list of these haplotypes and polymorphisms associated with severe RSV disease was recently provided by Miyairi and DeVincenzo (2008), T Tregoning and Schwarze (2010) and Zeng *et al* (2011). They include such cytokine genes as IL-4, IL-8, IL-10, IL-13 and IL-18, and receptor genes like CCR5, CD14, CX3CR1, IL4RA and TLR4. Confirmation of these associations may provide the basis for identifying, from birth, otherwise healthy infants who are at risk of severe RSV disease. It would also provide the rationale for including such infants in the high risk groups that currently benefit from the immunoprophylaxis provided by palivizumab, an anti-RSV F monoclonal antibody administered to high risk infants before and during the RSV season. However, much work remains to be done to validate these genetic associations.

3.3 Environmental factors

The environment also plays a role on the extent of RSV infection as some of these factors can impact on the exposure to the virus or lung functions. Thus, day care attendance or crowded living conditions (siblings) are recognised risk factors for RSV hospitalisation, lower respiratory tract involvement (Rietveld, 2003) and admission to ICU (DeVincenzo *et al*, 2005). This association with disease severity is likely a consequence of the high infectivity of the virus. A recent survey of RSV infection in the community attempted to define several significant predictors of RSV LRTI (Houben *et al*, 2010). As expected, they included day care and siblings but other factors such as a high parental education level, a date of birth outside RSV season (April to September) and, surprisingly, a birth weight >4 kg. However, no association was identified with maternal smoking. Thus, this study concluded that the risk of RSV-LRTI was 10 times higher for children who attended day care, had older siblings, had high parental education level, a birth weight >4 kg and were born between April and September. A comprehensive review of the literature on RSV disease risk factors by Simoes

(2003) drew similar conclusions regarding day care attendance and crowding/siblings as important environmental factors for severe RSV-LRTI. However, in contrast to Houben *et al* (2010), birth during the first half of the RSV season (September to November) was found to be a risk factor.

4. Conclusions

In this chapter we have attempted to provide a comprehensive overview of the consequences of RSV infection in humans, with particular emphasis on severe disease characteristics in otherwise healthy infants. There is a general consensus that RSV disease is a multifactorial phenomenon implicating virus, host and environmental elements. However, the mechanisms by which RSV causes disease in humans remain largely unknown. Ethical and clinical constraints provide challenging obstacles to elucidating these mechanisms directly in human subjects. Animal models of RSV disease have undoubtedly roles to play in addressing specific concepts relating to RSV pathogenesis, e.g., gene knockout studies, which cannot be undertaken in humans. However, as they are generally only capable of modelling some aspects of RSV pathogenesis in humans, extrapolating data derived from animal models to human RSV disease has been less than spectacular to date. In our opinion, ultimate success in deciphering the molecular mechanisms of RSV disease in humans is likely to come from studies directly in humans and/or in authentic *ex-vivo/in-vitro* human tissue models. A comprehensive understanding of RSV pathogenesis in humans is the first step in addressing these issues and, ultimately, to finding prophylactic and/or therapeutic solutions for this devastating viral pathogen.

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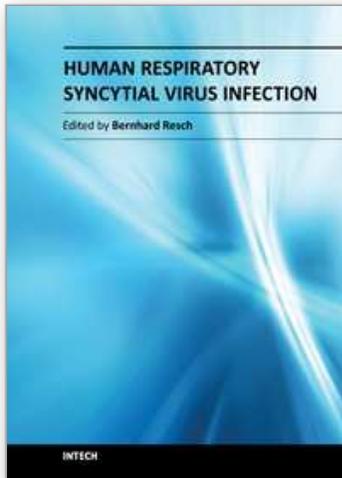
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In this online Open Access book on "Human RSV Infections", several distinguished authors contribute their experience in respiratory syncytial virology. A major focus lies on the fascinating pathophysiology of RSV and represents recent and actual work on different mechanisms involved in the complex pathogenesis of the virus. The second section elucidates epidemiologic and diagnostic aspects of RSV infection covering a more clinical view of RSV disease. At last, treatment modalities including the search for a vaccine that is still not in sight are discussed and conclude this book, thus building up a circle that runs from experimental models of RSV related lung disease over clinical aspects of disease to the latest news of therapeutic and prophylactic approaches to human RSV infection.

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