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# Interferon lambda 1–3 expression in infants hospitalized for RSV or HRV associated bronchiolitis

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**Summary** *Objectives:* The airway expression of type III interferons (IFNs) was evaluated in infants hospitalized for respiratory syncytial virus (RSV) or rhinovirus (HRV) bronchiolitis. As an additional objective we sought to determine whether a different expression of IFN lambda 1–3 was associated with different harboring viruses, the clinical course of bronchiolitis or with the levels of well established IFN stimulated genes (ISGs), such as mixovirus resistance A (MxA) and ISG56.
*Methods:* The analysis was undertaken in 118 infants with RSV or HRV bronchiolitis. Nasopharyngeal washes were collected for virological studies and molecular analysis of type III IFN responses.
*Results:* RSV elicited higher levels of IFN lambda subtypes when compared with HRV. A similar expression of type III IFN was found in RSVA or RSVB infected infants and in those infected with HRVA or HRVC viruses. Results also indicate that IFN lambda 1 and IFN lambda 2–3 levels were correlated with each other and with MxA and ISG56-mRNAs. In addition, a positive correlation exists between the IFN lambda1 levels and the clinical score index during RSV infection. In particular, higher IFN lambda 1 levels are associated to an increase of respiratory rate.
*Conclusions:* These findings show that differences in the IFN lambda 1–3 levels in infants with RSV or HRV infections are present and that the expression of IFN lambda 1 correlates with the severity of RSV bronchiolitis.


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## Introduction

Bronchiolitis is a disorder most commonly caused in infants by viral lower respiratory tract infections; it is characterized by acute inflammation, edema and necrosis of epithelial cells lining small airways, increased mucus production, and bronchospasm.[1] The most common virus causing bronchiolitis is the respiratory syncytial virus (RSV).[2,3] Other viruses identified as causing bronchiolitis are rhinovirus (HRV), human metapneumovirus, bocavirus, and parainfluenza.[4] In particular, HRV has been recently shown to infect the lower airway as well[5] and confirmed to be the second most frequent cause of bronchiolitis.[6]

It has been demonstrated that the combination of both host and viral factors profoundly influence the severity of viral associated bronchiolitis.[7–9] However, it is not yet clear whether the different subtypes of RSV (A and B) or HRV species (A, B, and C) cause different grades of bronchiolitis severity.[10,11] Furthermore, the role of the innate immune response, in the pathogenesis of severe RSV or HRV disease is still to be defined in detail.[7–9]

Among the main players of antiviral innate immune response, the type I interferons (IFNs), IFN alpha and beta, are considered cytokines crucial for anti-viral resistance and represent an early antiviral host defense mechanism against viral infections.[12] In 2003, a novel class of antiviral cytokines was discovered, characterized and classified as type III IFNs: IFN lambda1/IL-29, IFN lambda 2/IL-28A, and IFN lambda 3/IL-28B.[13] At the amino acid level IFN lambda2 and lambda3 are highly similar having 96% sequence identity while IFN lambda1 shares approximately 81% sequence identity with IFN lambda2 and lambda3.[13] The Type III IFNs possess antiviral properties similar to those of type I IFNs but appear to be expressed especially by epithelial cells and consequently exert host protection primarily at epithelial surfaces.[14–16]

Despite the fact that it is known that IFN lambda contributes to the control of viral infections in epithelial cells of respiratory tract[17–19] and that the presence of single nucleotide polymorphism around IFN lambda 3 (IL-28B) can increase the risk of hospitalization for bronchiolitis at early age,[20] the IFN lambda 1–3 expression in the respiratory tracts of hospitalized infants with RSV or HRV infections has never been addressed.

Hence, considering the importance of the IFN lambda in protecting the airway tract from virus infections,[17–19] we hypothesized that the heterogeneity of IFN lambda 1–3 levels could, at least in part, explain the broad clinical spectrum of RSV or HRV bronchiolitis. Therefore, we evaluated whether there was a difference in the gene expression of IFN lambda 1–3 subtypes between infants with a clinical diagnosis of RSV associated acute bronchiolitis and those with HRV infection. The same analysis was also performed between RSV or HRV subtypes. In addition, to characterize the activation of type III IFNs in the airway tract of infants with RSV or HRV infections, we evaluated whether there was a coordinate activation between IFNs lambda and that of MxA and IFN-stimulated gene (ISG) 56, which are well known markers of type I and III IFN antiviral activity.[12] Finally, to further characterize the above issues, we also assessed whether a correlation between IFN lambda 1–3 levels and demographic, virological and clinical parameters in RSV and HRV infected infants actually exist.

## Patients and methods

### Study population

A total of 118 infants with single RSV or HRV infection were retrospectively selected from a total of 250 infants admitted with a clinical diagnosis of acute bronchiolitis during three epidemic seasons (2008–2011) to the Paediatric Department of Policlinico Umberto I Hospital. The study was approved by the ethics committees and informed consent was obtained from the infant's parents.

Bronchiolitis was diagnosed from the presence of a history of upper respiratory tract infection followed by the acute onset of respiratory distress with cough, tachypnea, retraction, and diffuse crackles on auscultation (wheezing alone was not considered sufficient cause for inclusion in the study). The exclusion criteria were underlying chronic disease (such as cystic fibrosis, chronic pulmonary disease, congenital heart disease, and immunodeficiency) and recurrent (more than one) wheezing episodes.[4,21]

The severity of the illness was assessed clinically on the following four indications, each of which was assigned a score within the range 0–8.[4] In particular, on admission to hospital, the clinical severity was assigned to each infant, based on respiratory rate ($<$45 breaths/min = 0, 45–60 breaths/min = 1, $>$60 breaths/min = 2) arterial oxygen saturation in room air ($>$95% = 0, 95–90% = 1, $<$90% = 2), presence of retractions (none = 0, present = 1, present + nasal fare = 2), and ability to feed (normal = 0, reduced = 1, endovenous = 2).

### Specimen collection

Nasopharyngeal washings were collected in the first 48 h after admission to the hospital from infants suffering from acute bronchiolitis, and an aliquot was tested for viruses as previously described.[22] In particular nasopharyngeal washings were obtained with 3 ml of sterile saline physiological solution injected into each nostril and collected with a syringe. All samples were delivered on ice within 1–2 h to the virology laboratory and on arrival, if needed, they were vortexed with beads to solve mucus. They were divided into two aliquots: one was treated for nucleic acid extraction and viral detection; the second was centrifuged at 2000 rpm for 10 min, and each cell pellet was resuspended in 1 ml of phenol and guanidine isothiocyanate reagent (Trizol, Gibco-BRL, NY) and frozen at −80 °C for gene expression analysis.

### PCR assays for respiratory viruses

A panel of reverse transcription-PCR (RT-PCR) or nested PCR assays, some in a multiplex format, were used for the detection of 14 respiratory viruses, including RSV; influenza viruses A and B; coronaviruses OC43, 229E, NL63, and HKU1; metapneumovirus; adenovirus; HRV; and parainfluenza