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Immune monitoring of children with respiratory syncytial virus infection

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Asuncion Mejias

*Author for correspondence:
Department of Pediatrics,
Center for Vaccines and
Immunity
and
Department of Pediatrics,
Division of Pediatric Infectious
Diseases, The Research
Institute at Nationwide
Children's Hospital, The Ohio
State University School of
Medicine, Columbus, OH,
USA
Tel.: +1 614 722 5830
Fax: +1 614 722 3680
asuncion.mejias@nationwidechildrens.org*



Mark W Hall

*Department of Pediatrics,
Center for Clinical and
Translational Research
and
Department of Pediatrics,
Division of Critical Care
Medicine, The Research
Institute at Nationwide
Children's Hospital,
Department of Pediatrics, The
Ohio State University School
of Medicine, Columbus, OH,
USA*



Octavio Ramilo

*Department of Pediatrics,
Center for Vaccines and
Immunity
and
Department of Pediatrics,
Division of Pediatric Infectious
Diseases, The Research
Institute at Nationwide
Children's Hospital, The Ohio
State University School of
Medicine, Columbus, OH,
USA*

“...weak, rather than intact or robust, innate immune responses are associated with enhanced respiratory syncytial virus disease severity ... Immunomonitoring of children with respiratory syncytial virus lower respiratory tract infection ... could have important clinical implications.”

Respiratory syncytial virus (RSV) is the main cause of viral lower respiratory tract infection (LRTI) and leads to hospitalization in infants and young children in developed and developing countries [1]. In 2005, 3.4 million children were hospitalized for RSV LRTI worldwide. However, the burden of RSV disease is significantly larger in the outpatient setting, where it is associated with considerable acute and long-term morbidity [2]. Moreover, RSV is a significant pathogen in the elderly and in immunocompromised patients. By 2 years of age, 95% of all children have been infected with RSV at least once, yet reinfections are common as protective immunity is neither complete nor sustained. Epidemiologic studies have identified selected groups of infants (premature infants, children with chronic lung or congenital heart disease, immunodeficiency and, more recently, children with comorbidities) at high risk for severe disease and mortality. However, the majority of children hospitalized with RSV LRTI are previously healthy with no known risk factors for severe disease [3,4]. Of those hospitalized infants, 10–20% will develop a disease severe enough to require admission to the intensive care unit (ICU). Despite its impact on children's health, an effective vaccine or specific therapy is lacking, which is largely due to our limited understanding of the immune response to RSV and how it relates to clinical disease severity, and also due to the challenges of

performing studies in infants, the main target population for this infection.

Studies and clinical practice have shown that there is great variability in the severity of the disease among infected children [2]. Indeed, in the clinical setting it is impossible to predict, based on physical examination and currently available diagnostic tools, which patients will show progressively worse disease and require hospitalization and even ICU care, and which patients can be discharged safely. The difficulties in determining the likelihood of disease progression have led investigators to search for clinical risk factors and/or biomarkers, such as cytokines, which can help in predicting RSV disease severity, with no definitive results. In addition, the pressure to achieve a rapid resolution of symptoms has commonly led medical practitioners to take an overcautious attitude. As a result, many patients undergo unnecessary treatment with antibiotics, steroids or inhaled bronchodilators, despite evidence from controlled studies and meta-analysis showing that these therapies are not very effective. Obviously, this is a flawed approach that, besides driving up healthcare costs, facilitates the development of antimicrobial resistance and can be associated with further impairment of the host immune response.

It has been proposed that the combination of both viral factors and the host immune response probably contributes to the severity of RSV disease. Several studies have attempted to correlate RSV

loads measured in the respiratory tract by culture or real time-PCR with disease severity. Although most of these studies did not identify a clear association between RSV load and clinical disease severity, a few found a significant correlation between these two parameters [5,6]. It should be mentioned that these studies included heterogeneous patient populations and patients of different ages at different stages of their infection, probably introducing confounders in the analyses. The effect of different viral subtypes (RSV A and B), as well as that of viral genotypes, on the severity of the illness has also been studied. While some studies identified a significant correlation between RSV type A and disease severity, others failed to confirm those findings [7]. Moreover, during typical RSV outbreaks, which in temperate climates each year predictively last from late fall through to early spring, a limited number of RSV strains tend to circulate. Thus, it remains unclear why previously healthy children infected with the same viral strain and similar viral loads display such a wide variation of clinical manifestations, suggesting that an abnormal host immune response or host immune 'insufficiency' greatly contributes to the different disease phenotypes.

The importance of the innate immune response in the pathogenesis of severe RSV disease is being increasingly recognized. Early studies showed the presence of a robust inflammatory response in the airway of children with RSV LRTI with the production of proinflammatory cytokines and chemokines such as TNF- α , IL-6, IL-8, MIP-1 α and IFN- γ [8]. Recently, different groups of investigators, including our own, found decreased concentrations of inflammatory cytokines and other markers of cell injury, such as LDH, in the respiratory tract in the more severe forms of RSV disease [9–12]. These observations represent a paradigm shift and suggest that weak, rather than intact or robust innate immune responses, are associated with enhanced disease severity.

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The data regarding blood cytokine responses in children with RSV used as a tool to monitor clinical disease is limited, but encouraging. Two different studies showed that blood monocyte IL-12 and TLR-8-mediated TNF- α production were inversely correlated with disease severity in children with RSV LRTI [13,14]. We recently characterized innate immune functional responses in a cohort of 66 previously healthy infants hospitalized with a first episode of RSV LRTI during the 2010–2011 respiratory season. A nasal wash and a blood sample were obtained within 24 h of admission. The nasal wash sample was obtained to confirm and quantitate RSV loads, and whole blood was collected to measure cytokine concentrations before and after lipopolysaccharide (LPS) stimulation. Unstimulated plasma innate cytokines including IL-6, IL-8 and also IL-10 were modestly increased in infants with RSV LRTI compared with healthy controls, and this was independent of the severity of the disease. On the other

hand, children with RSV LRTI requiring ICU treatment had a significantly lower production capacity of TNF- α after LPS stimulation compared with infants with less severe RSV bronchiolitis hospitalized in the regular inpatient floor, and with healthy controls. Lower TNF- α production capacity independently predicted longer duration of hospitalization after adjusting for age, gender, days of symptoms at enrollment, presence of fever and RSV loads [15]. This insufficient or hyporesponsive state of the innate immune response has been associated with poor outcomes in critically ill children and adults with sepsis, and has been termed immunoparalysis in its most severe form. Innate immune dysfunction is characterized by reduced expression of major histocompatibility complex class II (HLA-DR) in monocytes and/or reduced capacity of whole blood to produce TNF- α upon stimulation with LPS (TLR-4 agonist). *Ex vivo* LPS-induced TNF- α production capacity is used as a readout of the overall ability of monocytes to respond to a new challenge, rather than reflecting a specific deficit in TNF- α production itself. Altogether, these results suggest that children with severe RSV infection exhibit defective innate immune responses. However, it is not known whether children who develop severe RSV LRTI are born with an already impaired innate immune response and RSV just uncovers their abnormal immune system, or if it is RSV by itself that causes such a profound suppression of the immune response. This will require studies including sequential samples.

On the other hand, genome-wide transcriptional profiles of the host response to disease have provided new key evidence about disease pathogenesis. The limited data available in infants with RSV infection using this unbiased methodology also suggest the existence of an impaired immune response in the more severe forms of RSV LRTI, and emphasizes the value of blood host gene signatures as a novel tool to better understand the pathogenesis of RSV infection [16–18]. In addition, with this novel approach, we can now correlate genomic data with clinical parameters of disease severity. In fact, we can derive a molecular disease severity score (genomic score) that correlates with clinical parameters of severity such as a clinical disease severity score, duration of supplemental oxygen and duration of hospitalization. Thus, this genomic score helps to objectively stratify patients based on the severity of the disease [19]. The practicality of this molecular disease severity score for RSV bronchiolitis will have tremendous implications in the clinical setting. Not only will it help to triage patients when they first present to the Emergency Department or pediatric office, but it will also be extremely useful to monitor clinical changes during the course of the disease, with the ultimate goal of predicting clinical outcomes. Currently, we have the tools needed to apply this methodology into the clinical setting and studies are underway to evaluate the value of transcriptional profiling for assessing RSV disease severity.

Immunomonitoring of children with RSV LRTI and other respiratory viral infections at the time of hospitalization could have important clinical implications [20]. Certain therapies, such as corticosteroids, are still widely misused in an effort to blunt the proinflammatory response to RSV. However, these data suggest that children with the most severe forms of RSV disease may in

fact be already immunosuppressed at the time of ICU admission, raising the possibility of worsening this immunosuppression with the addition of commonly prescribed steroids. Moreover, if the temporal relationship between immune suppression and clinical worsening is confirmed in subsequent studies, prospective immune monitoring using either TNF- α production or blood transcriptome analyses may become helpful tools to identify children with bronchiolitis at high risk for progressing to severe disease in the outpatient and inpatient settings. Finally, it also suggests the potential for the use of immune-stimulant agents such as IFN- γ or GM-CSF in this population. These drugs have been

used with some success in reversing innate immune suppression in critically ill adults and children.

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