

Soluble interleukin-2 receptor (sCD25) and interleukin-10 plasma concentrations are associated with severity of primary respiratory syncytial virus (RSV) infection

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ABSTRACT. The role of the immune response in the severity of RSV infection was examined by determining plasma concentrations of interferon- γ (IFN- γ), interleukin-10 (IL-10), interleukin-2 receptor (sCD25) and soluble tumor necrosis factor receptor II (sTNFR-II) in 196, previously healthy infants, during acute and convalescence phases of primary RSV infection. The results were analyzed separately for days 1-4 (early) and days 5-7 (late) of symptoms before sample collection and according to disease severity (105 hypoxic, 91 non-hypoxic). Significant associations between plasma levels and severity were found in early samples only. IL-10 and sCD25 concentrations were higher ($p=0.01$, each) in hypoxic compared with non-hypoxic infants, whereas no differences were observed in IFN- γ and sTNFR-II levels between the groups. Early sCD25 levels correlated positively with IL-10 concentrations ($p=0.0003$; $r=0.401$). Amongst the hypoxic infants, the number of days of oxygen supplementation correlated positively with early IL-10 levels ($p=0.009$; $r=0.495$) and negatively with the IFN- γ /IL-10 ratio ($p=0.007$; $r=0.495$). IFN- γ levels were significantly higher in the acute phase than during convalescence for hypoxic and non-hypoxic infants, while IL-10 levels were significantly higher in the acute phase only in hypoxic infants for days 1-4 (early; $p=0.0007$). sCD25 concentrations were elevated only in hypoxic infants at days 1-4 of the acute phase ($p=0.002$), whereas sTNFR-II levels did not vary between acute and convalescence phases, independent of severity and time point of sampling. We found no association between plasma levels during the convalescence phase and the severity of the RSV infection.

Keywords: respiratory syncytial virus, severity, interleukin-10, sCD25

Viral bronchiolitis is the leading cause of hospitalization of infants in the developed world and an important cause of mortality among children [1]. Respiratory syncytial virus (RSV), a member of the *Paramyxoviridae* family of RNA viruses, is associated with the majority of these cases [2]. RSV causes repeated infections throughout life that are especially serious in the elderly and in cardiac, pulmonary, and immunocompromised patients [3-5]. In infants, certain underlying conditions including prematurity, bronchopulmonary dysplasia, congenital heart disease and immunosuppression increase the risk of contracting and developing severe RSV disease [6]. Although 70 to 80% of previously healthy infants are infected with RSV in the first year of life, only a small proportion requires hospitalization due to hypoxia [6]. Airway size, passively acquired maternal IgG antibody, viral genotype and environmental factors such as household crowding are some determinants involved in this variation in clinical response to RSV [7-9]. Early vaccine trials using formalin-inactivated RSV as immunogen, resulted in enhanced disease in vaccinated

infants, indicating a role of the secondary immune response in the severity of RSV illness (reviewed in [10]). During recent years, the role of the host immune response, particularly cell-mediated immunity, in the pathogenesis of primary RSV infection has been demonstrated [11]. Cellular immunity to RSV involves both innate and adaptive immune responses, and includes natural killer (NK) cells, macrophages, and CD4⁺ and CD8⁺ T cells and cytokines. Cytokines are involved in cell-cell communication and activation, and those involved have been classified into Th1, Th2 and Th3 subfamilies. Type 1 cytokines such as IFN- γ , tumor necrosis factor (TNF)- β , interleukin (IL)-2, IL-12, and IL-18, promote cell-mediated immunity and are required for effective responses to intracellular pathogens including viruses [12]. Type 2 cytokines, such as IL-4, IL-5, IL-6 and IL-13, promote humoral immunity and mediate allergic responses [13]. Th3 type cytokines IL-10 and transforming growth factor (TGF)- β downregulate both Th1 and Th2 responses establishing a balance between cytokine effector functions [14, 15]. Deviation of

the immune response towards the type 2 pattern results in the increased severity of certain infectious and inflammatory diseases [16, 17], while increased production of Th3 cytokines can induce anergy [14, 15].

In studies of RSV infection of mice, the pattern of the cytokine response to infection has provided important information about the relationship between immunity and disease pathogenesis [18]. For example, RSV challenge of formalin-inactivated RSV (FI-RSV) vaccinated mice or mice immunized with recombinant viruses expressing the RSV G glycoprotein, was shown to result in a predominantly Th2-3 type immune response characterized by enhanced expression of IL-4, IL-5, IL-10 and enhanced pulmonary eosinophilia [19-22]. These studies in mice suggest that the enhanced disease observed in FI-RSV vaccine trials in infants may have been related to a skewed Th2-3 type cytokine response [23, 24].

It has been suggested that similar immune mechanisms mediate the severity of primary RSV infection in humans. Previous studies have shown significantly higher amounts of the chemokine MIP-1 α in the airways of infants with hypoxic RSV bronchiolitis compared with less severe forms of infection, while no association was seen between the production of Th2 cytokines IL-4, IL-5 and IL-13 and severity [25]. Conversely, nasopharyngeal samples from atopic infants showed excess type 2/deficient type 1-cytokine responses when they developed acute bronchiolitis, rather than upper respiratory infection alone [26]. These studies differ in their conclusions because of differences in their design, population of infants studied and time points during infection during which the cytokines have been analyzed. Studies in mice have shown that IFN- γ promotes RSV clearance in airways and, in humans, peripheral blood mononuclear cells (PBMCs) from infants with severe RSV bronchiolitis harbor lower levels of IFN- γ mRNA when compared to those with a milder clinical course [27, 28].

IL-10 is a Th3 cytokine with opposite effects to IFN- γ , but its role in RSV infection is not completely understood. It has been reported that IL-10 production is associated with severity of RSV infection; however, other reports do not demonstrate such an association [7].

In certain viral infections, the magnitude of immune activation is associated with the severity of the infection. For example, elevation of plasma levels of two markers of immune activation, such as soluble interleukin-2 receptor (sCD25) and soluble tumor necrosis factor receptor-II (sTNFR-II), is strongly correlated with tissue damage and disease progression [31, 32]. Interestingly, these markers were shown to be elevated in serum and bronchoalveolar lavage fluid of patients with extrinsic allergic alveolitis (EAA) and asthma, indicating a role for these molecules in airway inflammatory diseases [33-35]. Although it has been postulated that the severity of RSV infection is associated with immune mechanisms (chemokine production, Th2 imbalance), the role of the magnitude of the activation of the immune response in the severity of RSV infection has not been explored.

We therefore performed a study to investigate whether the severity of RSV infection is associated concomitantly with the magnitude of the immune activation and the Th1/Th3 imbalance of cytokine responses. In a randomly selected

population of previously healthy infants, we examined immune activation by analyzing plasma concentrations of soluble interleukin 2-receptor (sCD25) and soluble tumor necrosis factor receptor II (sTNFR-II) in infants with their first, RSV positive, respiratory infection, in samples taken at identical intervals from the onset of symptoms. In the same way, the type of immune responses was analyzed by determining IFN- γ and IL-10 plasma levels. Severity was defined by pulse oxymetry, clinical findings and the duration of supplementary oxygen requirement.

PATIENTS AND METHODS

Patients

The study protocol was approved by the institutional review boards of the Calvo Mackenna Hospital, University of Chile Faculty of Medicine, and the National Foundation for Development of Science and Technology-CHILE (FONDECYT). Previously healthy, term infants, less than one year old and seen within the first seven days of their first, proven RSV-positive infection, were consecutively enrolled into the study during the 2001 and 2002 RSV epidemics, both from the emergency room and the ward for infants at the Calvo Mackenna Hospital, Santiago. In all cases, informed consent was obtained from the guardian. RSV infection was diagnosed by detection of viral antigens in nasopharyngeal aspirates using ELISA (Becton Dickinson). The examination of the infant at enrollment included a pulse oxymetry reading. If the result was 95% or above in room air, the infant was considered to be non-hypoxic and was managed as an outpatient. If oxygen saturation was below 95% the infant was considered to be hypoxic, and was hospitalized and followed daily. In hospitalized infants, oxygen was administered until saturation in room air was 95% and the patient was discharged the following day. Outpatients were interviewed three weeks after enrollment to check that they did not need hospitalization. Within the non-hypoxic patients, two groups displaying increasing severity were distinguished: those with upper respiratory tract infection (URTI) only, and those with chest retractions/and or expiratory wheezing (lower respiratory infection, LRTI). Within the hypoxic infants, two groups displaying increasing severity were distinguished according to the length of the supplementary oxygen requirement. The less severe group of hospitalized infants had a duration of supplementary oxygen administration of less than the average of six days, and the more severe group of six days or more. Due to ethical considerations, we were not allowed to obtain blood samples from a control group composed of non-hospitalized, healthy, non-RSV-infected infants. However, we were able to obtain a blood sample during the convalescence phase (4 weeks after the onset of symptoms) from the same infants studied during the acute phase of RSV infection. Concentrations during the convalescence phase, in the absence of respiratory symptoms, were taken as values close to normal plasma levels of cytokines and soluble receptors.

Sample collection

Two, 2-3 mL samples of whole blood were collected into heparinized tubes on enrollment. The samples were kept

attached to a cold pack until delivered to the laboratory within 2 hours. Blood was centrifuged twice at 1500 rpm for 10 minutes at 4 °C, and plasma was 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 from 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. The results were analyzed according to duration of symptoms up to the day during which the blood sample was obtained, separately for days 1 to 4, and days 5 to 7 after the first day of symptoms. The first day of symptoms was counted as the first day of cough or nasal discharge and the day the sample was taken was omitted.

Statistical analysis

Statistical analysis was performed with the aid of Stat-View[®]5 software. Because cytokine and soluble receptors data showed skewing from the normal distribution, statistical analyses were performed after logarithmic (base 10) transformation of data, which established a normal distribution. If ELISA did not detect a particular cytokine in a specimen, the value of the lower limit of detection of that assay was used for statistical analysis. The comparisons between the hypoxic and non-hypoxic patients, and acute and convalescence phases levels were made using the t-test (continuous variables) and χ^2 (categories). To enable comparisons with other studies, we provide the geometric mean and median values. The relationships between oxygen saturation and days of supplementary oxygen administration, and the concentrations of the cytokines or immune response markers were explored by simple regression. Likewise, simple regression was used to examine the relationship between individual cytokines or immune response markers. The relationship of the concentrations of the cytokines and immune response markers with the four clinical categories of progressive severity was examined both by analysis of variance (ANOVA) and Spearman correlation coefficient. For all analyses, a P value below 0.05 was considered significant.

RESULTS

Patients and plasma cytokines

The patient characteristics at enrollment are presented in table 1. Patients were interviewed during the convalescence phase (median value 27 days after the beginning of symptoms; range 21-97 days), and a second blood sample was obtained. Following examination, no outpatient required hospitalization. Among the hypoxic infants, no patient required mechanical ventilation and no fatal cases were observed in any group. Cytokine levels in the acute phase of RSV infection were detectable in a proportion of patients. Interferon- γ was below the detection limit of the assay in 2/58, taken on days 1-4 samples (both outpatients), and in 1/57 samples taken on days 5-7 (hospitalized patient). Interleukin-10 was below the detection limit of the assay in 24/76 samples taken on days 1-4 (17 outpatients and 7 hospitalized), and in 28/78 samples taken on days 5-7 (8 outpatients and 20 hospitalized). Differences in the proportion of samples with detectable levels of IFN- γ and IL-10 between outpatients and hospitalized cases were not statistically significant (not shown).

Plasma concentrations of cytokines and soluble receptors according to disease severity are summarized in table 2. The number of samples analyzed for each marker is shown for each group of patients. Plasma IFN- γ levels were not significantly different between hypoxic and non-hypoxic infants. In contrast, IL-10 levels in samples from days 1-4 of symptoms were significantly higher in hypoxic infants compared with outpatients ($p=0.01$). The IFN- γ /IL-10 ratio between hypoxic versus non-hypoxic cases was not significantly different.

As for soluble receptors of immune activation, sCD25 levels were significantly higher in hypoxic patients com-

Table 1
Patients characteristics

Characteristic	Outpatients	Hospitalized	P values
N	91	105	
Age, months, mean	2.4	2.7	0.451
Females, N (%)	39 (43)	33 (31)	0.876
Pulse oxymetry, %	96.2	91.7	<0.0001
Cytokine sample was taken on			<0.0001
Day 1-4 of symptoms*, N (%)	60 (66)	36 (34)	
Day 5-7 of symptoms, N (%)	31 (34)	69 (66)	

* Nasal discharge, cough or both.

Table 2
Plasma levels of interferon- γ (IFN- γ), interleukin-10 (IL-10), soluble interleukin 2 receptor (sCD25) and soluble tumor necrosis factor receptor type II (TNFR-II) in infants infected with RSV

Marker	Days 1-4 of symptoms			Days 5-7 of symptoms		
	Non-hypoxic	Hypoxic	P value	Non-hypoxic	Hypoxic	P value
IFN- γ pg/mL	27.1 (28.6, 36)	41.5 (47.2, 22)	0.10	29.3 (36.2, 20)	25.6 (23.0, 31)	0.59
IL-10pg/mL	13.5 (10.1, 49)	20.9 (23.9, 27)	0.01	12.0 (8.9, 35)	13.5 (12.2, 33)	0.78
IFN- γ /IL-10	1.72 (1.6, 36)	1.92 (1.81, 22)	0.89	2.08 (2.06, 20)	1.65 (1.67, 30)	0.25
sCD25ng/mL	13.5 (14.2, 49)	16.1 (15.1, 26)	0.01	12.6 (11.5, 25)	14.3 (14.3, 53)	0.06
sTNFRng/mL	10.7 (13.0, 49)	10.9 (12.7, 26)	0.87	11.8 (13.9, 25)	11.0 (12.9, 53)	0.62

Values are geometric mean concentrations and, in parenthesis, the median values and the number of cases per group. Plasma samples were collected on days 1-4 and days 5-7 of symptoms. Original concentrations were normalized by logarithmic transformation (Log_{10}) and groups were compared by the t-test.

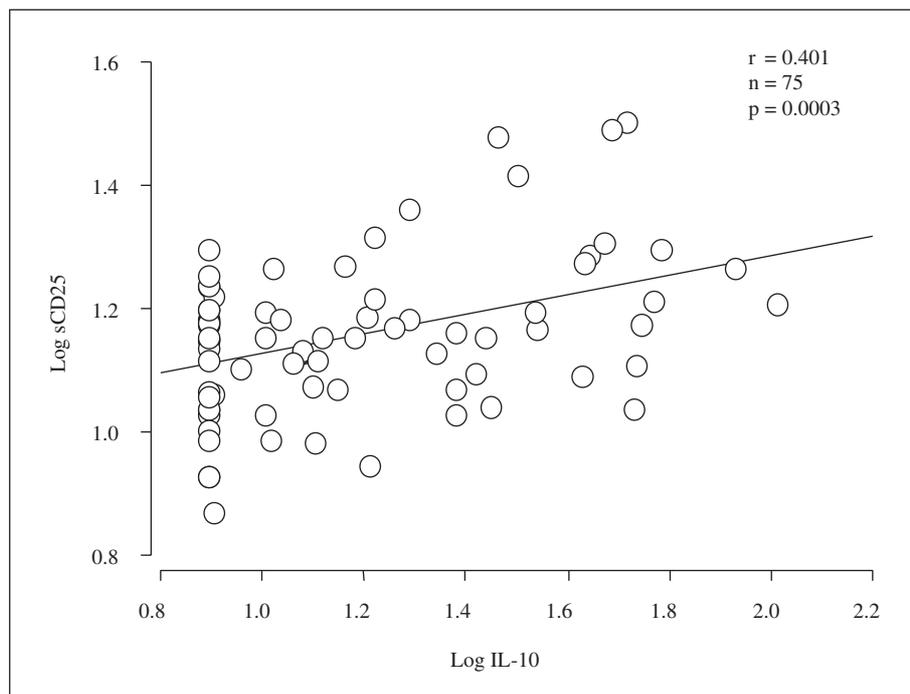


Figure 1

Positive correlation of sCD25 and IL-10 plasma levels. Values represent \log_{10} of concentrations in samples obtained Days 1-4 of symptoms.

pared with non-hypoxic cases, in samples taken on days 1-4 ($p=0.01$). In addition, the sCD25 concentrations from days 5-7 of symptoms showed a statistically significant trend toward being higher in hypoxic patients ($p=0.06$) compared to non-hypoxic cases. In all cases, we observed a significantly positive correlation ($p=0.0003$, $r=0.401$) between circulating levels of sCD25 and IL-10 in samples taken from days 1 to 4 of symptoms (*figure 1*).

In contrast to sCD25, sTNFR-II levels in plasma were similar in hypoxic and non-hypoxic patients ($p>0.05$). When all samples, i.e. 1 to 7 days, were considered for comparison between both groups of infants, only sCD25 levels were seen to be significantly higher in hypoxic patients ($p=0.01$, not shown).

Plasma cytokine and soluble receptors levels in acute and convalescence phases of RSV infection according to disease severity, are shown in *table 3*. In early and late samples, IFN- γ levels were significantly higher in the acute phase than during convalescence for both hypoxic and non-hypoxic infants. IL-10 levels were significantly higher in the acute phase than during convalescence only in hypoxic cases of days 1-4 (early; $p=0.0007$). In non-hypoxic infants of days 1-4, and in all cases of days 5-7 (late), IL-10 levels were not significantly different when acute and convalescence phases were compared. Considering all of the infants studied, the IFN- γ /IL-10 ratio was significantly higher in the acute phase of infection than during convalescence. Plasma sCD25 concentrations were significantly higher in the acute phase only in early samples (days 1-4) of hypoxic cases ($p=0.002$), whereas levels in non-hypoxic infants were similar in both phases of infection, independent of the number of days prior to sample collection. sTNFR-II levels did not vary between acute and convalescence phases, independent of severity and time point of sampling. We did not find associations

between severity and plasma concentration of any marker measured in the convalescence phase (data not shown).

Cytokines and soluble receptors in plasma according to form of illness

Differences in early (1-4 days of symptoms) plasma concentrations of cytokines and soluble receptors between clinical categories are shown in *figure 2*. sCD25 levels were significantly higher in the most hypoxic group of infants when compared to the URTI and wheezing groups ($p=0.04$ and 0.01 , respectively). IL-10 concentrations were significantly higher in the most hypoxic group in comparison to the URTI group ($p=0.003$). A clearly positive correlation was found between IL-10 levels and increasing severity of RSV disease (Spearman coefficient $p=0.002$). The IFN- γ /IL-10 ratio was significantly lower in the non-hypoxic wheezing group and the most hypoxic cases, in comparison to infants with less than six days of hypoxia ($p=0.049$ and 0.02 , respectively). When we compared clinical categories considering plasma levels of samples taken days 5-7 after onset of symptoms (*figure 3*), only sCD25 concentrations showed significant differences, being higher in hypoxic infants in comparison to the URTI group ($p=0.02$). We found no significant differences ($p>0.05$) in IFN- γ and sTNFR-II levels between the clinical categories (not shown).

Relationship between cytokines and degree of hypoxia

We also determined the relationship between the concentration of each cytokine and soluble receptor in plasma with the degree of hypoxia as determined in hypoxic infants by the number of days during which oxygen supplementation was needed. We found a positive correlation between IL-10 levels and days of oxygen supplementa-

Table 3
Plasma cytokine and soluble receptors levels in acute and convalescence phases of RSV infection according to disease severity

Marker	Samples	Acute	Convalescence	P
INF- γ (pg/mL)	<i>Days 1-4</i>			
	Hypoxic	41.5 (47.3; 22)	13.2 (9.7; 30)	0.004
	Non-hypoxic	27.1 (28.6; 36)	13.8 (7.1; 41)	0.025
	<i>Days 5-7</i>			
IL-10 (pg/mL)	Hypoxic	25.6 (23.0; 37)	11.4 (10.1; 51)	0.0008
	Non-hypoxic	29.3 (36.2; 20)	13.3 (12.0; 24)	0.012
	<i>Days 1-4</i>			
	Hypoxic	20.9 (23.9; 27)	13.4 (13.2; 30)	0.0007
INF- γ /IL-10	Non-hypoxic	13.5 (10.1; 49)	11.0 (7.8; 41)	0.528
	<i>Days 5-7</i>			
	Hypoxic	13.5 (12.2; 53)	12.4 (8.9; 51)	0.6293
	Non-hypoxic	13.0 (8.9; 25)	13.7 (7.8; 24)	0.386
sCD25 (ng/mL)	<i>Days 1-4</i>			
	Hypoxic	1.92 (1.81; 22)	0.99 (0.60; 30)	0.032
	Non-hypoxic	1.72 (1.60; 36)	1.26 (0.70; 41)	0.06
	<i>Days 5-7</i>			
sTNFR-II (ng/mL)	Hypoxic	1.65 (1.67; 37)	0.91 (0.84; 51)	0.007
	Non-hypoxic	2.08 (2.06; 20)	0.97(0.83; 24)	0.036
	<i>Days 1-4</i>			
	Hypoxic	16.1 (15.1; 26)	12.5 (12.1; 29)	0.002
sCD25 (ng/mL)	Non-hypoxic	13.5 (14.2; 49)	12.8 (12.5; 41)	0.75
	<i>Days 5-7</i>			
	Hypoxic	14.3 (14.3; 53)	12.6 (12.3; 51)	0.06
	Non-hypoxic	12.6 (11.5; 25)	12.3 (12.1; 24)	0.161
sTNFR-II (ng/mL)	<i>Days 1-4</i>			
	Hypoxic	10.9 (12.7; 26)	9.9 (12.2; 29)	0.766
	Non-hypoxic	10.7 (13.0; 41)	10.3 (11.8; 41)	0.595
	<i>Days 5-7</i>			
sCD25 (ng/mL)	Hypoxic	11.0 (12.9; 53)	11.9 (12.7; 51)	0.42
	Non-hypoxic	11.8 (13.9; 25)	11.5 (12.1; 24)	0.609

Values are geometric mean concentrations and, in parenthesis, the median values and number of cases. Original concentrations were normalized by logarithmic transformation (Log_{10}) and groups were compared by the t-test.

tation ($p=0.009$, $r=0.495$; *figure 4A*). Moreover, the IFN- γ /IL-10 ratio correlated inversely with the number of days of oxygen supplementation ($p=0.007$, $r= - 0.561$; *figure 4B*). There was no significant association between sCD25 levels and days of supplementary oxygen requirement ($p>0.05$, data not shown).

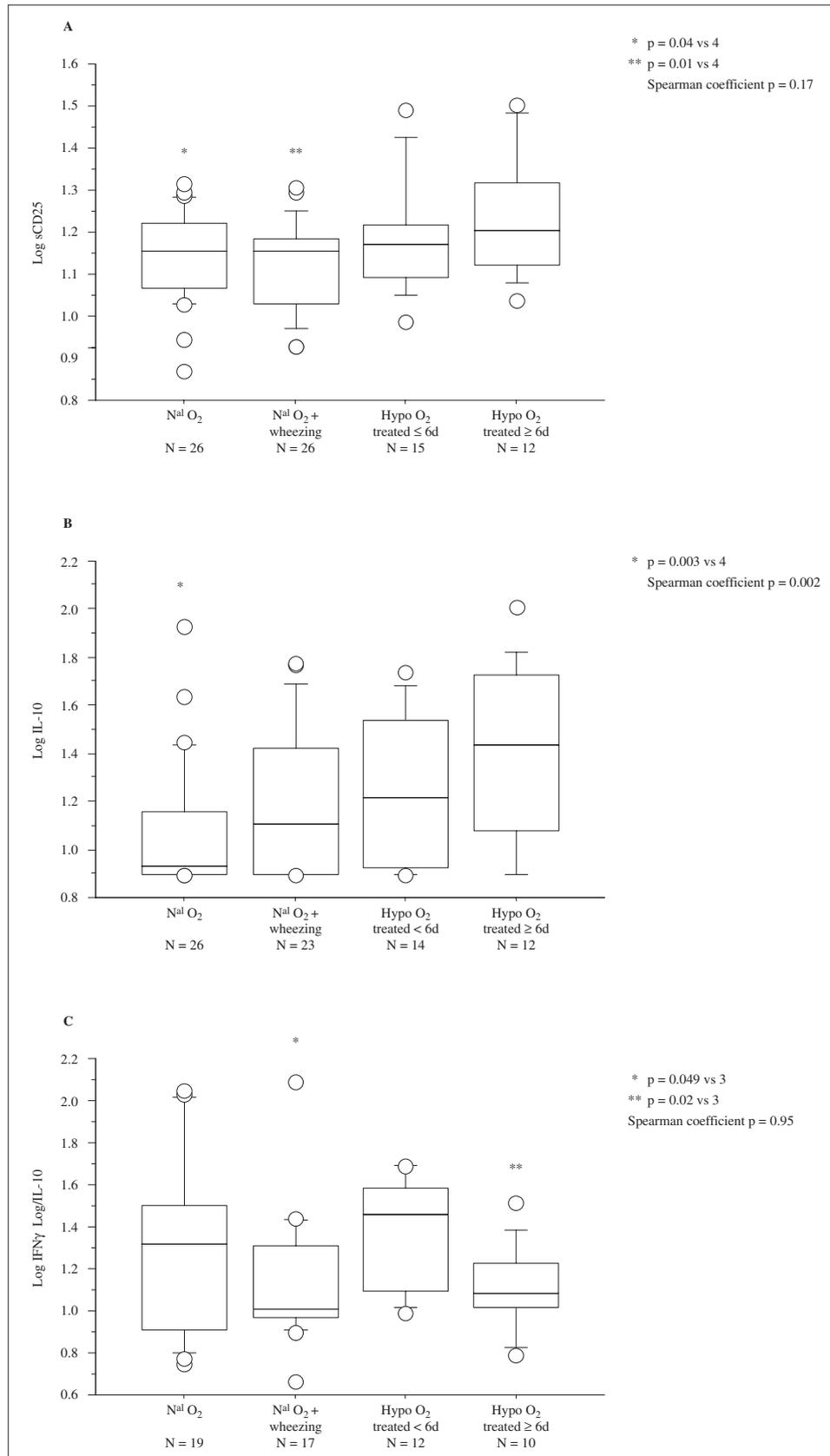
DISCUSSION

Several lines of evidence indicate that the severity of RSV infection is associated with host cytokine responses. In this paper, we have demonstrated that the severity of primary RSV infection is associated with the enhanced magnitude of immune activation and increased IL-10 production. Since cytokine responses to acute respiratory syncytial virus infection are known to fluctuate during the first days of infection, with varying time courses depending on the cytokines measured [26], we compared cytokine and receptor levels between samples obtained the same number of days after onset of symptoms of infection. Association of immune markers with severity was mainly seen early in infection, i.e., 1-4 days after onset of symptoms, and not seen in the convalescence phase. The relation between immune activation and RSV severity is manifested in plasma sCD25 levels. sCD25 concentrations were significantly elevated in infants who required hospitalization due

to hypoxia, whereas levels in non-hypoxic infants were normal. Elevated levels of sCD25 in hypoxic infants decreased significantly during the convalescence phase, in the absence of symptoms.

sCD25 is considered to be 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 [36]. sCD25 serum levels are elevated in the acute phase of RSV infection and in immune-mediated lung pathologies, such as extrinsic allergic alveolitis and asthma [34, 35, 37]. In view of our results, it is possible to argue that a greater T cell activation is involved in the greater severity of RSV infection. In this case, enhanced T cell activation may have been produced by a higher antigen load, considering that higher nasopharyngeal RSV titers or impaired RSV clearance correlate with disease severity in RSV infection in previously healthy infants [38].

In an earlier report, we showed that levels of the immune activation marker sTNFR-II are also elevated in the acute phase of RSV infection [39]. In this study however, we did not observe a relationship between plasma levels of sTNFR-II and severity of RSV illness. This observation suggests that only certain molecules of immune activation play a role in the severity of RSV disease, or that our samples were taken at a time point during which concentrations of this marker are not associated with a clinical

**Figure 2**

Levels of IL-10, IFN- γ and sCD25 in plasma samples obtained Days 1-4 of symptoms in RSV-infected infants of clinical categories with increasing severity. (1) Non-hypoxic, upper respiratory tract infection; (2) Non-hypoxic wheezing; (3) Hypoxic bronchiolitis, required less than six days of oxygen supplementation; (4) Hypoxic bronchiolitis, required six or more days of oxygen supplementation. N indicates the number of cases per group. The box contains 50% of the results, the line within the box in the median value of \log_{10} of individual concentrations and the dots are results outside the median plus two standard deviations (vertical lines). Comparisons among groups were made by analysis of variance (ANOVA). Spearman correlation coefficients represent correlation between ranks of severity and results of cytokine concentrations. IL-10 levels correlated positively with increasing severity of RSV disease (Spearman coefficient $p=0.002$).

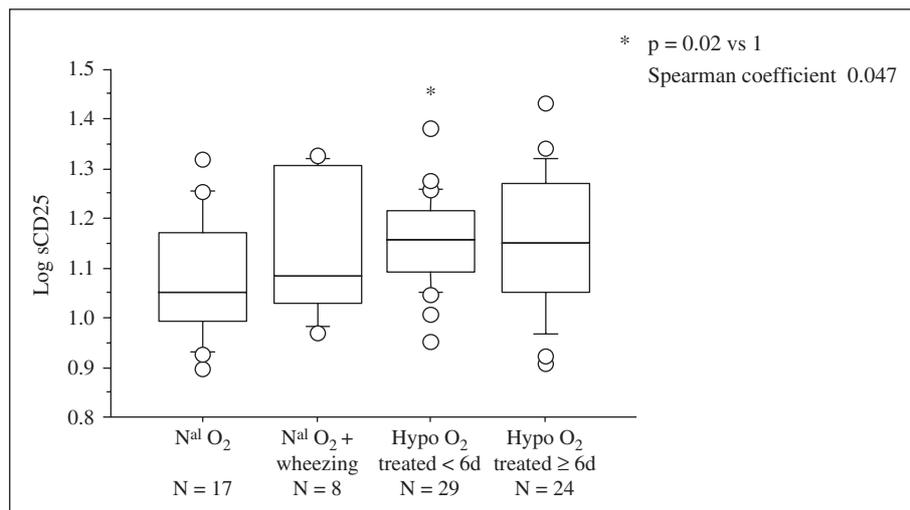


Figure 3

Levels of sCD25 days in plasma samples obtained Days 5-7 of symptoms in RSV-infected infants of clinical categories with increasing severity. (1) Non-hypoxic, upper respiratory tract infection; (2) Non-hypoxic wheezing; (3) Hypoxic bronchiolitis, required less than six days of oxygen supplementation; (4) Hypoxic bronchiolitis, required six or more days of oxygen supplementation. n indicates the number of cases per group. The box contains 50% of the results, the line within the box in the median value of \log_{10} of individual concentrations and the dots are results outside the median plus two standard deviations (vertical lines). Comparisons among groups were made by analysis of variance (ANOVA). Spearman correlation coefficients represent correlation between ranks of severity and results of cytokine concentrations. sCD25 levels correlated positively with increasing severity of RSV disease (Spearman coefficient $p=0.047$).

response. In the current study, we observed that sTNFR-II concentrations did not vary between acute and convalescence phases. Probably, plasma levels of this soluble receptor normalize later than the time point at which we determined the convalescence values (median 27 days after onset of symptoms).

IL-10 is a product of various cell types including Th3 cells and monocytes, and possesses a wide range of activities [40]. IL-10 can inhibit IFN- γ synthesis by suppressing IL-12 production by activated dendritic cells and macrophages [40]. In these cells, it can down regulate MHC class I- and II-associated antigen presentation, affecting cellular immune responses [40]. In our current study, we found an association between IL-10 levels and the severity of RSV infection. IL-10 levels were elevated only in hypoxic infants early in infection (days 1-4) and correlated negatively with length of oxygen supplementation. Increased IL-10 plasma concentrations in these hypoxic infants normalized during the convalescence phase. It is possible that IL-10 production in RSV infection inhibits IFN- γ synthesis, thus impairing viral clearance. In addition, IL-10 could down-regulate anti-RSV T cell immunity. It has been shown that T cell responses in infants are protective against RSV infection and that subjects with deficiencies in cellular immune responses develop severe clinical forms of RSV infection [3, 41]. Interestingly, we found a positive correlation between IL-10 and sCD25 levels, with the highest concentrations in the most severely affected group of patients for both markers. It has been shown that production of IL-10 during activation of T lymphocytes propels these cells into a state of anergy or hyporesponsiveness, without exerting their effector functions [40]. This hyporesponsiveness state could explain the impaired virus clearance reported for the more severe forms of RSV disease [38]. Our results help to broaden our knowledge of the role of IL-10 in RSV infection. Previous studies had shown that increased IL-10 levels produced by PBMCs obtained during the convalescence phase of RSV infection correlated

with the development of recurrent wheezing after one-year of infection [42]. Our study provides the first evidence that heightened IL-10 production correlates with severity of the acute phase of primary RSV infection. Our results are supported by previous observations in which IL-10/IL-12 ratios in nasopharyngeal aspirates were higher in URTI than in bronchiolitis [26]. In addition, a genetic polymorphism in the IL-10 gene promoter has been associated with severity of RSV infection [43]. In a previous study, IL-10 levels in nasopharyngeal aspirates from RSV-infected infants were higher in intubated patients compared with non-intubated cases [29]. However, infants requiring mechanical ventilation during RSV infection have been shown to exhibit several defects in cellular immunity [44]. Therefore, it may not be possible to distinguish clearly, if reduction of levels of a particular cytokine in these patients is a trait associated with their concurrent immune defects or a specific response to RSV infection. Moreover, other studies have found no differences in IL-10 production in NPA between intubated and non-intubated patients with RSV infection [30].

IFN- γ is a Th1 type cytokine involved in many immune processes such as macrophage activation, enhancement of antigen processing and presentation, and inhibition of development of Th2-type responses [45]. IFN- γ also exerts direct antiviral activity against many viruses, including RSV [27, 45]. The protective role of IFN- γ in human RSV infection has been previously demonstrated. For example, IFN- γ production is lower in URTI cases than in bronchiolitis, and lower levels of IFN- γ mRNA are found in PBMCs from infants with severe RSV bronchiolitis compared to those with a milder clinical course [28]. In another study, infection in a population of infants without a family history of atopy, percentages of IFN- γ -producing, CD8+ T cells were higher in infants with mild RSV disease compared to severe cases [46]. In those same infants, IFN- γ plasma levels were not significantly different between infants with mild and severe RSV disease [46]. In our

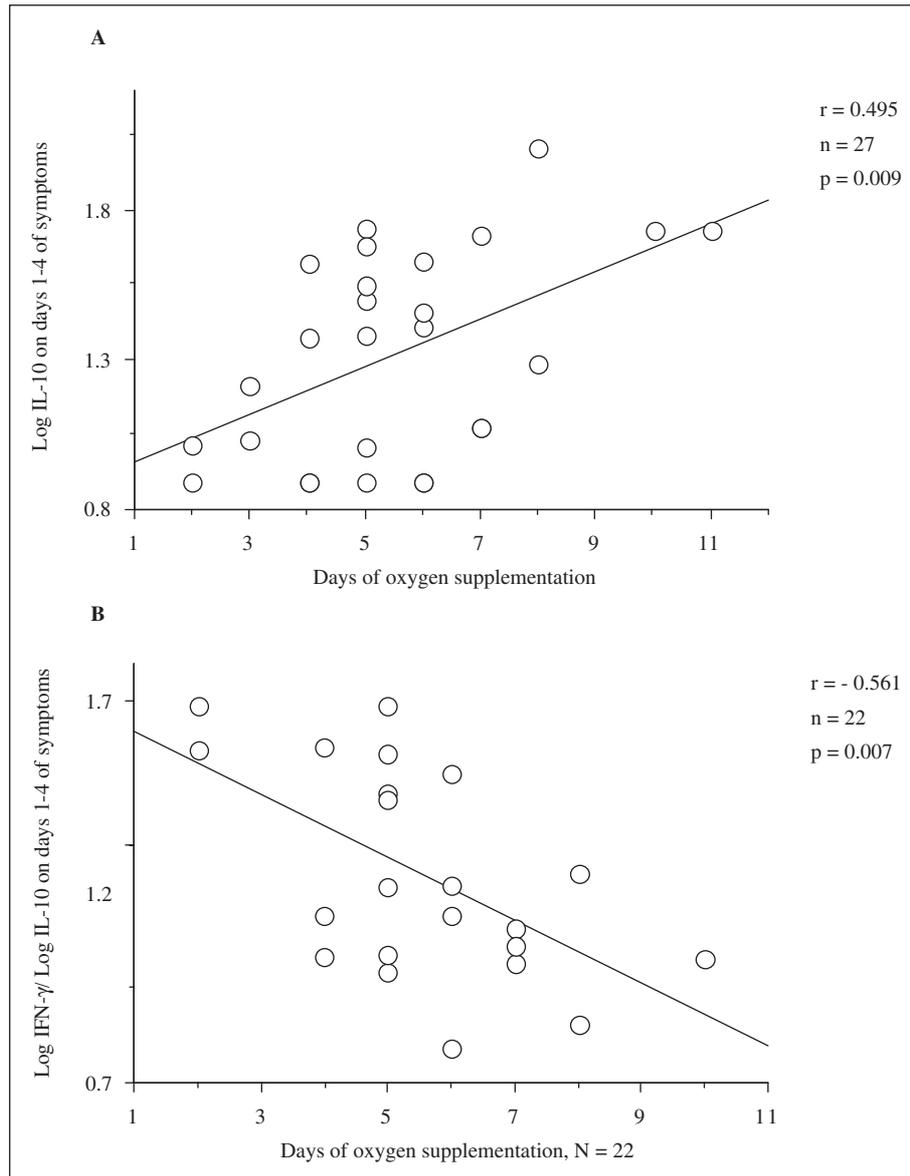


Figure 4

Relationship of the interleukin 10 (A) and interferon- γ /IL-10 ratio (B) in plasma to the degree of hypoxia, as determined by length of supplementary oxygen requirement in hospitalized infants with respiratory syncytial virus bronchiolitis. Points represent \log_{10} of cytokine concentrations Days 1-4 or ratios vs. days of oxygen supplementation.

study, we found that IFN- γ plasma levels were higher in the acute than in the convalescence phase, but we did not find an association between IFN- γ levels and RSV severity. Taken together, these observations suggest that protective role of IFN- γ in RSV infection may not be demonstrable by examining plasma concentrations, but becomes evident when intracellular production of IFN- γ is determined in stimulated PBMCs.

Despite of this, we found that the IFN- γ /IL-10 ratio (Th1/Th3 ratio) in plasma is associated with severity of RSV disease. We observed a significant, inverse correlation between IFN- γ /IL-10 ratios and duration of oxygen supplementation. Intriguingly, the IFN- γ /IL-10 ratio was significantly lower in infants with wheezing when compared to infants with URTI and those with mild bronchiolitis. It is possible that infants with wheezing are among those non-hypoxic, RSV-infected infants predisposed to produce low amounts of IFN- γ but comparatively high IL-10 levels. Wheezing exhibits clinical similarities with

asthma, a pathology that is known to be associated with low production of IFN- γ [47]. In addition, IL-10 production is increased in infants with recurrent wheezing following RSV infection [42].

In a previous study, we found that IL-10 plasma levels during the acute phase of RSV infection were similar to concentrations observed in a control group of non-virus-infected infants [39]. It is possible that older age and the underlying pathology in the control group of that study (infants up to 2 years old hospitalized for elective surgery), and the lack of categorization of RSV cases according to duration of symptoms prior to sample collection, may explain why we did not previously detect an association between IL-10 levels and RSV disease. Future prospective studies could help us to better define basal concentrations and more subtle variations in levels of cytokines, such as IL-10 and IFN- γ , which are difficult to demonstrate in a cross-sectional analysis. For instance, we have currently studied previously healthy infants younger than one year

old and found that IL-10 plasma levels in hypoxic infants, early in the acute phase of RSV infection, are higher than those observed in the same infants during the convalescence phase. This observation further supports our current finding that IL-10 production is involved in disease severity in infants acutely infected with RSV.

Early IL-10 and sCD25 plasma concentrations may have a significant predictive value for severe disease (hypoxia). Because the range of the results in this study is wide, the usefulness of these indicators should be tested further in a prospective clinical trial. Such indicators would be effective tools for determining the patients most in need of specific treatment, when available. To date, there is no safe, anti-RSV vaccine and treatment is expensive and mainly reserved for infants with underlying risk factors for severity [49, 50]. A number of studies have indicated that inhibition of IL-10 production or IL-10 receptor activity is an effective therapeutic strategy for treatment of infectious or tumor pathologies in which IL-10 plays a central role in the pathogenesis [51, 52]. Our results suggest that an approach aimed in blocking IL-10 effects could be beneficial in RSV infection. The understanding of immunological mechanisms involved in the severity of RSV disease may be useful for designing potential immunotherapy against this infection.

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