Respiratory Syncytial Virus Persistence

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Introduction
Respiratory syncytial virus (RSV) is a prevalent and highly infectious virus, more so than any other respiratory virus. Worldwide, it is a very important pathogen, causing acute upper- and lower-respiratory-tract infections (URT and LRT, respectively), especially in infants and young children [1-6].

Morbidity and Mortality
RSV is considered to be the most frequent cause of pneumonia and bronchiolitis, with bronchiolitis being the single most common cause for the hospitalization of infants [1,5-8]. RSV is an important cause of morbidity and mortality in the elderly and the immunocompromised. In the elderly it is the second leading cause of viral death, with a reported 5.5% annual incidence; in high-risk adult populations [5,6,9,10] the incidence rises to 10%.

Recent estimates made by the World Health Organization (WHO) indicate that, worldwide, RSV causes 64 million cases of morbidity and 160,000 deaths each year and accounts for more than 60% of acute LRT infection in children and more than 80% in infants [6-8,11].

RSV Infection
Clinical manifestations
Infection by virus starts in the nose and/or the eyes by the introduction of viral particles in an aerosol or by direct contact with fluids containing the virus [1,6,7,12,13]. Once the virus has penetrated the cells of the epithelium, replication of the virus takes place in the nasopharynx; the incubation period is 4–5 days, although in some cases it is longer [7,12,14]. Normally, infection is confined to the superficial cells of the respiratory epithelium and is restricted to the respiratory epithelium [1,4,7,11]. Virus can be spread to the lower respiratory tract (LRT), probably through aspiration of secretions, although the possibility of transmission by cell-to-cell fusion cannot be discarded [4,6,14]. The inflammatory response associated with this infection is extremely complex [15], and involves the release of multiple cytokines and chemokines from epithelium cells and from infiltrating immune cells, local neuro-immune interactions, and mast-cell degranulation with variable release of leukotrienes [16-19].

Epidemiology
LRT infections caused by RSV occur epidemically every year [6-11]. The seasonal appearance of these epidemics seems to vary with latitude, altitude, and climate, with the epidemic pattern tending to occur in clusters. The season in which RSV epidemics occur depends on geographic location and altitude. Although varying from continent to continent, outbreaks usually begin in coastal areas [1,6].

Seasonal RSV outbreaks occur each year throughout the world, with the peak and duration of an outbreak varying from one year to the next. Outbreaks occur during the winter months: in the northern hemisphere, the annual epidemics normally start in November, peak in January and February, and end in May; in the southern hemisphere, the epidemic season runs from May through September. In the tropics, epidemics peak during the rainy season [1,6,11].

Long term sequelae
Young children who have recovered from severe bronchiolitis often develop chronic and recurrent respiratory problems [2,6,19], and appear prone to early allergic sensitization [2,3,6]. The link between RSV infection and the development of sequelae (wheezing, asthma [19-23], and chronic obstructive pulmonary disease (COPD) has been clearly established in several well controlled prospective epidemiological studies [24-26]. RSV bronchiolitis in infancy has been reported to be an important risk factor for subsequent respiratory complication [23,27-31].

RSV Persistence
Because RSV does not produce vigorous immune response that allows reinfection [1,6,7,15], it is likely RSV may alter the immunity response as a strategy to permit persistence in host cells [15,32,33].

In humans
The effects of the sequelae of severe RSV disease may be explained, in part, by viral persistence, with the RSV infection causing an alteration of the airway structure and/or inducing an aberrant immune response [25,29,30]. Continuous stimulation of the immune system by persistent virus infections may cause chronic inflammation or alter the expression of immunoregulatory molecules [35,36], such outcomes may explain the clinical manifestations that persist long after acute viral infection. Infected epithelial cells and macrophages secrete cytokines, chemokines, and other factors that attract lymphocytes and other cells to the site of infection, thus resulting in airway inflammation [37,38].

The seasonality of RSV infection with little activity in summer month suggests that after acute infection the reservoir for RSV is the host and that, under suitable conditions, viral reactivation may result in re-infection and recurrence of the natural cycle [32]. Because, to date, no animal reservoir of RSV has been demonstrated [1,6], viral persistence in humans may be involved in maintaining the virus during inter-epidemic periods.

Although RSV persistence in humans has not been demonstrated, some observations indicate that persistent infections may occur in humans: 1) the presence of RSV antigen in bone biopsies and in cells cultured from patients with Paget disease was detected by using immunohistological assays [39]; 2) shedding of infective RSV was observed in immunocompromised patients [40]; 3) RSV was isolated repeatedly from the nasopharynx of apparently healthy children [41]; 4) RSV nucleic acid was detected in archival postmortem lung tissue from infants, who had died during the summer, without apparent clinical disease having been reported, thus suggesting that the virus...
can persist in lungs after acute infection [42]; and 5) individuals with COPD have been reported to have persistent RSV infection [24,25,43].

Taken together, the findings from these studies suggest the possibility that acute RSV infection in the winter, with subsequent resolution of symptoms, could be followed by chronic persistence of RSV in human lung tissue [14,32]. Furthermore, viral recovery and infectivity might be triggered through suppression of adaptive immunity, thus resulting in re-infection and recurrence of the RSV season the following year [6]. This interpretation of the cycle of RSV infection would be particularly applicable in elderly and COPD patients [5,44].

In animal models

RSV has been reported to persist in experimentally infected guinea pigs and mice. Viral proteins and genomic RNA were found in the lungs of infected guinea pigs at least 60 days after inoculation [45], which presented persistent airway abnormalities, hyper-reactivity and eosinophilia [46-48].

In mice, RSV latency and persistence, has been evidenced by the presence of genomic RNA or messenger RNA, respectively. In RSV infected BALB/c mice, viral genome and messenger RNA, was found in lung homogenates for more than 100 days after acute infection and all signs were resolved [49,50]. By T cells depletion, infective virus at low levels was obtained, suggesting that RSV persists at low-grade replication [49]. Moreover, RSV infection induced airway long-term disease, characterized by chronic inflammation and hyper-reactivity [51].

In vitro

RSV is relatively non-lytic in most human and animal epithelial and immune cell types and easily establishes stable, persistent infections in cell lines by infection with RSV Long strain or temperature-sensitive mutants. These cultures are characterized by low titers of extracellular infective virus and by a high percentage (90-100%), of infected cells presenting viral antigen, with no formation of syncytia and no apparent cellular cytopathology [52-57].

Viral persistence in cell lines experimentally infected with virus provides an excellent system to investigate changes in the expression profiles of virus and host genes by due to the continuous replication of viral genes in a cell by altering non-essential functions ["luxus activities"] viral chronicity can selectively disorder the functions of the infected cell without destroying it [58].

RSV persistent infection in human and mouse cell lines alters gene expression in these cells. The rate of surface viral proteins are severity reduced in persistently RSV infected cells (BCH-4) of BALB/c, compared to that of internal viral proteins and lytically infected human larynx epithelial (HEp-2) cells [59].

DNA microarray analyses of persistently infected cell lines, mouse macrophage-like P388D1 [60], and Hep-2 [61], showed that RSV persistence subverted apoptotic pathways through up- and down-regulation of expression of cellular apoptotic genes. Expression of anti-apoptotic proteins, Bcl-2, Bcl-X, and X-linked inhibitor of apoptosis protein (XIAP), was enhanced [60], and TNF-receptor-associated factor 1 [TRAF1] [61], and baculoviral IAP [60]. Moreover, in both cell lines, the mRNA levels of caspase 3, 8, and 9 were reduced with respect to those of non-infected cells [60,61]. The activity of caspase-3 was reduced and that of caspase-9 could not be detected in infected P388D1 macrophages [60].

RSV persistence in HEp-2 and in P388D1 induced significant changes in the expression of interleukins and chemokines, compared with that in non-infected cells. Increases in the expression of interleukins IL-1b and IL-6 [36], and of chemokines CCL3, MIP-1a, CCL3L1, CCL5 [RANTES], CXCL10 [IP-10], and MIP-2 were reported [61].

The pattern of chemokine expression may determine the nature of the pulmonary cellular infiltrate and, hence, the extent of inflammation [62]. In addition, the up-and/or down-regulation of some cytokines and interleukins has been shown to favor the establishment of virus persistence [63,64].

Conclusion

Mounting evidence, obtained in prospective studies in humans, suggests that RSV may persist latently or at a low level of replication in immunologically privileged sites in the lung and that RSV persistence may be associated with the link between RSV LRT infection and sequelae of airway hyper-reactivity. Moreover, RSV persistence has been suggested as an aggravating agent in patients with stable COPD and in those suffering exacerbations. Because no animal reservoir has been described for this virus, RSV persistence in humans has been proposed to explain not only these sequelae, but also virus survival between seasonal epidemics.

Results of studies in animals experimentally infected with RSV not only have documented instances of RSV persistence, but also have led to the hypothesis that RSV persists at low replication levels in the lung.

Persistence of RSV in immune and non-immune cell lines of both humans and animals has been reported and significant changes in the gene expression of pro-inflammatory interleukins and chemokines of the host have been observed. Persistent RSV infection of the lung may lead to prolonged inflammation and chronic respiratory disease. A continuous and/or unbalanced production of some cytokines may contribute to such a scenario.

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