

Effect of dexamethasone on respiratory syncytial virus-induced lung inflammation in children: results of a randomized, placebo controlled clinical trial

Somers CC, Ahmad N, Mejias A, Buckingham SC, Carubelli C, Katz K, Leos N, Gomez AM, DeVincenzo JP, Ramilo O, Jafri HS. Effect of dexamethasone on respiratory syncytial virus-induced lung inflammation in children: results of a randomized, placebo controlled clinical trial.

Pediatr Allergy Immunol 2009.

© 2009 The Authors

Journal compilation © 2009 Blackwell Munksgaard

Inflammatory mediators play a major role in the pathogenesis of respiratory syncytial virus (RSV) infection. The objective of this study was to evaluate the effect of i.v. dexamethasone on cytokine concentrations in tracheal aspirates (TA) of children with severe RSV disease and to correlate them with disease severity. Twenty-five cytokines were measured in TA obtained from children < 2 yr old intubated for severe RSV disease, and enrolled in a double-blind study of i.v. dexamethasone (0.5 mg/kg; n = 22) vs. placebo (n = 19). Cytokine concentrations, measured at baseline and days 1 and 5 post-randomization using a multiplex assay, were compared within both treatment groups and correlated with: (i) tracheal white blood cell counts, (ii) tracheal RSV loads by culture and (iii) parameters of disease severity, including number of days of requirement for mechanical ventilation, intensive care unit (ICU), and hospitalization. At baseline interleukin (IL)-13 and IL-15 concentrations were significantly higher in the dexamethasone treatment group. On day 1 post-treatment, only MCP-1, eotaxin and IL-6 concentrations were significantly different but higher in the placebo group. On day 5: IL-13, IL-7, IL-8 and MIP-1 α concentrations were higher in dexamethasone-treated patients. In both groups MIP-1 β inversely correlated with the days of ventilator support; MIP-1 α , MIP-1 β and eotaxin inversely correlated with ICU days; and IL-6 inversely correlated with hospitalization regardless of the treatment assigned. Systemic administration of dexamethasone did not have a consistent effect on TA concentrations of pro-inflammatory cytokines. This may help explain, at least in part, the lack of clinical benefit of steroid treatment in children with severe RSV bronchiolitis.

Cynthia C. Somers¹, Naveed Ahmad², Asuncion Mejias¹, Steve C. Buckingham³, Cecilia Carubelli¹, Kathy Katz¹, Nora Leos², Ana M. Gomez¹, John P. DeVincenzo³, Octavio Ramilo¹ and Hasan S. Jafri¹

¹University of Texas Southwestern Medical Center, Dallas, TX, ²Children's Medical Center, Dallas, TX, ³University of Tennessee Health Science Center, Memphis, TN, USA

Key words: cytokines; bronchiolitis; corticosteroids; children; respiratory syncytial virus

Hasan S. Jafri, MD, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA
Tel.: 301 398 4431
Fax: 301 398 9431
E-mail: jafrih@medimmune.com

Accepted 12 January 2009

Bronchiolitis is the leading cause of hospitalization for infants in the United States (1). It is associated with respiratory syncytial virus (RSV) infection in up to 70% of cases (2), accounting for more than 126,000 admissions annually (3). Treatment for acute RSV disease remains

controversial. Indeed, treatment with bronchodilators or with the antiviral agent ribavirin have not demonstrated a consistent benefit and their routine use is discouraged (4–7).

The pathogenesis of RSV infection and the specific factors that determine disease severity

remain to be fully characterized. Numerous studies have evaluated the role of corticosteroids for treatment of bronchiolitis with the anticipation to help ameliorate bronchiolar oedema and airway hyper-reactivity by virtue of their anti-inflammatory effects. Their clinical efficacy has been a matter of debate for decades, and although some studies found beneficial effects (8, 9), most large, randomized, placebo-controlled trials have failed to demonstrate a significant clinical benefit (10–15). Despite the large number of studies conducted to date, data concerning the specific reasons that could explain the lack of clinical benefit of systemic steroids in severe RSV infections are scarce.

We have previously reported that treatment of infants intubated with severe RSV bronchiolitis with i.v. dexamethasone did not improve clinical outcomes, while it delayed the normal decline of RSV loads measured in the lower respiratory tract when compared with placebo (10). Not much is known, however, about the effect of systemic administration of glucocorticoids on the RSV-induced cytokine profile in the respiratory tract of children with acute bronchiolitis and how this effect relates to disease pathogenesis. The aim of the current study was to characterize the effect of dexamethasone on the cytokine and chemokine responses induced by RSV over time in the respiratory tract of children with severe RSV disease to improve our understanding of how corticosteroids affect the immunopathogenesis of RSV. We also investigated the correlations between cytokine/chemokine concentrations and the clinical markers of disease severity previously evaluated in the original study (10).

Methods

Study design

Twenty-five cytokines were measured in tracheal aspirate (TA) samples obtained on days 0, 1 and 5 of hospitalization. Samples were collected from patients during the 1997–98 and 1998–99 RSV seasons in a randomized, double-blind, placebo-controlled trial of dexamethasone for children intubated for severe RSV infection. Briefly, 41 children were randomized to receive either dexamethasone sodium phosphate ($n = 22$; 0.5 mg/kg/dose) or an equal volume of normal saline ($n = 19$; placebo) administered intravenously every 12 h for 4 days as previously described (10). This study was conducted at Children's Medical Center, Dallas, TX and Le Bonheur Children's Hospital, Memphis, TN (10).

Collection of tracheal aspirates and cytokine concentrations measurement

Tracheal aspirates were obtained from all study patients immediately after randomization, before they received the first dose of study drug (day 0) and then at intervals of 24 ± 3 h on study days 1 and 5. TA were obtained using the same standardized method at both study sites as previously described (10, 16). Parallel aliquots of respiratory secretions were frozen immediately after collection on dry ice and stored at -80°C until tested. All cytokines and chemokines were tested using the LuminexTM multiplex assay system (Biosource International, Inc., Camarillo, CA, USA) according to manufacturer's instructions (17). Assays for the cytokines measured had lower limits of detection ranging from 3 to 30 pg/ml. For statistical analysis, samples with optical density readings below the limit of the standard regression curve were assigned the limit of detection level.

The following cytokines, and soluble receptors were measured in tracheal aspirate samples

Cytokines. Interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p40, IL-13, IL-15, IL-17, tumor necrosis factor- α (TNF- α), γ -interferon (IFN)- γ , IFN- α and granulocyte macrophage colony stimulating factor (GM-CSF).

Cytokine-receptors. Interleukin-1 receptor (IL-1R) and IL-2R.

Chemokines. Interleukin-8, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , interferon-inducible protein (IP-10), monokine induced by interferon- γ (MIG), eotaxin, regulated upon activation, normal T-cell expressed, and secreted (RANTES) and monocyte chemoattractant protein (MCP)-1.

Changes in cytokine concentrations in TA obtained at baseline, days 1 and 5 were first evaluated over time in both treatment groups combined and then individually within each treatment group (dexamethasone vs. placebo). In addition, differences in cytokine concentrations in TA between the two treatment groups were assessed at each time point.

Clinical and laboratory correlations

Parameters of clinical disease severity, quantitative RSV cultures and white blood cell (WBC) counts measured in TA were analysed by Spearman's test to determine the correlation with

cytokine concentrations at baseline (day 0), days 1 and 5. Parameters of disease severity included number of days of mechanical ventilation, days of intensive care unit (ICU) and total hospital days (10). Cultures and WBC counts were performed locally at each site on fresh specimens as previously described (10, 16).

Statistical analysis

Descriptive analyses were performed using frequency distributions, proportions, means/medians, standard deviations and percentiles. Normality of the data was tested using Kolmogorov–Smirnov and Shapiro–Wilk tests. Group comparisons were performed using Z-tests for the proportions and *t*-tests or Mann–Whitney *U*-tests, as required, for the continuous level measurements. Cytokine concentrations were compared using their raw values at each time-point separately, and delta values calculated by subtracting baseline concentrations from days 1 to 5 concentrations. These raw and delta values were compared between dexamethasone and placebo groups. The *p*-values obtained from these tests were used to identify the cytokines that were differentially expressed in the two treatment groups. These selected cytokines were further explored for their expression pattern over three time points (days 0, 1 and 5). Because the purpose of these analyses was to find the cytokines of interest, the *p*-values were not corrected for multiple comparisons. Friedman tests and Wilcoxon's signed rank tests were performed to test the change over time. Linear correlations were determined by using Spearman's analyses. Correlation coefficients <0.4 were considered weak, 0.4 – 0.6 as moderate and >0.6 as strong correlations. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) statistical software.

Results

Study population

Baseline characteristics of the study patients have been previously reported (10) and are summarized in Table 1. No subjects in either group had history of reactive airway disease, RSV infection or wheezing episodes prior to the current RSV hospitalization. One patient in the dexamethasone group had received a single infusion of RSV immune globulin 45 days before enrollment; no other subjects had received RSV immune globulin or palivizumab before enrollment.

Table 1. Baseline characteristics of the study infants

	Placebo group (n = 19)	Dexamethasone group (n = 22)	<i>p</i> -value *
Mean age (days)	44	60	0.3
Male (%)	58	64	0.9†
Birth weight (mean; g)	2898	2732	0.5‡
Gestational age (mean; wk)	37.1	36.5	0.9
Symptoms§ (mean, days)	3.6	4.2	0.2‡
Fever¶	1.3 (1.3)	1 (1.6)	0.5
Rhinorrhea	3.5 (2.4)	4.1 (4.5)	0.1
Cough	2.9 (1.7)	3.8 (2.3)	0.3
Difficulty breathing	2 (1.3)	2.2 (1.4)	0.6
Tobacco exposure (%)	26	29	0.8
Consumed breast milk (%)	45	44	0.9

Baseline characteristics of study infants are reported as medians (25–75% percentiles). Table adapted from previous publication (10).

*Mann–Whitney rank sum test.

†Z-test.

‡*t*-test.

§Duration of symptoms prior to randomization.

¶Presence of fever (temperature ≥ 38.5).

Cytokine concentrations in tracheal aspirates

Cytokine concentrations were analysed using both raw data as well as using delta values, as described in the statistical analysis section. However, as very similar results were seen using both approaches, only raw concentration analyses are included in the present manuscript (Table 2). On day 5, cytokine concentrations were measured in the subset of patients that remained intubated ($n = 11$ in the placebo group and $n = 10$ in the dexamethasone treatment group, who remain intubated). Our analysis included a total of 98 samples.

Dynamics of cytokine concentrations in tracheal aspirates. Change in cytokines concentrations in both treatment groups combined. We observed a temporal variation in different cytokine concentrations in TA in relation to the duration of treatment in both groups (placebo and dexamethasone). Except for IL-4 concentrations that significantly increased from days 0 to 5 ($p = 0.002$) in both groups combined, the rest of the cytokines/chemokines evaluated significantly decreased over time in both treatment groups, from days 0 to 1 and 5 and from days 1 to 5.

Days 0–5: We found a significant decrease ($p < 0.05$) from days 0 to 5 for the following cytokines: IL-6, IL-7, IL-10, IL-12, IL-17, eotaxin, IFN- γ , MCP-1, MIP-1 α and MIG.

Days 0–1: IL-1, IL-8, MIP-1 β and RANTES concentrations significantly decreased from days 0 to 1 and remained low through day 5 in both groups ($p < 0.05$).

Table 2. Cytokine concentrations in tracheal aspirates

Cytokines (pg/ml)	Day 0			Day 1			Day 5		
	Placebo N = 19		p-value	Placebo N = 19		p-value	Placebo N = 11		p-value
	Median (25th–75th)	Dexamethasone Median (25th–75th)		Median (25th–75th)	Dexamethasone Median (25th–75th)		Median (25th–75th)	Dexamethasone Median (25th–75th)	
IL-1	266.5 (132.6–353.4)	230.6 (139.0–471.2)	0.7	189.4 (114.5–348.2)	217.6 (75.7–296.9)	0.38	264.7 (82.7–328.5)	293.9 (67.2–483.3)	0.53
IL-2	11.3 (5.9–17.1)	16.5 (5.3–26.5)	0.26	19.4 (8.3–40.1)	27.1 (8.6–42.3)	0.75	17 (13.3–37.4)	29.6 (9.2–45.7)	0.92
IL-1R	7616.5 (2746–21679)	7616.5 (3216–15817)	0.84	11266.4 (7200–14309)	10368.8 (5806–19990)	1	5321.2 (3235–7399)	12410 (5368–19614)	0.06
IL-2R	436.5 (363–608)	456.3 (340–605)	0.67	501.1 (393–577)	484.5 (289–579.4)	0.6	387.8 (363–500)	454.3 (336–556.4)	0.77
IL-4	5 (5–5)	5 (5–5)	1	38 (5–38.5)	37.1 (5–39.2)	0.89	37.4 (37.0–37.7)	21.3 (5–38)	0.39
IL-6	724 (294.7–1270.3)	752.2 (239.3–1207.1)	0.94	419.9 (347.5–718.7)	212.5 (86.1–480.1)	0.006	184.6 (50.5–433.4)	470.4 (136.4–745.7)	0.13
IL-7	167.1 (161.8–174.3)	169.1 (169.0–176.1)	0.76	90.1 (82.8–159.8)	91.1 (84.3–150.1)	0.74	77.8 (77.4–89.3)	125.1 (88.6–164.2)	0.01
IL-8	11842 (6790.0–24592)	14270 (5547–23587)	0.86	6647 (3694.2–10990.5)	4582 (2577–9224)	0.17	2456.5 (704.2–6889.7)	10511 (6786–24482)	0.03
IL-10	132.4 (106.8–151.1)	146.6 (113.8–169.9)	0.24	111.7 (101.5–139.0)	119.8 (108–143.5)	0.33	95.7 (76.7–106.7)	107.9 (95.8–143.5)	0.09
IL-12	409.2 (209.8–678.4)	659.5 (326.2–945.6)	0.17	151.8 (91–473.2)	111.4 (71.5–471.05)	0.22	41.4 (10.2–86.5)	79.7 (21.5–92.9)	0.43
IL-13	31.2 (30.67–31.22)	31.22 (31.22–31.78)	0.003	31.2 (30.6–31.7)	31.2 (30.6–59.6)	0.49	7.3 (7.3–51.2)	35.5 (30.9–60.2)	0.01
IL-15	33.96 (31.92–37.04)	37.04 (33.96–43.28)	0.02	82.8 (33.9–102.7)	75.7 (33.1–132.4)	0.8	52.5 (46.0–80.1)	56 (35.5–93.5)	0.61
IL-17	64.3 (57.2–69.5)	63 (57.2–72.1)	0.97	54.6 (10.2–60.6)	58.4 (20.3–63.0)	0.35	10.9 (10.2–59.0)	62 (43.4–66.6)	0.07
IP-10	569.6 (141.1–905.1)	156.8 (21.9–1218.5)	0.81	360.5 (202.2–1568.4)	162.9 (24.3–1228.8)	0.07	99.6 (11.0–134.5)	14.6 (12.6–76.5)	0.94
MIG	137.5 (84.0–589.1)	203.3 (93.7–455.2)	0.54	136.7 (102.6–226.9)	108.5 (84.8–168.0)	0.2	92.5 (82.8–137.7)	110.2 (79–194.3)	0.39
MIP-1 α	282.6 (142.5–571.8)	207.5 (136.0–744.5)	0.81	177.4 (132.5–314.5)	103.7 (87.2–220.0)	0.12	83.2 (44.8–90.5)	187 (81.5–680.0)	0.03
MIP-1 β	661 (118.8–1812.4)	681.3 (202.5–1752.5)	0.68	715.1 (314.8–850.2)	443.5 (240.9–949.5)	0.23	325.5 (161.7–389.0)	458.8 (69.5–1750.0)	0.29
RANTES	76.1 (52.9–153.1)	73.2 (49.3–128.1)	0.64	62.4 (49.3–82.9)	59.4 (42.7–90.4)	0.81	40.8 (16.4–80.3)	64.8 (42.4–81.3)	0.35
TNF- α	10 (10–53.6)	17 (10–78.1)	0.47	59.3 (30–79.7)	55.1 (10–205.2)	0.96	24.6 (10.2–67.6)	31.4 (10–484.4)	0.9
IFN- α	115.8 (108.7–127.0)	115.8 (108–134.9)	0.7	241.6 (119.2–338.9)	230.4 (120.9–309.9)	0.77	253.4 (192.8–324.1)	153 (105.4–230.4)	0.82
IFN- γ	14.1 (10.8–18.4)	22.2 (11.4–35.9)	0.3	16.3 (2.90–23.9)	13.3 (5.8–26.8)	0.88	2.9 (2.9–20.8)	13.5 (5.0–21.4)	0.07
MCP-1	2965 (815.1–5770.4)	5082 (2463.9–7634.9)	0.14	1626.2 (895.5–3472.8)	516.9 (161.1–955.3)	0.001	470.9 (271.4–810.6)	546.7 (188.8–1010.4)	0.84
Eotaxin	16.9 (8.4–32.4)	6.3 (5–11.7)	0.12	8.4 (5–13.1)	5.2 (4.8–9.2)	0.02	5 (5–8.0)	5.1 (4.7–6.6)	0.84
GM-CSF	65.5 (64.0–67.0)	64 (61.1–69.8)	0.15	64 (35.5–66.2)	203.9 (67–224.4)	0.78	43.6 (26.1–63.3)	64 (61.1–214.2)	0.1

Tracheal aspirates from 41 infants intubated with RSV bronchiolitis were collected on days 0, 1 and 5 and measured for cytokine concentrations with a multiplex bead assay. Values are expressed as medians (25–75% percentiles). IL-5 was below the limit of detection at all time points. The cytokine lower limits of detection were: IL-1 (15 pg/ml), IL-2 (6 pg/ml), IL-1R (30 pg/ml), IL-2R (30 pg/ml), IL-4 (5 pg/ml), IL-5 (3 pg/ml), IL-7 (10 pg/ml), IL-8 (3 pg/ml), IL-10 (5 pg/ml), IL-12p40 (15 pg/ml), IL-13 (7 pg/ml), IL-15 (10 pg/ml), IL-17 (10 pg/ml), TNF- α (10 pg/ml), IFN- γ (5 pg/ml), IFN- α (15 pg/ml), GM-CSF (15 pg/ml), MIP-1 α (10 pg/ml), MIP-1 β (10 pg/ml), IP-10 (5 pg/ml), MIG (4 pg/ml), eotaxin (4 pg/ml), RANTES (15 pg/ml) and MCP-1 (10 pg/ml). Statistically significant ($p < 0.05$) results are presented in bold font.

Days 1–5: In both groups, the concentrations of GM-CSF, TNF- α , IFN- α , IL-2, IL-13, IL-15 and IP-10 peaked on day 1 post-treatment and subsequently decreased significantly by day 5 ($p < 0.05$).

Change in cytokines concentrations over time, within each group. When we analysed changes in cytokines over time within each treatment group separately, we only found differences from days 0 to 1; MCP-1 ($p < 0.001$) and MIG ($p = 0.03$) had decreased over time and TNF- α concentrations significantly increased ($p = 0.03$) only in the dexamethasone group, compared with the placebo group.

Differences in cytokine concentrations in tracheal aspirates between the two treatment groups at each time point. Cytokine concentrations were measured at baseline (day 0) and days 1 and 5 (Table 2). IL-5 concentrations were below the level of detection in both groups at all time-points evaluated.

Day 0: On admission, concentrations of two cytokines, IL-15 and IL-13, were significantly higher in the dexamethasone group vs. placebo ($p < 0.05$) (Table 2).

Day 1: Cytokine concentrations were similar in both groups except for MCP-1, and IL-6 which were significantly lower in the dexamethasone treated group (Fig. 1). Concentrations of eotaxin were also significantly lower in the dexamethasone group. However, they were close to the limit of detection of the assay, which makes it difficult to interpret the significance of this finding.

Day 5: IL-7, IL-13, IL-8 and MIP-1 α concentrations were higher in patients treated with dexamethasone (Fig. 2). There were fewer samples studied on day 5 (11 in the placebo and 10 in steroid group) as mechanical ventilation was discontinued in several subjects, therefore the statistical power of the analysis may be affected by the smaller sample size at this time point.

Correlations between cytokine concentrations and viral loads and WBC counts measured in tracheal aspirates. On day 2, after randomization RSV loads were significantly higher in the dexamethasone group (10). We found a number of cytokines that significantly correlated with RSV loads in both groups. RSV loads in TA significantly correlated with concentrations of IL-6 ($r = 0.43$; $p = 0.008$) and IL-7 ($r = 0.5$; $p < 0.001$) on day 0, and with IL-7 ($r = 0.62$; $p < 0.001$), IL-17 ($r = 0.45$; $p = 0.005$), IFN- γ ($r = 0.50$; $p = 0.001$) and GM-CSF ($r = 0.34$; $p = 0.023$) on day 1. On the other hand, viral loads were inversely correlated with IL-1 β

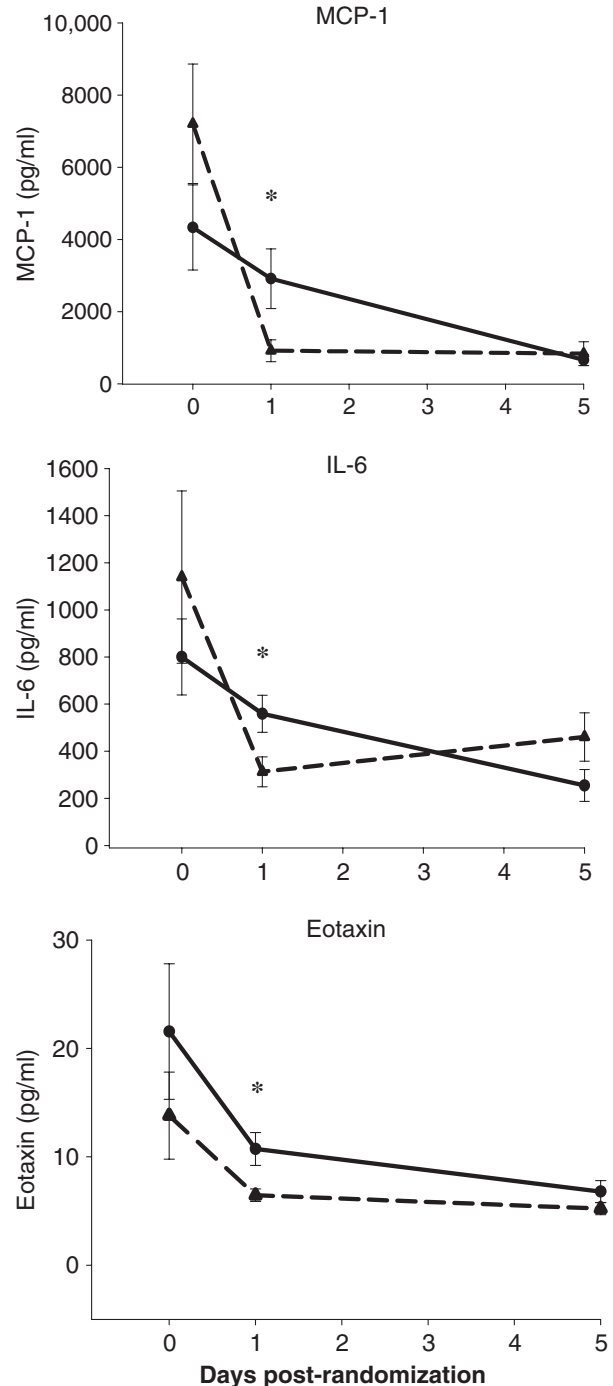


Fig. 1. Cytokine concentrations in tracheal aspirates on day 1. Among the 25 cytokines measured in tracheal aspirates only MCP-1, IL-6 and eotaxin were significantly different between the placebo ($n = 19$) and the group that received dexamethasone ($n = 22$). These 3 cytokines were significantly decreased in the group that received dexamethasone. Data expressed in column mean $\pm$ s.e. *Mann-Whitney test ($p < 0.05$).

($r = -0.44$; $p = 0.013$), IL-4 ($r = -0.5$; $p < .001$) and IFN- α ($r = -0.56$; $p < 0.001$) concentrations on day 1.

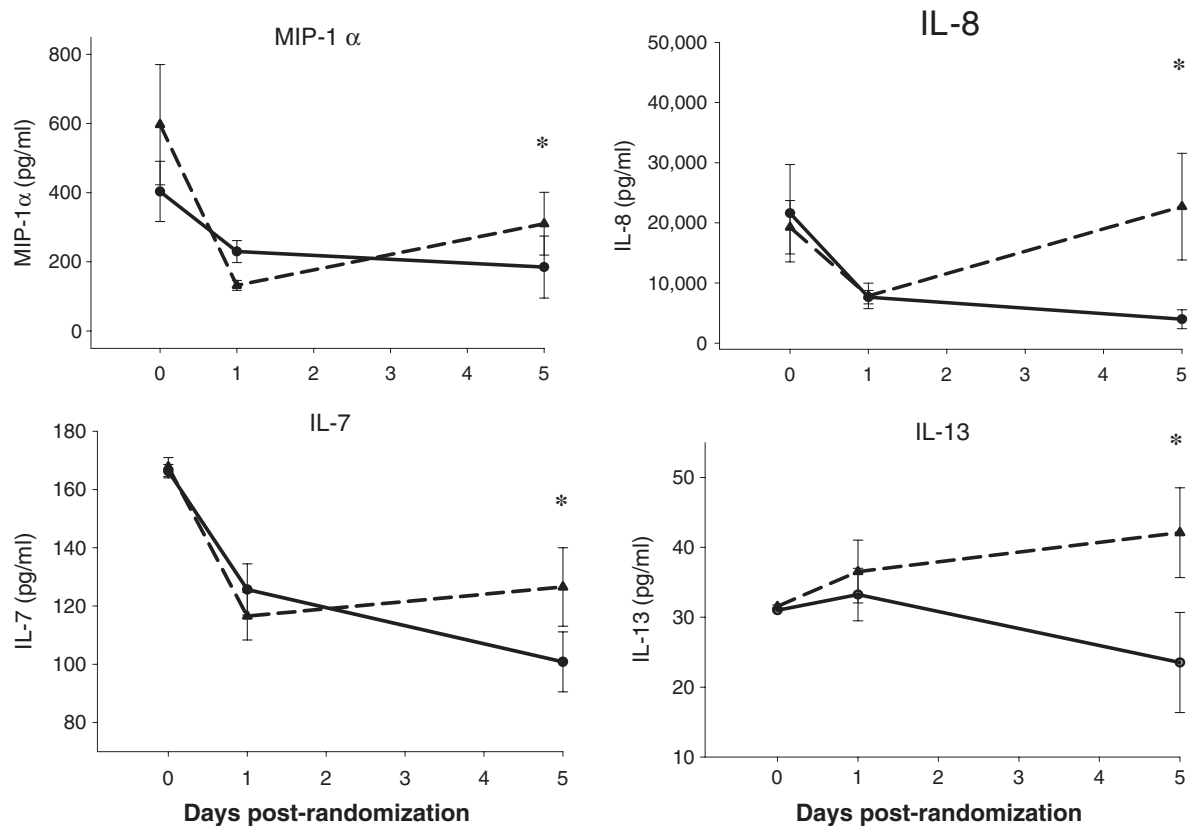


Fig. 2. Cytokine concentrations in tracheal aspirates on day 5. Concentrations of MIP-1 α , IL-8, IL-7 and IL-13 were significantly more elevated in the group that received dexamethasone as compared with the placebo group. Data expressed in column mean $\pm$ s.e. *Mann-Whitney test ($p < 0.05$).

No significant differences were observed in WBC counts or absolute neutrophil counts in TAs on any study day in any treatment group. However, when each treatment group was analysed separately, there was a significant decrease in WBC counts in TA over time in children assigned to placebo ($p = 0.02$); in contrast, this was not observed in the dexamethasone-treated patients. WBC counts were significantly correlated with IL-10 ($r = 0.5$; $p = 0.002$), IL-8 ($r = 0.7$; $p < 0.001$) and MIP-1 α ($r = 0.6$; $p < 0.001$) concentrations in both groups at all time points.

Correlations between cytokine concentrations in tracheal aspirates and clinical parameters of RSV disease severity. Infants in the placebo and dexamethasone groups did not significantly differ in their median ventilator days, ICU days or hospital days (10).

Length of mechanical ventilator support. In both treatment groups there was a weak and inverse correlation between MIP-1 β concentrations in TA on day 1 and days of mechanical ventilator support ($r = -0.3$; $p = 0.048$).

Length of ICU stay. In both groups, there was a weak and inverse correlation between tracheal concentrations of MIP-1 α ($r = -0.33$; $p = 0.04$), MIP-1 β ($r = -0.37$; $p = 0.02$) and eotaxin ($r = -0.35$; $p = 0.02$) on day 1 and the length of ICU stay.

Length of hospitalization. In both groups, the concentrations of IL-6 ($r = -0.4$; $p = 0.008$) on day 0 were moderately correlated with length of hospitalization.

Discussion

Studies suggest that the pattern of cytokine production elicited by RSV affects the balance between virus replication and disease pathogenesis, that ultimately determines the manifestations of the disease (18). We have previously reported that treatment with i.v. dexamethasone resulted in a significant delay in the decline of RSV loads in TA of children intubated for severe RSV infection. In addition, we did not observe any significant differences in the clinical outcomes evaluated (10). We took advantage of this well characterized population of intubated

children to explore the relative importance and role of different cytokines in this severe form of RSV disease and how they were impacted by therapy with steroids. In agreement with the clinical and virological findings previously described, we did not observe a consistent effect of dexamethasone in the cytokine profile analysed. Most cytokines showed similar concentrations between the two groups after one day of treatment (day 1) except for MCP-1, eotaxin and IL-6, which were higher in the placebo group. In contrast, the concentrations of IL-7, IL-8, IL-13 and MIP-1 α were significantly higher in the dexamethasone-treated group after 5 days of treatment. Thus, treatment with corticosteroids had variable effects depending on the cytokine examined and the timing of that measurement.

Several lines of evidence suggest that the inflammatory process in response to RSV infection plays a critical role in the pathogenesis of the disease. Elevated concentrations of pro-inflammatory cytokines and chemokines which likely help mediate recruitment and activation of inflammatory cells, such as IL-6, IL-8, MIP-1 α , MIP-1 β , MCP-1, IP-10, RANTES, eotaxin, IL-10 and IFN- γ have been found in respiratory secretions of infants with RSV infection (16, 19–23). We observed a mild inverse correlation between MIP-1 α , MIP-1 β and eotaxin concentrations and disease severity as defined by days of ICU stay and mechanical ventilation suggesting that the magnitude of the inflammatory response may play a protective role in the setting of acute RSV infection. Consistent with our findings, recent reports have shown that children with mild RSV bronchiolitis have a more robust cytokine response than those with a more severe form of the disease (16, 24, 25). Sheeran et al. (16) found that TA concentrations of RANTES, IL-8 and IL-10 were inversely correlated with clinical markers of disease severity in children intubated with RSV. More recently Bennet et al. (24), also reported that concentrations of several cytokines including IL-6, IL-8, IFN- γ , MIP-1 β and IL-10 in nasopharyngeal secretions were inversely correlated with hypoxia in a group of 63 non-intubated children <2 yr old with bronchiolitis (16, 22).

It could be speculated that the increased concentrations of IL-7, IL-8, IL-13 and MIP-1 α in the respiratory secretions of the dexamethasone-treated patients could be related to the higher viral loads present and the lack of significant decrease in WBC counts in TA observed in these patients. A direct correlation between WBC counts and cytokine concentrations has been previously described in less severe

RSV infection (25). We confirmed a direct correlation between the concentrations of IL-8, IL-10 and MIP-1 α , and WBC counts in TA from patients with severe disease irrespective of the treatment assigned.

Our study complements previous studies that analysed the effects of steroids on clinical outcomes in acute RSV infection (14) and provides at least a partial explanation for the lack of effective anti-inflammatory effect and clinical benefit of corticosteroids for severe RSV disease. Thus, taken together the lack of clinical benefit, the delayed decline of RSV loads and the inconsistent anti-inflammatory effect, we suggest that systemic steroid therapy is not indicated in children with severe acute RSV lower respiratory tract infection. Efforts should be concentrated on identifying and evaluating alternative agents with antiviral effect, with or without immunomodulatory activity for treatment of acute RSV infection in infants.

There are several factors to consider as we interpret our findings. First, it remains unclear whether the effects exerted by dexamethasone as seen in our study because of glucocorticoid class effect or a dexamethasone-specific effect. The findings by Bulow et al. (26) support the former concept, because in their study prednisolone had no effect on clinical outcomes of RSV infection. Secondly, when analysing the effects of a therapeutic agent, the route of administration can strongly influence the outcomes. Nevertheless, in agreement with our observations, when dexamethasone was administered by other routes such as inhalational or oral, it did not affect clinical disease severity scores and oxygen requirement in children with acute RSV infection (13, 14).

Our study has several limitations. The timing of the administration of dexamethasone could have played a critical role in the observed results. It is possible that dexamethasone failed to exert potent enough anti-inflammatory effect to alter the pathology because it was introduced too late in the course of the disease. Indeed, the mean disease duration prior to dexamethasone administration was 4 days. Nevertheless, considering that patients with acute RSV infection frequently present with non-specific upper respiratory tract symptoms that could last a few days, it would be difficult to conduct a study where steroids are instituted before the disease becomes overtly evident. In addition, it could be argued that the reported p-values were not corrected for the multiple comparisons performed; therefore, some of the significances could be explained by random chance. But as mentioned earlier, the

primary goal of this study was to identify the cytokines that would be differently expressed in the two treatment arms and to further explore their role over time; therefore, correcting them for multiple comparisons was deemed unnecessary. Nevertheless, if such correction was made, most of the significances would be lost and it will further support our conclusion of the lack of consistent effect of dexamethasone on the cytokine profile induced by RSV. Furthermore, we previously measured the concentrations of a limited number of cytokines (IL-8, TNF- α and RANTES) using conventional ELISA and we observed similar results with a lack of significant and consistent effect of dexamethasone therapy on these cytokines (27).

In conclusion, treatment with dexamethasone failed to induce a consistent anti-inflammatory effect in children with severe acute RSV infection as manifested by the lack of significant effect on the concentrations of the cytokines and chemokines that are thought to play a major role in orchestrating the inflammatory response induced by RSV. Additional studies to evaluate novel antiviral and/or anti-inflammatory agents for treatment for RSV bronchiolitis in children are warranted.

References

- LEADER S, KOHLHASE K. Respiratory syncytial virus-coded pediatric hospitalizations, 1997 to 1999. *Pediatr Infect Dis J*. 2002; 21: 629–32.
- HENRICKSON KJ, HOOVER S, KEHL KS, HUA W. National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatr Infect Dis J*. 2004; 23: S11–8.
- SHAY DK, HOLMAN RC, NEWMAN RD, LIU LL, STOUT JW, ANDERSON LJ. Bronchiolitis-associated hospitalizations among US children, 1980–1996. *JAMA*. 1999; 282: 1440–6.
- REIJONEN T, KORPPI M, PITKAKANGAS S, TENHOLA S, REMES K. The clinical efficacy of nebulized racemic epinephrine and albuterol in acute bronchiolitis. *Arch Pediatr Adolesc Med*. 1995; 149: 686–92.
- AMERICAN ACADEMY OF PEDIATRICS. Respiratory syncytial virus. In: PICKERING LK, BAKER CJ, LONG SS, McMILLAN JA, eds. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics, 2006: 27.
- DAVISON C, VENTRE KM, LUCHETTI M, RANDOLPH AG. Efficacy of interventions for bronchiolitis in critically ill infants: a systematic review and meta-analysis. *Pediatr Crit Care Med*. 2004; 5: 482–9.
- HARIPRAKASH S, ALEXANDER J, CARROLL W, et al. Randomized controlled trial of nebulized adrenaline in acute bronchiolitis. *Pediatr Allergy Immunol*. 2003; 14: 134–9.
- DAUGBJERG P, BRENOE E, FORCHHAMMER H, et al. A comparison between nebulized terbutaline, nebulized corticosteroid and systemic corticosteroid for acute wheezing in children up to 18 months of age. *Acta Paediatr*. 1993; 82: 547–51.
- GARRISON MM, CHRISTAKIS DA, HARVEY E, CUMMINGS P, DAVIS RL. Systemic corticosteroids in infant bronchiolitis: A meta-analysis. *Pediatrics*. 2000; 105: E44.
- BUCKINGHAM SC, JAFRI HS, BUSH AJ, et al. A randomized, double-blind, placebo-controlled trial of dexamethasone in severe respiratory syncytial virus (RSV) infection: effects on RSV quantity and clinical outcome. *J Infect Dis*. 2002; 185: 1222–8.
- ROOSEVELT G, SHEEHAN K, GRUPP-PHELAN J, TANZ RR, LISTERICK R. Dexamethasone in bronchiolitis: a randomized controlled trial. *Lancet*. 1996; 348: 292–5.
- VAN WOENSEL JB, VAN AALDEREN WM, DE WEERD W, et al. Dexamethasone for treatment of patients mechanically ventilated for lower respiratory tract infection caused by respiratory syncytial virus. *Thorax*. 2003; 58: 383–7.
- BENTUR L, SHOSEYOV D, FEIGENBAUM D, GORICHOVSKY Y, BIBI H. Dexamethasone inhalations in RSV bronchiolitis: a double-blind, placebo-controlled study. *Acta Paediatr*. 2005; 94: 866–71.
- CORNELI HM, ZORC JJ, MAHAJAN P, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. *N Engl J Med*. 2007; 357: 331–9.
- LODRUP CARLSEN KC, CARLSEN KH, NIKANDER K, et al. Nebulized budesonide after hospitalization for recurrent bronchial obstruction in children younger than 18 months. *Pediatr Allergy Immunol*. 2001; 12: 159–65.
- SHEERAN P, JAFRI H, CARUBELLI C, et al. Elevated cytokine concentrations in the nasopharyngeal and tracheal secretions of children with respiratory syncytial virus disease. *Pediatr Infect Dis J*. 1999; 18: 115–22.
- SZODORAY P, ALEX P, BRUN JG, CENTOLA M, JONSSON R. Circulating cytokines in primary Sjogren's syndrome determined by a multiplex cytokine array system. *Scand J Immunol*. 2004; 59: 592–9.
- ALVAREZ R, HARROD KS, SHIEH WJ, ZAKI S, TRIPP RA. Human metapneumovirus persists in BALB/c mice despite the presence of neutralizing antibodies. *J Virol*. 2004; 78: 14003–11.
- McNAMARA PS, FLANAGAN BF, HART CA, SMYTH RL. Production of chemokines in the lungs of infants with severe respiratory syncytial virus bronchiolitis. *J Infect Dis*. 2005; 191: 1225–32.
- WELLIVER RC, GAROFALO RP, OGRA PL. Beta-chemokines, but neither T helper type 1 nor T helper type 2 cytokines, correlate with severity of illness during respiratory syncytial virus infection. *Pediatr Infect Dis J*. 2002; 21: 457–61.
- BONT L, HEIJNEN CJ, KAVELAARS A, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. *Am J Respir Crit Care Med*. 2000; 161: 1518–23.
- BONT L, HEIJNEN CJ, KAVELAARS A, et al. Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J Infect Dis*. 2001; 184: 355–8.
- BONVILLE CA, ROSENBERG HF, DOMACHOWSKIE JB. Macrophage inflammatory protein-1 α and RANTES are present in nasal secretions during ongoing upper respiratory tract infection. *Pediatr Allergy Immunol*. 1999; 10: 39–44.

24. BENNETT BL, GAROFALO RP, CRON SG, et al. Immunopathogenesis of respiratory syncytial virus bronchiolitis. *J Infect Dis.* 2007; 195: 1532–40.
25. WELLIVER TP, GAROFALO RP, HOSAKOTE Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis.* 2007; 195: 1126–36.
26. BULOW SM, NIR M, LEVIN E, et al. Prednisolone treatment of respiratory syncytial virus infection: a randomized controlled trial of 147 infants. *Pediatrics.* 1999; 104: e77.
27. JAFRI HS, CARUBELLI CM, BUCKINGHAM SC, et al. Impact of Dexamethasone Therapy on TNF- α , IL-8, and RANTES Concentrations in the Lower Respiratory Tract Secretions of Children Intubated for Respiratory Syncytial Virus (RSV) Disease. Abstract 1564. 2000 Pediatric Academic Societies meeting. Boston, MA, 2000.