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Interleukin (IL)-1β, IL-6 and IL-8 in nasal secretions: a common role for innate immunity in viral bronchial infection in infants?

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There is growing evidence to support a role for the immune response in lower respiratory tract infection (LRTI) due to viruses in children.[1,2] Respiratory syncytial virus (RSV) is the most frequent aetiological agent of LRTI, but other respiratory viruses (human metapneumovirus [hMPV], parainfluenza virus types 1, 2 and 3, influenza virus B, adenovirus types 1, 2 and 5, and mycoplasmas) can produce symptoms indistinguishable from those elicited by RSV.

Currently, however, there is controversy over the ability of the different viral agents to induce pro-inflammatory cytokines. Differences in the locally secreted cytokine profile in nasal washes between RSV and metapneumovirus infections have been described, as have similarities between RSV and influenza.[3] Additionally, immune function in neonates differs from that in adults.[4]

At birth, the immune system is not fully mature. Neonatal antigen-presenting cells tend to be deficient in interleukin (IL)-12. Furthermore, T cells secreting interferon (IFN)-γ (Th1 cells) are more likely to undergo apoptosis after antigen exposure. This might explain in part the Th2-skewed immunity in newborns. Neonatal immune cells appear unable to provide strong responses because neonatal antigen-presenting cells fail to up-regulate major histocompatibility complex (MHC) class II and co-stimulatory molecules. Antibody levels and classes are also different in early life to those found in adulthood.

Neonatal antibody is characterised by increasing levels of maternal immunoglobulin during the last trimester of pregnancy, which is replaced during the first year of life progressively by neonatal IgM, then IgG and finally IgA production. These special features of the neonatal immune system mean that young children are much more susceptible to infectious disease; however, with ageing, mortality due to infection decreases rapidly such that by the age of 10 years it is reduced 10- to 100-fold.[4]

Greater understanding of the immune response in LRTI is important for better understanding of the physiopathology of the condition, which may contribute to the development

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Table 1. Comparison of cytokine levels. Cytokine values are expressed as median and interquartile range.

	Group 1 (A) (n=8)	Group 2 (B) (n=14)	Controls for Group 1 (C) (n=6)	Controls for Group 2 (D) (n=11)	$P^{(a)}$	$P^{(b)}$	$P^{(c)}$
IL-6	502 (1033)	135 (296)	19 (33)	28 (34)	0.008*	0.001*	0.110
IL-8	8749 (8765)	3565 (3895)	540 (737)	913 (1479)	0.001*	0.001*	0.029*
IL-10	215 (457)	105 (206)	20 (114)	34 (64)	0.228	0.051	0.330
IL-2	33 (137)	16 (109)	12 (32)	12 (48)	0.414	0.687	0.714
IFNγ	36 (71)	14 (55)	3 (12)	14 (46)	0.345	0.536	0.868
IL-12p70	119 (283)	87 (114)	42 (108)	36 (200)	0.108	0.202	0.365
TNF-α	257 (295)	100 (266)	78 (198)	27 (154)	0.282	0.222	0.330
IL-1β	851 (5036)	704 (937)	104 (83)	124 (295)	0.013*	0.001*	0.664
IL-5	33 (63)	35 (75)	14 (36)	10 (17)	0.345	0.120	0.714
IL-4	18 (137)	40 (94)	22 (46)	18 (26)	0.573	0.095	0.664

P : Difference between groups (significance).
$P^{(a)}$: Difference between (A) and (C).
$P^{(b)}$: Difference between (B) and (D).
$P^{(c)}$: Difference between (A) and (B).
*$P<0.05$.

of better treatment strategies. This is particular important in severe cases of LRTI in young children.

This study examines the cytokine profile in nasal washes from children under the age of one year, diagnosed with severe LRTI that showed bronchial involvement and clinical findings of viral respiratory disease. A control group showing no respiratory disease is used for comparison.

The patient group comprised children up to the age of 12 months who needed hospitalisation in the paediatric department of the Hospital Clínico Universitario, Valladolid, Spain, from February to May 2005, all of whom had signs of viral bronchial illness (tachypnoea, prolonged expiratory time, wheezing, rales, chest retractions, dyspnoea of sudden appearance, fever).[5] Those children who showed acute and severe presentation were recruited for the study (n=22).

The decision to hospitalise was made independently by the attending physicians on the basis of clinical findings alone (e.g., respiratory distress requiring oxygen therapy, poor feeding with signs of dehydration and/or apnoea). Evaluation of clinical severity was performed at admission following the modified Wood`s Clinical Asthma Score (M-WCAS).[5]

Those children under one year old with no respiratory or inflammatory pathology admitted during the observation period were included in the study as age-matched controls. In all cases, parent or other legal permission was requested prior to sample extraction and this was recorded in the patient's history.

Nasopharyngeal aspirate samples were obtained within 24 hours of admission to hospital by gently flushing the infants' nostrils with 4 mL sterile saline solution. Secretions were divided into aliquots, snap frozen immediately in dry ice and then stored at –70°C until tested.

Viral presence was diagnosed in infected infants and excluded in controls on nasopharyngeal aspirates by direct immunofluorescence staining (Imagen, Dako, Denmark) for RSV, adenovirus, parainfluenza types 1, 2 and 3, and influenza A and B after viral culture on MDCK, LLCMK2, A549 and Hep2 cells.

After centrifugation of nasal samples at 5000 rpm in a microfugue (Eppendorf), cytokine levels were determined in the supernatants using the FlowCytomix multiplex human Th1/Th2 10plex kit (Bender) and five-colour flow cytometry (Cytomics, Beckman Coulter).

Table 2. Characteristics of patients in Groups 1 and 2. Values are expressed as median and interquartile range (except Gender and Prematurity).

	Group 1 (n=8)	Group 2 (n=14)	P value
Age (months)	1.5 (2.2)	7 (5.25)	0.005*
Gender (male/female)	3/5	8/6	0.440
Severity (M-WCAS score)	4 (2.1)	3.25 (3.75)	0.330
Days in hospital	6.5 (3.5)	4 (3.5)	0.188
Weight (grams)	4510 (2655)	7200 (2877)	0.008*
C reactive protein (mg/dL)	4.2 (8.9)	8.95 (37.85)	0.297
Prematurity (term/premature)	6/2	9/5	0.604

P : Difference between groups (significance). *$P<0.05$.

Differences in infant characteristics and levels of cytokines were analysed for significance by a non-parametric test (Mann-Whitney). Children with clinical signs of viral bronchial infection who were RSV-positive (Group 1, n=8, age median [IQR]: 1.5 [2.2] months) were compared with an age-matched control group (n=6, age median [IQR]: 2 [1] months). The differences in ages between the RSV-positive group and the control group were not statistically significant by the Mann-Whitney test (P=0.852).

The group of children with clinical signs of viral bronchial infection but negative for the screened viruses (Group 2, n=14, age median [IQR]: 7 [5.2] months) was also compared with an age-matched control group (n=11, age median [IQR]: 4 [4] months). Once again, the differences in ages between Group 2 and the control group were not statistically significant by the Mann-Whitney test (P=0.536). The χ^2 test was used to compare the effects of prematurity and gender.

Respiratory syncytial virus was isolated in eight children, while 14 children were negative for all the viruses screened. A common pattern in the profile of nasal cytokine secretion in young children suffering from severe LRTI was apparent. When the children in Group 1 were compared with its age-matched control group, IL-1β, IL-8 and IL-6 levels were higher in the former ($P=0.008$, $P=0.001$ and $P=0.013$, respectively; Table 1). When similar comparison was made between Group 2 and its age-matched control group, IL-1β, IL-8 and IL-6 levels were also higher in the former ($P=0.001$, Table 1). In addition, when Groups 1 and 2 were compared, IL-8 levels were significantly higher in Group 1 patients ($P=0.029$, Table 1). As shown in Table 2, differences in the M-WCAS score for severity between Groups 1 and 2 were not statistically significant; thus, differences between cytokine levels in these groups could not be attributed to differences in clinical severity.

IL-1β, IL-8, and IL-6 are produced during the very early stages of infection.[6] IL-1β stimulates almost all local and systemic inflammatory responses. IL-6, which can be induced by IL-1β, is pyrogenic, induces the liberation of acute-phase reactants by the liver, and, in turn, switches off pro-inflammatory cytokine production. While IL-1β mediates the initial adhesive reaction of neutrophils to the endothelium, IL-8 appears to be essential for the directed migration of leucocytes into infected tissue. Thus, it is not unexpected that these cytokines play an important role independently of the causative viral agent in children under the age of one year (with an immature immune response and underdeveloped specific responses).

Laham *et al*. previously reported lower pro-inflammatory cytokine production in human metapneumovirus infection than in RSV and influenza infections.[3] On the basis of these differences, and given that these viruses lead to identical clinical manifestations, they concluded that innate inflammation is not critical for the induction of respiratory symptoms in viral respiratory diseases.

However, Laham's study compared only infected patients, and it is important to include non-respiratory pathology control groups in any study of cytokine secretion profiles. In the present study, despite the finding of higher levels of IL-8 in Group 1, the inflammation-related mediators (IL-1β, IL8, IL6) were clearly elevated in Groups 1 and 2, compared with their respective age-matched controls. Both groups showed clinical signs of viral infection, despite the fact that those in Group 2 were negative for the viruses screened. However, the virus culture methods employed are less sensitive than are polymerase chain reaction (PCR)-based methods.

The small number of patients studied here, together with the number of parameters measured, means that larger studies are required to limit the possible role of type 1 and type 2 statistical errors, and such studies are currently underway. Nevertheless, the immature immunological status of the studied children supports a possible role for these innate factors of immunity in RSV-mediated disease. Comparison of cytokine profiles between patients with mild and severe disease is also needed.

Why some children present with severe disease while others do not remains unexplained. However, genetics seems to explain, at least in part, the different clinical pictures (i.e., the presence of mutations in the Toll-like receptor family).[7] This particular aspect deserves further study.

Finally, IL-1β and IL6, together with the chemokine IL-8, have an important role in mediating viral clearance; however, they may also mediate immune-mediated pathogenesis, leading to exacerbation of the inflammatory response in the bronchial tree. Some authors have proposed immunomodulatory approaches to treat the inflammatory component in LRTI caused by RSV.[8,9]

On the basis of the results presented here, this approach deserves further study, not only in RSV infection but also in those patients with non-identified viral agents who show clinical criteria of viral LRTI (at least in children less than a year old).

In conclusion, the high levels of IL-1β, IL-6 and IL-8 in nasal aspirates reveal the important role played by innate immunity in bronchial viral disease in young children. This is independent of the causative viral agent

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