Plasma interferon-γ, interleukin-10 and soluble markers of immune activation in infants with primary adenovirus (ADV) and respiratory syncytial virus (RSV) infection

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ABSTRACT. Adenovirus (ADV) and respiratory syncytial virus (RSV) are etiological agents of acute respiratory tract infection in infants. Long-term prognosis of ADV infection includes severe lung damage, bronchiectasis and hyperlucent lung, while RSV infection is associated with development of recurrent wheezing and subsequent asthma. These differences may be related to differences in the primary immune responses elicited by these viruses. In this paper, we investigated the type of cytokine responses and the magnitude of immune activation in ADV and RSV infections in infants. We examined plasma concentrations of interferon-γ (IFN-γ), interleukin-10 (IL-10), soluble interleukin-2 receptor (sCD25) and soluble tumor necrosis factor receptor II (sTNFR-II) in previously healthy infants during the acute phase of primary ADV infection (n = 21) and RSV infection (n = 68), and in uninfected controls (n = 44). In ADV-infected infants, IFN-γ plasma levels were significantly higher than those observed in RSV cases and the control group ($p < 0.05$). RSV cases did not show any differences in IFN-γ plasma levels compared to the other groups. sCD25 levels were significantly higher in ADV- and RSV-infected infants than in controls ($p < 0.0001$), and higher in ADV than in RSV cases ($p < 0.05$). sTNFR-II levels were significantly higher in RSV- and ADV-infected infants than in controls ($p < 0.0001$, $p < 0.05$, respectively), and higher in RSV than in ADV infection ($p < 0.05$). No significant differences were observed in IL-10 plasma concentrations between the three groups. These results indicate that ADV and RSV infections in infants differ significantly with regard to the magnitude of production of interferon-gamma and soluble immune activation markers sCD25 and sTNFR-II. These immunological differences may be involved in the different clinical outcomes associated with these viral infections.

Keywords: IFN-γ, IL-10, sCD25, sTNFR-II, adenovirus, respiratory syncytial virus

Viruses are the most common causative agents of lower respiratory tract infections in young infants worldwide [1]. Among them, respiratory syncytial virus (RSV) and adenovirus (ADV) are responsible for 30 and 5% of hospitalized cases, respectively [2, 3].

The acute phase of ADV and RSV infection is characterized by bronchopneumonia and obstructive bronchial syndrome [2, 3]. However, some clinical manifestations may help to distinguish these two viral infections. For example, during the acute phase of ADV infection high grade and prolonged fever, systemic inflammatory response with development of solid organ damage, such as hepatitis, and shock-like syndrome are frequently observed [4-6]. After recovery, ADV infection may lead to the development of chronic sequelae such as hyperlucent lung, bronchiectasis and severe lung damage [7-9]. In contrast, RSV infection is restricted to the respiratory tract and is more frequently associated with bronchiolitis [10]. Sequelae of RSV infection are related to recurrent wheezing, allergic sensitization and asthma [11, 12]. Clinical differences between ADV and RSV infections may be related to the type of cytokine responses triggered by these viruses.

Cytokines are involved in cell-cell communication and have been classified into Th1, Th2 and Th3 subfamilies. Th1 cytokines include interleukin (IL)-2, IL-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, whereas the Th2 cytokines include IL-4, IL-5, IL-6 and IL-13 [13, 14]. The Th3 subfamily is composed of IL-10 and transforming growth factor (TGF)-β [15, 16]. Cytokine expression is tightly regulated, because the pattern of cytokine expression can determine the outcome of the immune response [13]. It has been shown that peripheral blood mononuclear cells (PBMCs) infected *in vitro* with ADV produce predominantly IFN-γ and small amounts of IL-10 [17]. In the other hand, RSV-infected PBMCs produced only IL-10 in response to the infection [17]. These observations suggest

that ADV induces predominantly a Th1 type response while RSV induces preferentially Th2 and Th3 type responses. These findings were obtained in healthy, non-infected, children older than 5 years, in whom immune responses to these viruses are more likely to be immune memory due to re-infections rather than primary responses [17].

Sequelae of ADV and RSV infection may appear shortly after primary infection, suggesting that primary immune responses to these viruses play a role in the chronic pathology [7-9, 11, 12]. Currently, little is known about the differences between the primary immune responses of ADV and RSV infections. It has been shown that serum concentrations of the proinflammatory cytokine IL-6 are higher in ADV-infected infants than RSV cases, while no differences have been found in serum concentrations of IFN-γ [4]. More recently, it has been described that ADV-infected infants have significantly higher percentages of IFN-γ-producing PBMCs in comparison to RSV-infected cases [18].

In other viral infections, such as dengue fever and Hepatitis B, early immune activation is strongly correlated with development and severity of virus-induced immunopathology [19, 20]. In both infections, immune activation was analyzed examining plasma concentrations of soluble interleukin-2 receptor (sCD25) and soluble tumor necrosis factor receptor-II (sTNFR-II) [19, 20]. These markers are elevated in serum and bronchoalveolar lavage fluid of patients with lung inflammatory diseases such as extrinsic allergic alveolitis (EAA) and asthma [21-23]. The potential differences in levels of these molecules between ADV and RSV infections have not been explored.

In order to broaden our knowledge of the primary immune responses in ADV and RSV infections, we compared plasma concentrations of soluble immune activation markers sCD25 and sTNFR-II and the cytokines IFN-γ and IL-10 during the acute phase of primary ADV and RSV infections in young infants.

PATIENTS AND METHODS

Patients and viral diagnosis

The study protocol was approved by the Institutional Review Boards of Roberto del Rio and San Juan de Dios Hospitals, and University of Chile Faculty of Medicine. Previously healthy, term infants younger than 2 years old, were consecutively enrolled into the study during the winter seasons of 2002 and 2003. Patients were seen during their first episode of lower respiratory tract infection (presumably primary infection), and within the first seven days of respiratory symptoms (nasal discharge, cough or both). Age- and sex-matched controls were hospitalized for elective surgeries and were not infected with respiratory viruses (RSV, ADV, influenza or parainfluenza virus) and showed no respiratory pathology. Informed consent was obtained from the parents or guardians of all study participants. Viral infection was diagnosed by detection of viral antigens in nasopharyngeal aspirates by either an immunofluorescence assay or by viral isolation in cell culture as described previously elsewhere [5, 7].

Blood sample collection

Samples of 2-3 mL of whole blood were collected into heparinized tubes during the first days of hospitalization. The samples were kept at cold and delivered to the laboratory during the same day. Blood was centrifuged at 1500 rpm for 10 minutes at 4°C, plasma collected, aliquoted and frozen at -70°C until analyzed.

Quantification of cytokines and immune activation markers

Cytokines and receptors were measured from blinded samples using commercial sandwich ELISA Sets (IL-10, IFN-γ, sCD25 purchased at Becton Dickinson Pharmingen; sTNFR-II purchased at R&D Systems). The lower limits of detection were as follows: IFN-γ, 4.7 pg/mL; IL-10, 7.4 pg/mL; sCD25, 7.4 pg/mL; and sTNFR-II, 7 pg/mL.

Statistical analysis

Our primary endpoint was to compare cytokine and immune activation marker concentrations among virus-infected and controls, and between ADV- and RSV-hospitalized infants. The STATA 7.0 software was used for statistical analysis. Because cytokine and soluble receptor data showed skewing from the normal distribution, statistical analyses were completed after logarithmic (base 10) transformation of data, which established a normal distribution. Samples with detectable levels of cytokines and soluble receptors were used for statistical analysis. Differences between illness groups were assessed using analysis of variance. To enable comparisons with other studies, we provide the geometric mean values after transformation back from the mean $\log_{10}$ value. For all analyses, a P value below 0.05 was considered significant.

RESULTS

One of our initial goals was to compare plasma cytokine and soluble receptor concentrations in acute and convalescence phases of infections. However, the majority of patients did not visit the hospital for collection of a second blood sample, and only data for the acute phase were available for analysis.

We enrolled a total of 21 ADV and 68 RSV cases, and 44 control subjects. In all cases of RSV infection, viral diagnosis was made by immunofluorescence assay, while ADV infection was diagnosed by viral isolation in 50% of cases. As for clinical findings, bronchiolitis was five times more frequent in RSV infection than in the ADV group (16/68 *versus* 1/21 cases, respectively). The proportions of samples with detectable levels of cytokines and soluble cytokine receptors are summarized in *table 1*. In a proportion of samples, IFN-γ and IL-10 plasma concentrations were below detection limit in all groups. In contrast, sCD25 and sTNFR-II were detectable in all samples. sCD25 was not measured in 2 cases (one ADV and one RSV) due to the small amount of blood available. Comparison among the three groups of infants revealed statistical differences in all of them, but not in IL-10. Log_{10} values of plasma concentrations of these mediators are summarized in *table 2*. The range, distribution and geo-

Table 1
Proportions of samples with detectable levels of cytokines and immune activation markers in the three groups

Marker	Control	ADV	RSV
IFN-γ	33/44*	18/21	47/68
IL-10	28/44	13/21	37/68
sCD25	44/44	20/20	67/67
sTNFR-II	44/44	21/21	68/68

* Number of samples with detectable levels/total number of samples analyzed per group.

Table 2
Plasma levels of cytokines and soluble markers of immune activation in patients and controls

Marker	ADV	RSV	Control
IFN-γ (pg/mL)	41,7[a]	23,4	21,9
IL-10 (pg/mL)	18,6	19,1	19,1
sCD25 (ng/mL)	15,1[a]	12,6[b]	8,3
sTNFR-II (ng/mL)	9,8[b]	11,2[c]	8,6

Values represent geometric mean concentrations. Original data were normalized after logarithmical (log_{10}) transformation and comparison of the groups were made by analysis of variance.
[a] $p < 0.05$ *versus* RSV and control group.
[b] $p < 0.05$ *versus* control group.
[c] $p < 0.05$ *versus* ADV and control group.

metric means of the concentrations of IFN-γ, sCD25 and sTNFR-II are represented in *figures 1* and *2*.
IFN-γ levels in ADV-infected infants were significantly higher when compared to controls ($p < 0.05$) and RSV cases ($p < 0.05$) *(figure 1)*. No differences were observed in IFN-γ levels between RSV and the control group. sCD25 plasma concentrations in ADV-infected infants were significantly higher compared to the control group

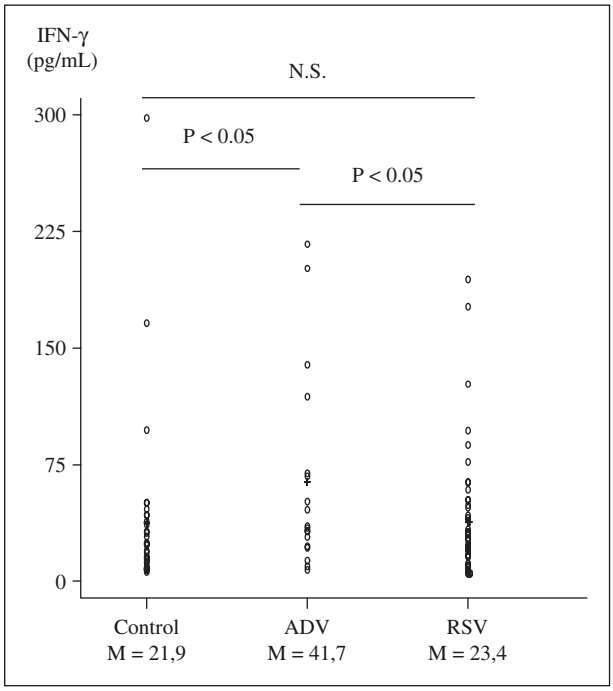

Figure 1
Plasma IFN-γ concentration in ADV and RSV infections, and non-virus- infected controls. N.S.: not significant. M: geometric mean concentration (pg/mL).

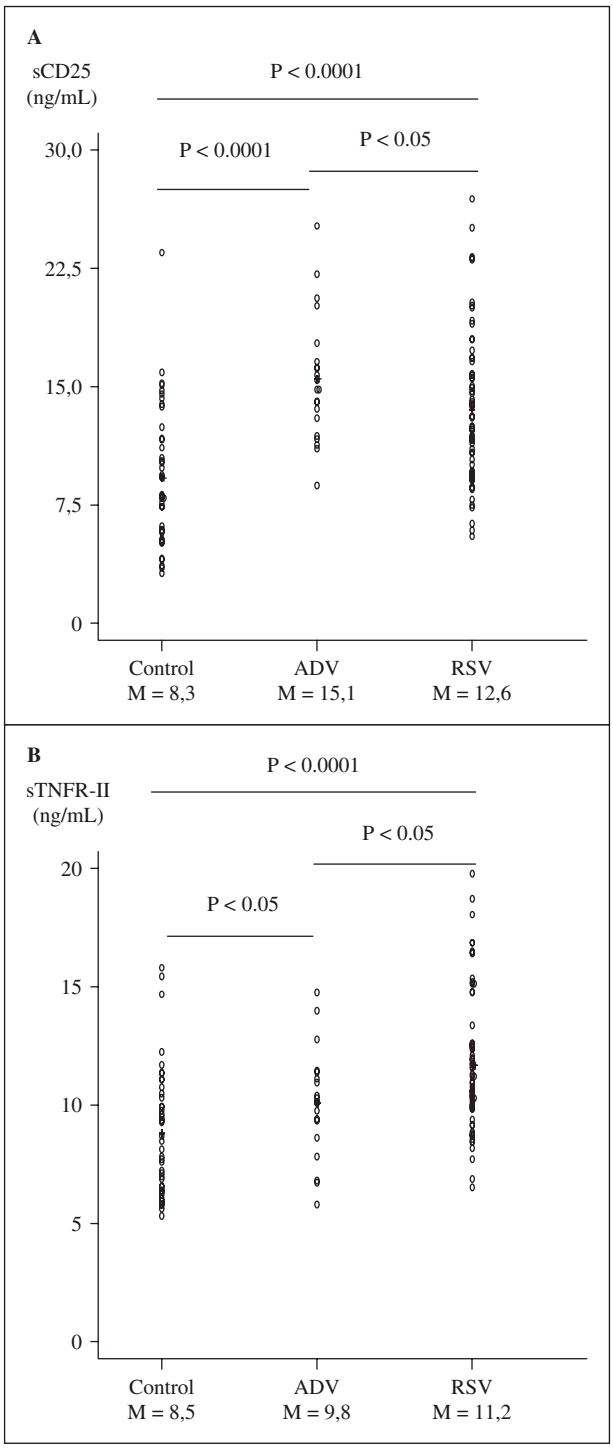

Figure 2
Plasma concentrations of soluble immune activation markers in ADV and RSV infections, and non-virus-infected controls. **A)** sCD25 levels; **B)** sTNFR-II levels. M: geometric mean concentration (ng/mL).

($p < 0.0001$) and RSV-infected infants ($p < 0.05$) *(figure 2A)*. sCD25 levels were also higher in RSV infection than the control group ($p < 0.0001$) *(figure 2A)*. sTNFR-II plasma concentrations were significantly higher in virus-infected infants ($p < 0.05$ for ADV and $p < 0.0001$ for RSV) when compared to the control group *(figure 2B)*. Moreover, sTNFR-II levels were higher in RSV cases than ADV-infected infants ($p < 0.05$) *(figure 2B)*.

DISCUSSION

Previous clinical studies on ADV and RSV infections have shown that acute illness is characterized by bronchopneumonia and obstructive bronchial syndrome, but RSV infection is more frequently associated with bronchiolitis [2, 3, 10]. Differences in primary immune responses elicited by these viruses may be related to different clinical manifestations. In ADV infection, systemic inflammatory manifestations of the acute phase are associated with increases in serum C-reactive protein and IL-6, which are not seen in RSV infection [4]. Besides these observations, little is known about the differences in primary immune responses between ADV and RSV infection in infants.

IFN-γ is a Th1 type cytokine involved in many immune processes such as macrophage activation, enhancement of antigen-processing and presentation, and direct antiviral activity [24]. Another relevant function of this cytokine is the inhibition of development of Th2-type responses [25, 26]. In this study, we observed that IFN-γ plasma levels in the acute phase of ADV infection are higher than in RSV infection. In line with our results, Aberle *et al.* have reported significantly higher percentages of IFN-γ-producing PBMCs in ADV-infected infants compared to RSV-infected cases [18]. A previous report by Kawasaki *et al.* did not find increases in serum IFN-γ levels in ADV-infected infants compared to RSV infection [4]. A possible explanation for this difference may be related to the prevalence of the ADV genotype in the populations studied. It is known that in our Chilean population, ADV 7h was the most frequent genotype isolated in hospitalized infants infected with ADV up to 1996, when ADV genotyping was discontinued [3]. Of interest, *in vitro* infection of PBMCs with different ADV genotypes has demonstrated that ADV 7h is more efficient than other ADV genotypes in inducing production of IFN-γ [17]. Future studies combining cytokine analysis and virus genotyping will help us to establish a role for ADV genotype in the severity and the type of immune response observed during primary ADV infection.

It has been found that peripheral blood, RSV-specific, T cells of infants can produce IFN-γ in response to *ex vivo* RSV stimulation [27, 28]. However, it has been demonstrated that G and/or SH proteins of the RSV envelope inhibit the production of IFN-γ in lungs of mice infected with the virus [29]. In the current study, we show that RSV-infected infants and healthy controls exhibit similar concentrations of IFN-γ in plasma. Aberle *et al.* found that percentages of IFN-γ-producing PBMCs stimulated with polyclonal activators did not differ between RSV-infected infants and controls and that IFN-γ production by these cells is lower in RSV infection when compared to other viral infections [18]. These observations are important since low production of IFN-γ by PBMCs in RSV infection is associated with development of a predominantly Th2-type response and subsequent asthma, a pathology driven by Th2 T cell responses [30-32].

In this study, we did a first analysis of IL-10 production in the acute phase of ADV in infants. We found no significant differences in comparison to RSV and control groups. The lack of differences in IL-10 plasma levels between RSV and control group agrees with previous studies by other investigators, in which LPS-stimulated monocytes, obtained during acute phase of RSV infection in infants, produce similar amounts of IL-10 when compared to healthy controls [33].

It has been shown that activation of the immune system is closely associated with the development and severity of tissue damage observed in certain viral infections, such as dengue and hepatitis B [19, 20]. Our current study is the first demonstration of the significant elevation of two, soluble immune activation markers, sCD25 and sTNFR-II, during the acute phase of ADV infection in infants, when compared to controls. As demonstrated in a previous report by others, sCD25 levels are elevated in RSV-infected infants [34]. Higher plasma concentrations of sCD25 in RSV-infected infants are associated with hypoxia and the need for hospitalization (J.A.F, unpublished results). Here, we found that elevation of sCD25 is significantly higher in ADV cases than in RSV-infected infants. Conversely, sTNFR-II is more elevated in RSV than in ADV infection. sCD25 is considered a marker of immune activation because it is expressed on the T lymphocyte membrane and is solubilized when T cells are stimulated by antigen or polyclonal activators [35]. Phytohemagglutinin-stimulated PBMCs, infected with ADV or RSV, were shown to decrease surface expression of CD25 on T lymphocytes, this effect being more pronounced in ADV infection [17]. It is possible that reduced CD25 surface expression on T cells may be the result of increases in proteolytic cleavage and solubilization of CD25 from the cell membrane. This hypothesis is supported by our results since we observed higher plasma levels of sCD25 in ADV-infected infants than in RSV cases. Tumor necrosis factor receptors (TNFRs) have been demonstrated to be expressed on a variety of cells and to be shed from the cell surface to form soluble TNFRs (sTNFR-I and sTNFRII), which retain the ability to bind TNF and inhibit the effects of TNF-α [36-40]. TNFR-II (p75/80 kDa, CD120b) is primarily expressed on hematopoietic lineage cells, including alveolar macrophages and lymphocytes [36, 37]. The finding that sCD25 levels are higher in ADV infection while sTNFR-II plasma concentrations are higher in RSV-infected infants suggests that these two viral infections differ in the pattern of activation of the immune system. In the current study, we found that the diagnosis of bronchiolitis was five times more frequent in RSV than in ADV infection. It is possible that the differences in plasma levels of IFN-γ, sCD25 and sTNFR-II between these infections may be related to these different clinical manifestations. Of interest is a prospective study conducted by Legg *et al.* who found that the development of bronchiolitis in RSV infection is associated with low production of IFN-γ [41]. Immune activation during the acute phase of ADV infection is accompanied by a marked production of IFN-γ, which in turn may inhibit development of Th2 type responses and subsequent asthma. Recently, it has been shown that RSV but not ADV infection associates with wheezing in infants [42]. This sharp difference supports a previous concept that certain viral infections in childhood have protective or harmful effects on wheezing disorders and asthma [43].

In conclusion, the current study demonstrates differences in the type of cytokine response and the pattern of immune activation between acute phases of primary ADV and RSV infections. These findings may help us understand the clinical spectrum of ADV and RSV infections and, possibly, other viral infections in infants.

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