T Helper 1/T Helper 2 Cytokine Imbalance in Respiratory Syncytial Virus Infection Is Associated With Increased Endogenous Plasma Cortisol

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ABSTRACT

OBJECTIVE. Cellular immunity has classically been described as the defense mechanism for viral infections. The development of cellular or humoral immune responses will depend on a repertoire of cytokines produced by numerous cells, including $CD4^+$ and $CD8^+$ T cells. These lymphocytes can be subdivided into 2 subsets, T helper 1 (Th1) and Th2, on the basis of the cytokine profiles they synthesize. Type 1 T cells produce interferon γ (IFN-γ), an essential cytokine in the viral cell-mediated immune response. Th2 cells selectively produce interleukin 4 (IL-4) and IL-5 that participate in the development of humoral immunity and have a prominent role in immediate-type hypersensitivity. An imbalance in the Th1/Th2 cytokine immune response has been related to pathogenesis of the respiratory syncytial virus (RSV) bronchiolitis and to the severity of the infection. Glucocorticosteroids have a role in inhibiting the IFN-γ response, acting directly on T cells or indirectly through IL-12. In this way, an increase in plasma cortisol would induce a decrease in the Th1 products with the imbalance between Th1/Th2 cytokines and a shift to Th2 response. We hypothesized that there is a relationship among endogenous cortisol response in acute RSV infection, severity of illness, and decreased Th1 cytokine response.

METHODS. We studied 42 infants under 12 months of age during an acute RSV infection. Twenty-one infants with a median age of 6 months had a severe illness and required hospitalization, whereas 21 with mild diseases with a median age of 7 months were under ambulatory control. All of them had bronchial obstruction evidenced by wheezing and/or hyperinflation on chest radiograph and positive RSV antigen detected by indirect immunofluorescence in nasopharyngeal aspirates. The control group included 21 infants in good health matched by age and gender with median age of 6 months that required blood tests for minor surgery. They were evaluated during a non-RSV epidemic period. Heparinized blood was collected on enrollment from all participating children at 9 AM for total leukocyte and differential cell count, determination of lymphocyte subsets, and for intracel-

Key Words
cytokines, respiratory syncytial virus, respiratory infectious diseases, lymphocyte markers, glucocorticoids

Abbreviations
RSV—respiratory syncytial virus
IFN—interferon
PBMC—peripheral blood mononuclear cell
Th—T helper
IL—interleukin
NPA—nasopharyngeal aspirate

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lular detection of cytokines in single cells; mononuclear cells were cultured to determine in the supernatant cytokine production. In addition, 1 mL of plasma was separated and kept frozen at −20°C for cortisol assay. In the supernatant of the cultured peripheral blood mononuclear cells (PBMCs), we quantified IL-12, IFN-γ, IL-4, IL-5, and IL-10. Lymphocyte phenotypes and $CD4^+$ and $CD8^+$ T cells with intracellular IL-4, IL-10, and IFN-γ were analyzed by triple-color immunofluorescence of single cells on a FACScan flow cytometer.

RESULTS. Infants with severe illness had significantly higher plasma cortisol levels than infants with mild disease, and in both groups of infected infants, those were higher than in the control group. A significantly decreased IL-12 and IFN-γ production by PBMCs and a fall in the percentage of $CD4^+$ T cells expressing IFN-γ were observed only in the severely affected infants. IL-12 concentrations were 2 pg/mL in severe illness versus 13 pg/mL in mildly infected infants and 12 pg/mL in controls. PBMCs from infants with severe illness produced less IFN-γ than mildly infected infants and than controls when compared with severe illness. No differences between the 3 groups of infants were observed during the acute phase of the disease for IL-4, IL-5, and IL-10. IL-12 and IFN-γ production had an inverse correlation with plasma cortisol levels. During severe RSV bronchiolitis, infants developed lymphopenia, and significantly lower eosinophil counts and percentages and absolute counts of $CD4^+$ and $CD8^+$ T cells. Eighty days postinfection, all values had returned to normal levels.

CONCLUSIONS. In this study, we demonstrate that during the acute phase of RSV infection, there is an increase in the level of plasma cortisol that is parallel to the decrease in IL-12 and IFN-γ production. These findings suggest an association between increased plasma cortisol and a decreased Th1-type response. The increase in plasma cortisol was greater in infants with the more severe symptomatology in association with a lower level of IL-12 and IFN-γ production. The potential causative role of endogenous cortisol in the imbalance of the Th1/Th2 response observed during severe RSV infection requires additional investigation. Our results suggest that the immunologic changes observed in the more severely ill patients may be partially explained by the increased levels of plasma cortisol. This finding should be taken into consideration when systemic steroids are prescribed to infants infected with the RSV because there is still controversy regarding the efficacy of systemic steroid use in severe bronchiolitis.

RESPIRATORY SYNCYTIAL VIRUS (RSV) infection is one of the main causes of respiratory illness worldwide. Almost all children become infected with RSV within the first 2 years of life and ~2% require hospitalization.[1] The severity of the disease has been related to a depressed lymphoproliferative response with decreased interferon γ (IFN-γ) production by peripheral blood mononuclear cells (PBMCs),[2–5] suggesting a T helper 1 (Th1) involvement in the viral clearance during the processes of recovery. Acute RSV bronchiolitis elicits an imbalance in type 1/type 2 cytokines in both the airways secretions and PBMCs.[2–5] Type 1 T cells produce IFN-γ, an essential cytokine in cell-mediated immunity to viral infections. Interleukin 12 (IL-12), produced by monocytes and antigen-presenting cells, promote the differentiation of naive $CD4^+$ T cells to the Th1 phenotype and decrease the synthesis of the type 2 cytokines IL-4 and IL-10 by $CD4^+$ T cells.[6] Both $CD4^+$ (T-helper) and $CD8^+$ (T-cytotoxic) cells are now recognized to have similar capabilities for production of type 1 and type 2 cytokines.[7]

RSV-infected epithelial cells produce inflammatory cytokines such as tumor necrosis factor α, IL-1α, IL-1β, and IL-6[8,9] that potently stimulate cortisol secretion.[10] Glucocorticosteroids have a role in inhibiting the IFN-γ response, acting directly on T cells or indirectly through IL-12.[11,12] In this way, an increase in plasma cortisol would induce an imbalance between type 1/type 2 cells and their products with a shift toward a type 2 response.

The aim of this study was to determine if an increase in plasma cortisol occurred during acute episodes of RSV infection and whether its magnitude was related to the severity of the disease. Another aim was to determine if the variations in the plasma cortisol levels are associated with an imbalance in the Th1/Th2 response and to the severity of the infection. For these purposes, we measured the levels of plasma cortisol, the IL-12, IFN-γ, IL-4, IL-5, and IL-10 produced by in vitro–activated PBMCs, the percentages of $CD4^+$ and $CD8^+$ T cells expressing IFN-γ, IL-4, and IL-10, the total leukocyte and differential cell counts, and the lymphocyte phenotype subsets in 2 groups of infants with severe or with mild RSV infection and in a group of healthy, age-matched controls.

METHODS

Patients

We studied 42 infants under 12 months of age, 21 considered severe forms and the same number with mild disease associated with RSV infection; they were incorporated to the study during the epidemic periods of 2002 and 2003 (the months of July and August). All of them had respiratory distress with bronchial obstruction demonstrated by wheezing and/or hyperinflation on chest radiograph and positive RSV antigen detected by indirect immunofluorescence in nasopharyngeal aspirates (NPA). They had no history of any other significant pathologies before the RSV infection such as chronic lung disease, prematurity, congenital heart disease, and so on, and had not received any oral or inhaled steroids,

whereas most of them went on to receive inhaled $\beta 2$ agonists. Antecedents of parental and sibling asthma or sibling deaths or any chronic respiratory diseases were registered on enrollment. The clinical criteria applied to define severity on enrollment to the hospital was a Tal score[13] 2 or 3; this score takes into account the respiratory rate, intensity of wheezing, the presence of cyanosis, and the use of accessory of respiratory muscles. An additional criterion was the presence of oxygen requirement with saturation below 93%. The 21 infants (11 boys) admitted to the Roberto del Río Children's Hospital in Santiago, Chile, had a median age of 6 months (interquartile range: 5–9 months). Roberto del Río Children's Hospital and its satellite health centers provide medical care to the Northern Health District of Santiago (~1 million inhabitants). The population belongs mainly to the middle and middle-low strata according to the Graffar Scale adapted to the Chilean population by Alvarez et al[14,15]; it may be considered that this is a rather homogenous population and the infants studied were comparable from this point of view. Evolution of symptomatology at home at the time of consultation to the health center was 48 to 72 hours. None of the hospitalized patients required mechanical ventilation or antibiotic treatment and all had comparable oxygen requirements, length of stay, and duration of symptoms while hospitalized. On admission to the hospital, a complete physical examination was conducted and body temperature, pulse and respiratory rate, diuresis (by weight), and O_2 saturation with a finger oximeter were performed; these were repeated every 4 hours until discharged. Oxygen administration was decreased when saturation reached 95% or more, and it was suspended when the patient maintained saturation above 98%. This group was compared with 21 ambulatory symptomatic infants (11 boys) with a median age of 7 months (interquartile range: 6–7 months) detected at one of the health centers. A physical examination including the Tal score of 1 and oxygen saturation, which had to be above 93%, was performed; on the basis of these findings, it was considered that none of them required hospitalization and, furthermore, none was hospitalized during the evolution of the episode. Their mothers were advised to contact the health center if any of the following symptoms appeared or increased: high fever, restlessness, polypnea, audible wheezing, rejection of feedings, or chest retraction. On discharge from the study, all patients had a monthly follow-up for 3 months and then every 3 months to completion at 1 year; if symptoms of bronchial obstruction reappeared, additional controls were advised. The control group included 21 asymptomatic infants matched for age and gender with median age of 6 months (interquartile range: 4–9 months), who required blood tests as a requisite for minor surgery; their parents were contacted at the outpatient surgery clinic of the hospital. They were evaluated during a non-RSV epidemic period. The study was approved by the Ethics Committee of the Faculty of Medicine (University of Chile). The parents gave their informed consent for their infants to participate in the study after the aims and scope of the project had been explained to them.

Blood Collection

Heparinized blood (5 mL) was collected on enrollment from all participating children at 9 AM for total leukocyte and differential count, and determination of lymphocyte subsets and for intracellular detection of cytokines in single cells; these cells were cultured to determine in the supernatant cytokine production. One milliliter of plasma was separated and kept frozen at −20°C for cortisol assay.

Cortisol Assay

The concentration of plasma cortisol (ng/mL) was determined by radioimmunoassay using cortisol radioimmunoassay kits (Diagnostic System Laboratories, Inc, Webster, TX).

PBMC Isolation

PBMCs were separated by a density gradient (Histopaque 1077; Sigma-Aldrich, St Louis, MO), counted in a Neubauer chamber, and viability was assessed by Trypan blue dye exclusion.

Culture of Cells

Mononuclear cells from RSV and control infants were cultured in flat-bottomed 24-well culture plates (Nunc, Roskilde, Denmark) at a concentration of 1×10^6 cells per well in 600 μL of AIM-V medium in the presence or absence of phytohemagglutinin (5 μg/mL) and phytohemagglutinin (5 μg/mL) plus lipopolysaccharide (1.0 μg/mL) for 24 and 48 hours at 37°C with 5% CO_2. After culture, cells were centrifuged and viability was determined by Trypan blue dye exclusion. The supernatant was kept at −20°C before IL-12, IFN-γ, IL-4, IL-5, and IL-10 assay.

IL-12, IFN-γ, IL-4, IL-5, and IL-10 Assays

The concentrations of IL-12, IFN-γ, IL-4, IL-5, and IL-10 were determined in the centrifuged mononuclear cell supernatants using BD OptEIA enzyme-linked immunosorbent assay sets (BD Biosciences Pharmingen, San Diego, CA). The limit of detectability of these assays was ~2 pg/mL. Samples that registered above the standard curve were diluted and reanalyzed. When values were below the detection threshold, the minimum detectable level was assigned.

Detection of Intracellular Cytokine by Flow Cytometry

Intracellular detection of cytokines in single cells was performed in PBMCs activated for 4 hours with 25 ng/mL of

PMA plus 1 μg/mL of ionomycin (Sigma-Aldrich, St Louis, MO) in the presence of 10 μg/mL of Brefeldin-A (Sigma-Aldrich). Cells were analyzed by triple-color immunofluorescence of single cells on a FACScan flow cytometer (Becton-Dickinson, San Jose, CA) within 24 hours. Analysis was gated on CD3+ T cells.

Phenotyping of T-Lymphocyte Subpopulations

Using monoclonal antibodies to CD3/CD4 and CD3/CD8, T-lymphocyte subpopulations were measured by double-color flow cytometry (FACScan; Becton-Dickinson) using Lysis software.

Statistical Analysis

Medians and 25th to 75th percentile ranges were analyzed by using the Mann-Whitney *U* test for independent groups and the Wilcoxon signed-rank test for paired samples. Correlations were analyzed by Spearman's rank correlation test. $P < .05$ was accepted as statistically significant.

RESULTS

Levels of Plasma Cortisol

The levels of plasma cortisol from patients with RSV bronchiolitis, the mildly infected infants, and the controls group measured on enrollment are shown in Fig 1 (medians, 25th–75th percentile ranges, and extremes). Infants with acute RSV bronchiolitis had median levels of plasma cortisol of 174 ng/mL (135–280 ng/mL percentile ranges), significantly higher ($P < .03$) than mildly infected infants whose median levels of plasma cortisol were 144 ng/mL (98–187 ng/mL percentile ranges) and the control group ($P < .0001$) with a median of 69 ng/mL (44–84 ng/mL percentile ranges). Plasma cortisol levels in mildly RSV-infected infants were also significantly higher ($P < .0001$) than in the control group. After 80 days, when patients were asymptomatic, the levels of cortisol were similar to normal controls, with median values of 67 ng/mL (53–101 percentile ranges) and 68 ng/mL (53–92 percentile ranges) for infants who have had severe and mild infection, respectively.

Levels of IL-12 in the Supernatant of Phytohemagglutinin-Activated PBMCs

Only results obtained from phytohemagglutinin-stimulated PBMCs after 24 hours are shown because they were not significantly different from those obtained with phytohemagglutinin plus lipopolysaccharide or 48 hours.

The levels of IL-12 produced by phytohemagglutinin-stimulated PBMCs obtained from the RSV severely infected, mildly infected, and the normal control infants on enrollment are shown in Fig 2 (medians, 25th–75th percentile ranges, and extremes).

PBMCs from infants with severe bronchiolitis produced significantly less ($P < .0006$) IL-12 with medians of 2 pg/mL (2–5 pg/mL percentile ranges) than mildly infected infants with medians of 13 pg/mL (3–25 pg/mL percentile ranges) and the normal control group ($P < .0004$) with medians of 12 pg/mL (4–35 pg/mL percentile ranges). Eighty days later, the levels of IL-12 in infants who have had severe RSV infection had significantly ($P < .0001$) increased up to 16 pg/mL (5–

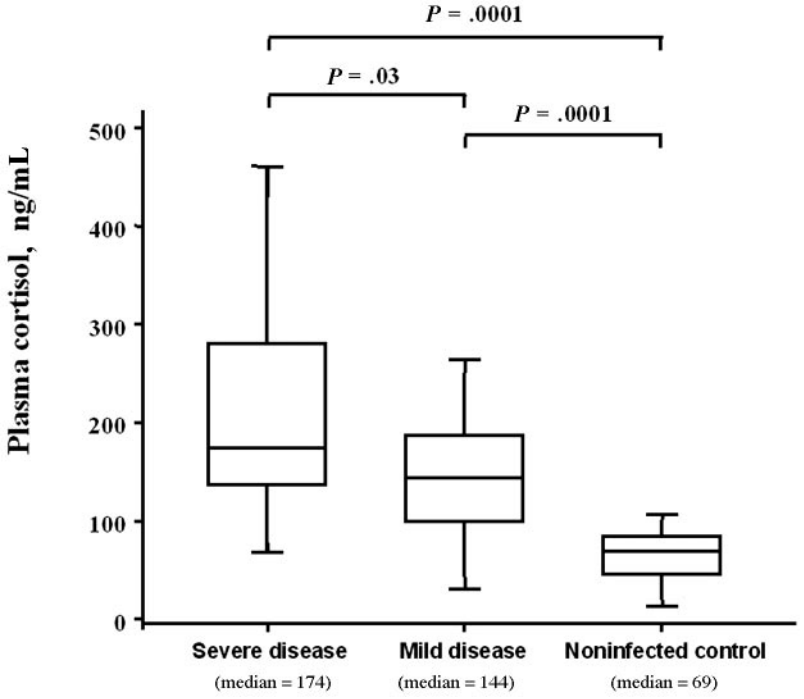

FIGURE 1
Levels of plasma cortisol (ng/mL) (21 subjects in each group), medians, 25th to 75th percentile ranges, and extremes from infants with RSV infection and severe disease, mild disease, and from the normal control group.

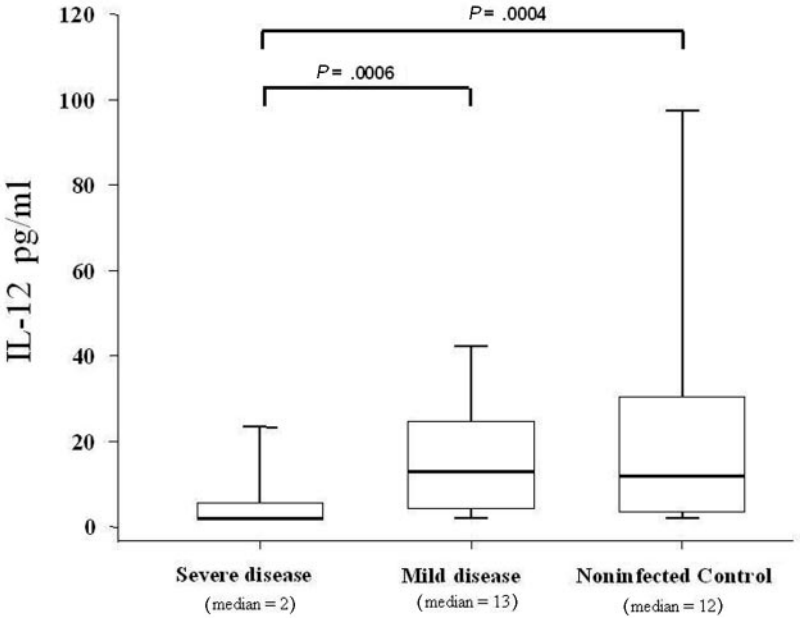

FIGURE 2
IL-12 (pg/mL) in the supernatant of 24-hour phytohemagglutinin-stimulated PBMCs obtained on enrollment from infants with RSV infection with severe and mild disease and from the normal control group. Data are presented as median (21 subjects in each group), 25th to 75th percentile ranges, and extremes.

33 pg/mL 25th–75th percentile ranges), whereas those who have had a mild infection had no significant differences with a median of 9 pg/mL (2–20 percentile ranges). The levels of IL-12 in 12 of 21 infants with severe bronchiolitis were below the limit of detectability.

Levels of IFN-γ in the Supernatant of Phytohemagglutinin-Activated PBMCs

The levels of IFN-γ produced by phytohemagglutinin-stimulated PBMCs obtained from the RSV severely infected, mildly infected, and the normal control infants on enrollment are shown in Fig 3 (medians, 25th–75th percentile ranges, and extremes).

PBMCs from infants with severe bronchiolitis produced significantly less IFN-γ ($P < .0001$) with medians of 10 pg/mL (2–88 percentile ranges) than mildly infected infants with medians of 423 pg/mL (91–769 percentile ranges) and the normal control group ($P < .0001$) with medians of 413 pg/mL (97–1076 percentile ranges). Eighty days later, the median values of IFN-γ obtained from infants who have had a severe disease had significantly ($P < .0014$) increased to 179 pg/mL (47–

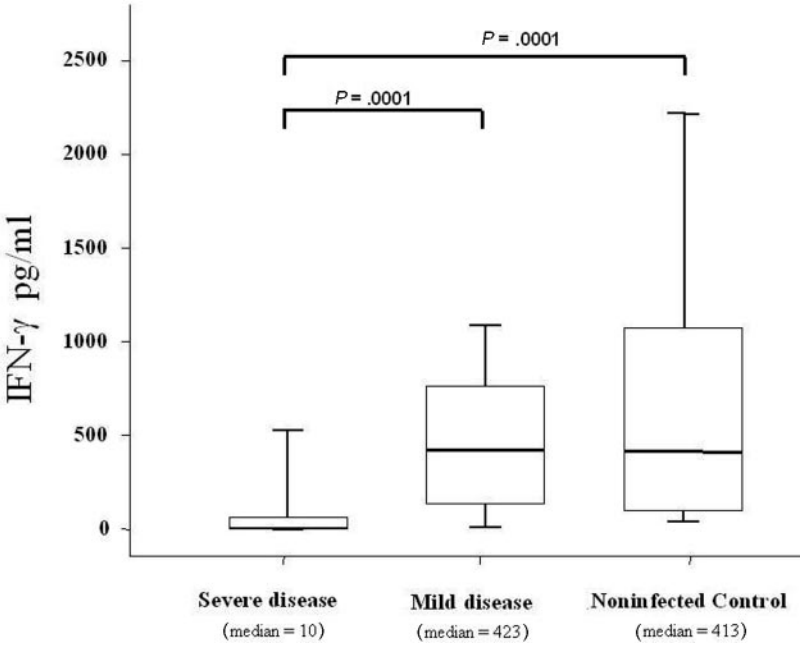

FIGURE 3
IFN-γ (pg/mL) in the supernatant of 24-hour phytohemagglutinin-stimulated PBMCs obtained on enrollment from infants with RSV infection with severe and mild disease and from the normal control group. Data are presented as median (21 subjects in each group), 25th to 75th percentile ranges, and extremes.

496 percentile ranges), whereas those infants with mild disease had no significant change with median values of 395 pg/mL (101–1047 percentile ranges).

The levels of IL-4, IL-5, and IL-10 during the acute phase of the disease were not significantly different between the groups (data not shown).

Percentage of CD4$^+$ and CD8$^+$ T Cells Producing IFN-γ in Infants With Bronchiolitis, Mild RSV Infection, and Controls

To determine if the decreased amounts of IFN-γ in the supernatant of stimulated PBMCs were the result of decreased number of T cells producing IFN-γ, we measured the percentage of CD4$^+$ and CD8$^+$ T cells expressing IFN-γ (Fig 3). The percentage of CD4$^+$ T cells expressing IFN-γ obtained from infants with bronchiolitis was 1.5% (1.0–2.4% percentile ranges), significantly less ($P < .04$) than from infants with mild infection 2.5% (1.5–3.4% percentile ranges) and from the control group ($P < .02$) in whom the percentage was 3.7% (2.0–4.7% percentile ranges). As for the percentage of CD8$^+$ T cells expressing IFN-γ, this was 3.7% (3.0–10.0% percentile ranges) in the severely ill infants, not significantly different from the infants with mild disease whose median was 4.4% (2.6–10.0% percentile range) and from the normal control group's value of 6.8% (1.7–10.0% percentile ranges). The percentage of CD4$^+$ T cells and CD8$^+$ T cells producing IL-4 and IL-10 were not significantly different when comparing the 3 groups (Fig 4).

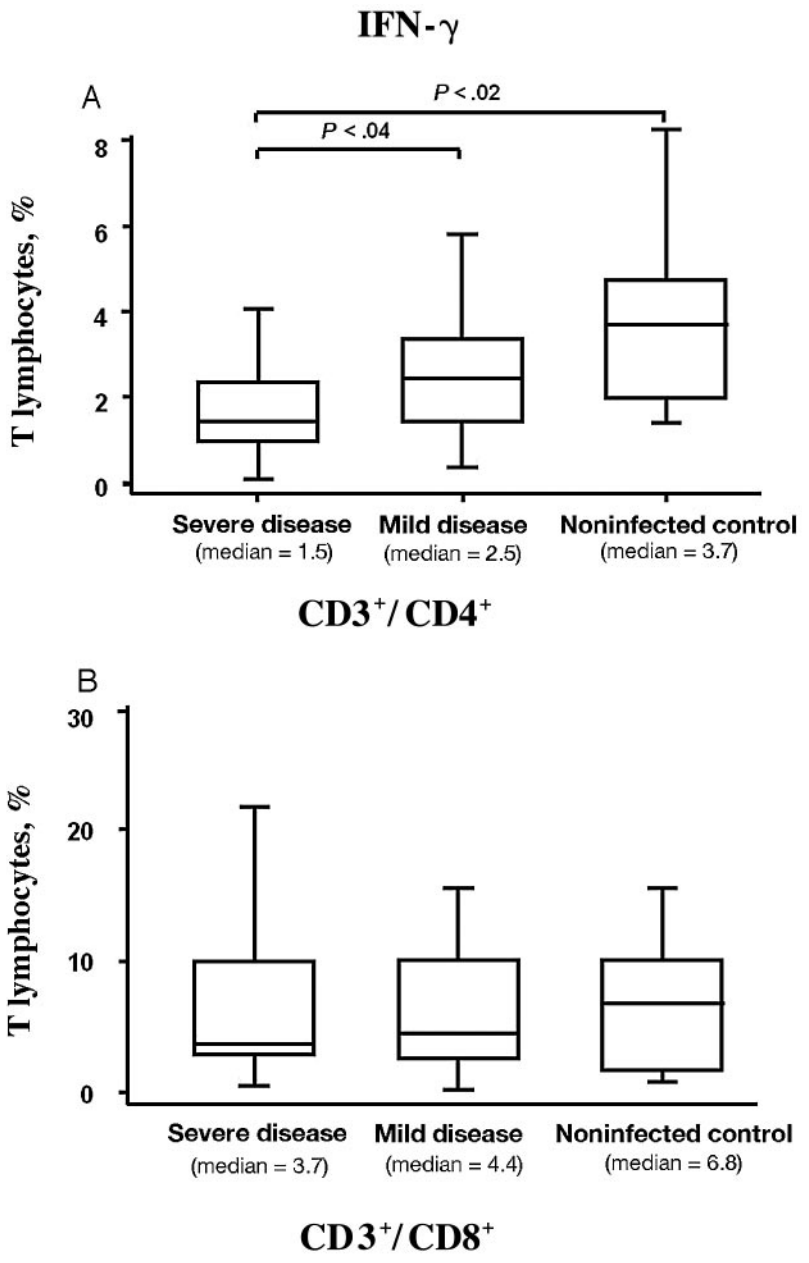

FIGURE 4
Percentage of CD3$^+$/CD4$^+$ and CD3$^+$/CD8$^+$ T cells expressing IFN-γ, medians, 25th to 75th percentile ranges, and extremes, from infants infected with RSV with severe and mild disease and from the normal control group.

Total leukocyte count, differential cell counts, and $CD4^+$, $CD8^+$ T-cell subpopulations, per mm^3, in patients during severe RSV bronchiolitis, mild illness, and the control group are shown in Table 1.

The total blood leukocyte and the differential cell counts, and the percentage of T cell subpopulations were determined to assess whether the lower percentages and absolute counts value of $CD4^+$ T cells producing IFN-γ could be explained by a decrease in the number of total $CD4^+$ T cells. During severe RSV bronchiolitis, the infants developed lymphopenia, lower eosinophil counts, and lower percentages and absolute values of $CD4^+$ T and $CD8^+$ T cells. These values were significantly lower than those from infants with mild illness and from normal controls. No changes in absolute values or the percentages of total leukocytes and monocytes were observed.

Correlation Between Plasma Cortisol and Levels of IFN-γ and IL-12

The levels of plasma cortisol obtained from all RSV-infected infants, either with severe bronchiolitis or mild disease, were correlated with the levels of IFN-γ and IL-12 obtained in the supernatant of stimulated PBMCs. A significantly inverse correlation was observed between the levels of plasma cortisol and the levels of IFN-γ ($r = -0.33$; $P < .031$) and IL-12 ($r = -0.41$; $P < .0069$). A highly significant positive correlation ($r = 0.64$; $P < .0001$) was found between the levels of IFN-γ and IL-12 produced by PBMCs.

DISCUSSION

In this study, we demonstrate that during the acute period of RSV infection, there is an increase in the level of plasma cortisol.[16] Although we found that this increment was greater in infants with more severe disease, there is some overlap between the groups. Most children had been ill for 2 to 3 days before the blood samples were obtained, and in consequence, it was not possible to determine when this increase started. When the blood tests were repeated (after 80 days), all infants were asymptomatic and their cortisol levels had returned to normal. Viral and bacterial infections are accompanied by increased glucocorticosteroid levels as a physiological response to inflammation,[17] but to our knowledge, changes of plasma cortisol levels in infants during mild and severe episodes of RSV infection have not been reported. Furthermore, there is still controversy with respect to the efficacy of systemic steroid use in infants with RSV bronchiolitis.[18–20] Although this increase in plasma cortisol was observed in infants with mild as well as in those with severe disease, the imbalance of the Th1/Th2 response, with a deficient type 1 response, was present only in those cases with severe bronchiolitis and higher levels of plasma cortisol. IFN-γ and IL-12 production by PBMCs was comparable in infants with mild disease and noninfected infants. In addition to the levels of plasma cortisol, the immunologic imbalance between the Th1 and Th2 patterns observed may also depend on glucocorticosteroid receptor gene polymorphism and on the activity of the glucocorticosteroid-metabolizing enzyme 11-hydroxysteroid dehydrogenase (11-β HSD) in every individual. Genetic variations in the response to plasma cortisol could explain the differences in severity and long-term manifestations of the infection.

Glucocorticosteroid decreases the synthesis of IFN-γ by acting directly on $CD4^+$ T cells and indirectly by inhibiting IL-12 production by monocytes.[11,12] We found that production of both cytokines by stimulated PBMCs was decreased and inversely correlated with the levels of plasma cortisol. This finding may indicate that cortisol was acting directly in both T lymphocytes and monocytes, inhibiting the production of IFN-γ and IL-12, respectively. It has been shown that IL-12 decreases during the acute phase of the RSV respiratory infection in infants and that this was inversely related to the severity of the disease when the outcome parameter was the duration of the mechanical ventilation.[21] Bont et al[22] attribute this result to the direct effect of RSV on IL-12 production by monocytes without considering the possibility that corticosteroids directly inhibit IL-12 production in human monocytes; our observations do not allow differentiation between the possible direct effects of the virus and the effect of cortisol. Because RSV infects mainly airway epithelial cells and viremia has never been demonstrated, we may speculate that the

TABLE 1 **Total White Blood Cells, Differential Cell Count, and $CD4^+$ and $CD8^+$ T-Cell Subset From Infants Infected With RSV With Severe and Mild Disease and Normal Controls**

Subjects	Severe Disease ($n = 21$)	Mild Disease ($n = 21$)	Normal Controls ($n = 21$)	$P < 0.05$[a]
Leukocytes	10 300 (8150–12 950)	12 850 (8950–14 000)	10 500 (8400–12 400)	NS
Lymphocytes	4606 (3384–6376)[a]	7373 (4807–10 065)	6300 (4880–7765)	vs mild disease and NC
Monocytes	600 (393–1050)	690 (432–989)	525 (284–691)	NS
Eosinophils	0 (0–0)[a]	240 (0–590)	166 (0–466)	vs mild disease and NC
$CD4^+$	1972 (1610–2773)[a]	3319 (1654–4730)	2850 (1841–3698)	vs mild disease and NC
$CD8^+$	585 (446–875)[a]	1140 (709–1830)	860 (757–1397)	vs mild disease and NC

Values are expressed as cells per mm^3 and as medians (interquartile range). NS indicates not significant; NC: normal controls.
[a] $P \leq .05$.

effects of RSV infection on peripheral blood cells may be produced by plasma cortisol. On the other hand, Legg et al[5] found that infants with RSV bronchiolitis had an imbalance in Th1/Th2 responses, but the viral load was comparable when compared with infants with upper RSV infection. This imbalance occurred in the first 24 to 48 hours and one could speculate that this represents a response to the increase in glucocorticosteroid levels.[5]

It is well known that IL-10, an antiinflammatory cytokine, strongly inhibits the production of a number of other cytokines by activated monocytes/macrophages, including IL-12.[23] In our study, the lower level of IL-12 found during RSV bronchiolitis was not the result of an increase in the amount of IL-10 produced by PBMCs nor of a decreased number of T cells producing IL-10. In addition, monocytes from RSV bronchiolitis were not different in percentages or absolute values from those of patients with mild illness and the controls. The decrease of IL-12 without changes of IL-10 production by PBMCs creates the imbalance in Th1/Th2 cytokines previously observed in severe RSV-infected patients.[2,5]

Th1/Th2 imbalance with a shift to a Th2 profile of cytokine production is further evidenced by increased endogenous cortisol production under stress conditions.[24,25] The significant differences in the plasma cortisol levels found between both groups of infants infected with RSV could be partially explained by the lower levels of oxygen saturation in infants with more severe illness, which may induce the stress that stimulates cortisol production. Furthermore, the individual variation in acute-phase inflammatory cytokine production by RSV-infected epithelial cells may also explain these differences.

The increase in plasma cortisol of RSV-infected infants showed a tendency ($P < .055$) to decrease absolute counts and the percentage of IFN-γ expressing $CD4^+$ T cells in peripheral blood (data not shown). Lymphopenia with changes in blood lymphocyte recirculation is a well-known effect of increased plasma cortisol.[26,27] In our study, lymphopenia may be a manifestation of the migration of T cells into the airways. Although neutrophils predominate in the bronchial secretions of children with RSV infection,[28] T-helper cells and eosinophils are also found.[29,30] Besides lymphocyte recirculation, apoptosis is a well-known mechanism of immunoregulation of T cells by glucocorticosteroids.[31] Apoptosis of peripheral blood T cells has been described in infants with acute RSV bronchiolitis.[32] We observed lymphopenia with decrease of $CD4^+$ and $CD8^+$ T cells, but did not observe changes in the percentages of $CD8^+$ T cells producing IFN-γ from RSV-infected infants when compared with normal controls. Moreover, in these infants, we did not find any correlation between the decrease of $CD4^+$ T lymphocytes and the decreased percentage of IFN-γ expressing $CD4^+$ T cells. These findings indicate that there is individual susceptibility in the subgroup of IFN-γ expressing helper and suppressor T cells that could be related to the different sensitivity of their glucocorticosteroid receptors. It was interesting to detect low eosinophil blood counts in the severely ill infants considering that these leukocytes are very sensitive to the effects of glucocorticosteroids.

Six RSV-infected infants who had been on systemic corticosteroids in the previous 24 hours were also studied (data not shown). Their levels of plasma cortisol were significantly higher (median: 1.092 ng/mL), their levels of IL-12 and IFN-γ were undetectable, and they had lower numbers of $CD4^+$ T cells with intracellular IFN-γ than the untreated patients. These results strongly lend support to the idea that the increase in plasma cortisol is related to the depressed Th1 response.

In this study, we found an inverse correlation between the increase in plasma cortisol during the acute phase of the infection and a Th1/Th2 imbalance of cytokine production by PBMCs associated with the severity of the disease. It is obvious that an association between increased levels of plasma cortisol and a decreased Th1 immune response does not prove causation. Furthermore, a cross-sectional study does not allow to establish causation relationships between increased plasma cortisol levels, imbalances of the Th1/Th2 responses, and the severity of the disease. However, our results strongly suggest that the immunologic changes observed in the more severely ill patients may be partly explained by the increased levels of plasma cortisol. This finding should be taken into consideration when systemic steroids are prescribed to RSV-infected infants. Whether these changes occur in other forms of respiratory infections, viral or bacterial, remains to be clarified.

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