

Newborn patients exhibit an unusual pattern of interleukin 10 and interferon γ serum levels in response to cardiac surgery

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Objective: The aim of this study was to determine the clinical significance of serum levels of interleukin 10 and interferon γ in pediatric patients undergoing cardiopulmonary bypass.

Methods: We divided the patients into 2 groups: 8 neonates and 19 nonnewborn children. Interleukin 10 and interferon γ serum levels were quantified before sternotomy, at admission to the pediatric intensive care unit (30 minutes postoperatively), 24 hours after the onset of the operation, and 3 days after the operation.

Results: Newborn patients displayed significantly greater amounts of serum interleukin 10 than older children, not only in regard to the peak level achieved but also at every postoperative time point analyzed. In contrast, no significant changes in interferon γ serum levels were observed in neonates at any time point, whereas nonnewborn pediatric patients showed a significant increase in interferon γ serum concentrations immediately after the operation. This unusual pattern of cytokine response in newborn patients was not associated with modifications in cortisol serum levels. Furthermore, although neonates had significantly different surgical and clinical variables than did the nonnewborn pediatric patients, the variation in interleukin 10 production in neonates could not be accounted for by differences in the magnitude of surgical injury. In the group of neonates, there were significant positive correlations between peak interleukin 10 serum levels and both partial pressure of arterial oxygen/fraction of inspired oxygen ratio and postoperative body weight gain.

Conclusions: Newborn patients undergoing cardiopulmonary bypass exhibit a distinctive biologic response pattern characterized by high levels of serum interleukin 10 without changes in serum interferon γ . This cytokine imbalance could have potential clinical implications.

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Surgical trauma represents a model of tissue injury and repair. In the pathogenesis of this process, both neuroendocrine and immunoinflammatory systems are involved at the local and systemic levels.¹⁻⁴ Major body injuries, including surgical intervention, evoke reproducible metabolic, hormonal, and immunologic responses that are recognized as the stress response.^{1,2} The intensity of this response appears to be

TABLE 1. Baseline clinical and surgical characteristics of pediatric patients

	Weight (kg)	Age (mo)	Sex	Diagnosis	Operation	Time on bypass (min)	Crossclamp time (min)	Minimum temperature (°C)
Neonates	2.8	0.5	M	TGA	Arterial switch	100	70	16
	2.7	0.3	M	TGA	Arterial switch	131	65	18
	3.3	0.3	F	TGA	Arterial switch	155	63	18
	3.9	0.4	F	TGA	Arterial switch	120	62*	18
	3.3	0.5	M	TGA	Arterial switch	90	50*	16
	3.5	0.2	M	TGA	Arterial switch	122	70*	15
	3.1	0.2	M	TGA	Arterial switch	114	60	18
	3.4	0.3	F	TGA	Arterial switch	126	66	16
Mean ± SD	3.25 ± 0.39	0.37 ± 0.10				119.75 ± 19.72	63.25 ± 6.43	16.88 ± 1.25
Nonneonates								
	5.7	11	M	VSD	VSD repair	58	36	30
	5.3	4.5	F	TGA	Mustard procedure	72	43*	25
	6.7	13	M	TOF	TOF repair	99	51	24
	7.3	7	M	VSD and ASD	VSD and ASD repair	66	38	28
	18	48	F	TA	Fontan procedure	75	44	28
	9.5	15	M	VSD	VSD repair	55	30	30
	10.9	22	M	TOF	TOF repair	82	40	28
	13.5	34	F	VSD	VSD repair	62	34	28
	8.1	10	F	VSD	VSD repair	60	32	28
	8.7	11	M	VSD	VSD repair	67	38	28
	8.8	14	F	TOF	TOF repair	85	46	28
	55	144	F	VSD and SAoS	VSD and SAoS repair	55	41	32
	18	60	M	ASD	ASD repair	40	20	33
	22	85	F	LV-RA shunt	LV-RA shunt repair	32	12	34
	21	73	F	ASD	ASD repair	30	5	30
	52	167	F	PSVS	PSVS repair	48	23	28
	20	57	F	ASD	ASD repair	45	22	33
	28	88	M	ASD	ASD repair	40	20	33
	32	107	M	ASD	ASD repair	34	15	33
Mean ± SD	18.45 ± 14.55	51.08 ± 48.64				58.16 ± 19.16	31.05 ± 12.76	29.53 ± 2.84

TGA, Transposition of the great arteries; VSD, ventricular septal defect; TOF, tetralogy of Fallot; ASD, atrial septal defect; TA, tricuspid atresia; SAoS, subaortic stenosis; LV, left ventricle; RA, right atrium; PSVS, pulmonary supra-valvular stenosis.

*Total circulatory arrest was performed.

proportionally related to the extension of tissue damage.^{4,5} This stress response may be necessary for recovery of tissue after challenge and therefore is of survival value.^{2,3,5} However, when the resultant physiologic response is extensive and sustained, it may become injurious and contribute to the critical progression of the disease and, ultimately, to the death of the patient.²⁻⁴

Cardiac surgery is one of the most severe surgical stresses to which a child can be exposed and is clinically associated with systemic responses that can influence the postoperative course.⁶⁻⁸ It is widely recognized that cardiopulmonary bypass (CPB) activates a number of inflammatory pathways, including molecules of the complement system,⁸ kallikrein cascade, eicosanoid pathway,⁹ and cellular elements of the immune system, including monocytes-

macrophages and T cells and polymorphonuclear neutrophils and platelets.^{8,10}

Cytokines are molecules involved in the regulation of the immune inflammatory response. These molecules are secreted by different cells, including lymphocytes and accessory cells, and act on either the cytokine-producing cell (autocrine actions) or on other immune and nonimmune target cells (paracrine actions).¹¹ Accessory cells produce interleukin (IL) 1 β , IL-6, IL-10, and tumor necrosis factor (TNF) α .¹¹ T lymphocytes are also relevant cytokine producers, and several patterns of secretion have been defined. T-helper 1 (T_H1) cells mainly secrete IL-2, interferon (IFN) γ , and lymphotoxin, whereas T_H2 cells induce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 secretion.^{12,13} The cytokine production of T_H1 and T_H2 lymphocytes appears to show

mutual functional downregulation. It has been claimed that IFN- γ suppresses the proliferation and function of T_H2 cells, and in contrast, IL-10 inhibits that of T_H1 lymphocytes.¹³

IL-10, a 35- to 40-kd protein, is considered a relevant anti-inflammatory cytokine. IL-10 shows an inhibitory effect on monocyte class II major histocompatibility complex expression and on the production of reactive nitrogen oxides.^{13,14} In addition, this molecule upregulates the expression of both IL-1 receptor antagonist and soluble membrane-bound TNF receptors (sTNFR-55 and sTNFR-75).^{14,15}

By contrast, IFN- γ is a potent proinflammatory molecule that upregulates the expression of major histocompatibility complex class II antigens on macrophages¹⁶ and increases the release of TNF- α and IL-1 by these cells.^{12,16} Furthermore, this cytokine promotes the differentiation of undefined T_H cells into activated T_H1 cells.¹³

There is increasing evidence that the characteristics of immune response to stress signals appear to be dependent on the age and nutritional status of the subject.^{17,18} The functional state of the immune system varies with age, including the pattern of production and response to cytokines.¹⁹⁻²³ In this regard, deficient production of IFN- γ has been observed in neonates.^{19,23,24} Also, it is worth noting that children usually show a more prolonged but less severe biologic response than neonates.^{17,18} On the other hand, pediatric patients with congenital heart disease are frequently undernourished,²⁵ with associated immune system disturbances.²⁶ Thus, it is possible to suggest that different patterns of IL-10 and IFN- γ responses to surgical stress might be found during the early stages of life.

On the basis of this hypothesis, we have performed a prospective descriptive study to determine serum concentrations of IL-10 and IFN- γ before and after cardiac surgery in neonates and nonnewborn pediatric patients undergoing hypothermic CPB. The potential clinical significance of the serum levels of these cytokines was also analyzed.

Methods

Patients

Twenty-seven children undergoing corrective surgical repair for congenital heart defects with hypothermic CPB were studied from June 1997 to November 1997 at Gregorio Marañón University Hospital in Madrid. Patients were consecutively studied in order of presentation. The patients included in this study had no clinical or microbiologic evidence of concomitant associated infectious disease and were not receiving immunosuppressive or anti-inflammatory drugs before surgical intervention.

The 27 patients were divided into 2 groups: neonates (8 patients) and nonneonates (19 patients, Table 1). Furthermore, children from the nonneonate group were analyzed separately on the basis of the presence or absence of congenital cyanotic heart disease.

The local ethics committee approved the study. A legal guardian for each patient gave informed consent for the experimental protocol.

Anesthetic and CPB Techniques

Patients weighing more than 10 kg were premedicated with midazolam (0.7 mg/kg, oral route). In all other patients anesthesia was induced without premedication. Induction was completed with etomidate (0.3 mg/kg), and tracheal intubation was facilitated with pancuronium (0.1 mg/kg). Anesthesia was maintained with oxygen, high-dose fentanyl (100 μ g/kg), and pancuronium. Central venous catheters were introduced through the internal jugular vein, and radial or femoral arterial catheters were placed.

CPB equipment consisted of a roller pump (Cobe Stöckert Instrument, Rungis, France) and an appropriately sized Dideco hollow-fiber oxygenator with an integral cardiotomy reservoir (Dideco, Mirandola, Italy). The tubing of the extracorporeal circuit was constructed of polyvinyl chloride (Cobe Laboratories, Ltd, Gloucester, United Kingdom). The priming solution was a mixture of 5% albumin in Plasmalyte A solution, and packed red blood cells were added to achieve a hematocrit level approximating the targeted temperature and falling within the range of 20% to 30%. Packed red blood cells were added to the priming solution in all patients.

Body temperature was monitored continuously with a nasopharyngeal and vesical probe. Moderate hypothermia was induced in nonnewborns (23°C-34°C). In newborn patients lower temperatures (15°C-18°C) were induced. Core cooling and rewarming was achieved with an oxygenator heat-exchanger and with a warming-cooling blanket to reach a vesical-nasopharyngeal temperature of 36.5°C before terminating CPB. A flow of 2.4 L/m² per minute was maintained, except where a period of low perfusion or circulatory arrest was considered necessary. Anticoagulation was achieved with an initial bolus of 300 IU/kg of heparin injected into the right atrium before cannulation, and repeated doses were administered when the activated clotting time was shorter than 400 seconds. Heparin was neutralized with an injection of protamine sulfate within 5 minutes after the end of perfusion. Myocardial protection was achieved by means of Abboplegisol solution (temperature 4°C), with an initial dose of 25 to 30 mL/kg. Conventional ultrafiltration during the rewarming phase of CPB was performed in all patients.

After cardiac surgery was completed, body temperature was allowed to recover, and aortic crossclamping was stopped. Twelve patients were separated from CPB during infusion of dopamine, 10 patients during infusion of dopamine and milrinone or dobutamine, and 5 patients without any vasoactive drug. CPB was discontinued when the hemodynamic state was stable, and systemic perfusion pressures between 50 and 70 mm Hg were maintained. Thereafter, the patients were transported to the pediatric intensive care unit (PICU) in stable circulatory condition. Surgical data (type of intervention, CPB and aortic crossclamping time, minimum temperature on bypass, and presence or absence of circulatory arrest) of each patient and each group's mean \pm SD are shown in Table 1.

PICU Management

At the arrival to the PICU, all patients began mechanical ventilation, ensuring adequate ventilation and oxygenation. They

TABLE 2. Postoperative clinical data in pediatric patients

	Post/pre BW (%)	Postoperative ICU length of stay (d)	Mechanical ventilation (d)	Postoperative blood loss at first day (mL/kg)	Dopamine >10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or epinephrine*	Postoperative infection*	$\text{PaO}_2/\text{FiO}_2$ at third hour	$\text{PaO}_2/\text{FiO}_2$ at 18 hours
Neonates	122.2 \pm 3.2	10.63 \pm 3.29	6.25 \pm 1.83	21.44 \pm 8.43	5/8	2/8	197.0 \pm 23.6	168.9 \pm 24.6
Nonneonates	106.4 \pm 5.6	5.84 \pm 4.07	2.24 \pm 3.10	19.13 \pm 10.51	7/19	2/19	374.3 \pm 90.3	328.7 \pm 133.2
<i>P</i> value	<.001	.007	.002	.588	.221	.558	<.001	.002

Data are shown as means \pm SD or *absolute frequency. *Post/pre BW*, Postoperative/preoperative body weight percentage ratio at 6 hours after admission into the PICU.

received vasoactive drugs (dopamine and/or milrinone and/or adrenaline) if needed. Sedation and analgesia were achieved with midazolam and fentanyl, which were slowly tapered before extubation. Blood transfusions were administered if needed. Total parenteral nutrition was begun if enteral nutrition was not initiated 72 hours after the operation. No immunosuppressive or anti-inflammatory drug was administered. Blood gas analysis was performed after induction of anesthesia, during CPB, after separation of CPB, and during PICU admission. Systemic arterial pressure, right atrial pressure, colloid or donor blood volumes administered, postoperative blood loss, duration of mechanical ventilation, and days of PICU admittance were also recorded. The ratio of arterial partial pressure of oxygen to fractional inspired oxygen concentration ($\text{PaO}_2/\text{FiO}_2$) was calculated at 1, 3, 6, 12, and 18 hours after admission to the PICU. The postoperative/preoperative body weight percentage ratio as an indicator of postoperative body weight gain was determined 6 hours after admission to the PICU.

Blood Samples

Blood samples for IL-10, IFN- γ , and cortisol determinations were taken as follows: shortly after anesthetic induction before sternotomy (T0), at admission to the PICU (30 minutes postoperatively, T1), 24 hours after onset of the operation (T2), and 3 days after the operation (T3). The serum samples were obtained in all children at each time point. Blood samples were collected in sterile vacuum tubes and immediately centrifuged (1500g for 20 minutes at 4°C). Three aliquots of serum were obtained from each sample and stored at -70°C until analysis. Simultaneous blood samples were taken as follows: for Coulter counter analysis of total leukocyte concentration, platelet count, and hemoglobin concentration; aspartate aminotransferase and alanine aminotransferase activities; creatinine and blood urea nitrogen concentrations; and hemostasis test (including fibrinogen, prothrombin time, and partial thromboplastin time). Serum cortisol levels were assayed by means of radioimmunoassay (Immulite 2000 Cortisol; Diagnostic Products Corporation, Los Angeles, Calif). The sensitivity of the radioimmunoassay for cortisol is 0.2 $\mu\text{g}/\text{dL}$.

Cytokine Analysis

The amount of IL-10 and IFN- γ present in serum samples was measured with an enzyme-linked immunosorbent assay (human IL-10, Bender Medsystems, Vienna, Austria; human IFN- γ , Endogen, Cambridge, Mass), as previously described.²⁷ In brief, known concentrations of recombinant human IL-10 or IFN- γ and

the experimental samples were added in duplicate and incubated in polystyrene microtiter plates coated with a murine monoclonal antibody against the appointed cytokine, followed by incubation with an enzyme-linked polyclonal antibody directed to the cytokine. Next, a substrate solution for the enzyme was added, and the color development was stopped by adding 2N H_2SO_4 . The absorbance was measured with a microtiter plate spectrophotometer. The amount of IL-10 or IFN- γ present in each sample was determined from a standard curve generated in each assay and expressed as picograms per milliliter. The sensitivity of the enzyme-linked immunosorbent assay for IL-10 is 1.5 pg/mL, and the sensitivity for IFN- γ is 5 pg/mL. The reproducibility of all measurements was within 10% in our laboratory.

Statistical Analysis

Statistical analysis was performed on personal computers with commercially available software (SPSS 8.0 software, Chicago, Ill). Results in the text and tables are expressed as means \pm SD or as absolute frequency. For the analysis of normally distributed data (as evidenced by the Kolmogorov-Smirnov test), we used the independent-samples *t* test for comparison of values between the groups. The covariate analysis for the repeated-measures data on each subject was performed by using the general linear model repeated-measures procedure with simple contrasts, in which the reference category was the first category (T0). The χ^2 test (with the Fisher exact test if necessary) was used in the analysis of the qualitative data. The Mann-Whitney *U* test was used for the analysis of nonnormally distributed data. Correlation was studied with the Pearson correlation test (analyzing cytokines and cortisol serum levels at T1).

Results

Postoperative Clinical Data

All patients survived during the study period. We found differences in several clinical parameters after the surgical aggression between neonates and nonneonate patients, with both the level of pulmonary dysfunction and the postoperative/preoperative body weight percentage ratio being significantly higher in neonates than in the other children (Table 2). Moreover, length of both intubation time and stay in the PICU were also significantly longer in neonates (Table 2). On the other hand, although not statistically significant, there was a clear trend for the group of newborns to require

TABLE 3. IL-10, IFN- γ , and cortisol serum levels in the pediatric patients before and after cardiac surgery

	Neonates (n = 8)	Nonneonates (n = 19)	P value
IL-10 (pg/mL)			
T0	1.87 \pm 0.51	1.89 \pm 0.88	.923
T1	52.79 \pm 18.98	17.47 \pm 7.36	.001
T2	8.91 \pm 1.92	2.96 \pm 1.55	<.001
T3	8.47 \pm 2.00	2.30 \pm 0.88	<.001
IFN- γ (pg/mL)			
T0	6.89 \pm 1.80	6.40 \pm 1.37	.447
T1	7.97 \pm 1.42	19.91 \pm 3.48	<.001
T2	6.22 \pm 1.30	6.37 \pm 1.13	.781
T3	7.26 \pm 1.36	6.03 \pm 1.26	.060
Cortisol (μ g/dL)			
T0	11.64 \pm 1.88	12.77 \pm 7.05	.522
T1	29.98 \pm 4.69	30.72 \pm 8.23	.815
T2	21.35 \pm 5.37	18.98 \pm 3.3	.276
T3	18.49 \pm 3.75	19.61 \pm 3.74	.483

Data are shown as means \pm SD of the serum levels in each pediatric group. T0, Before sternotomy; T1, at PICU arrival; T2, 24 hours after onset of the operation; T3, 3 days after the operation.

higher doses of vasoactive drugs and to have more infectious complications (Table 2).

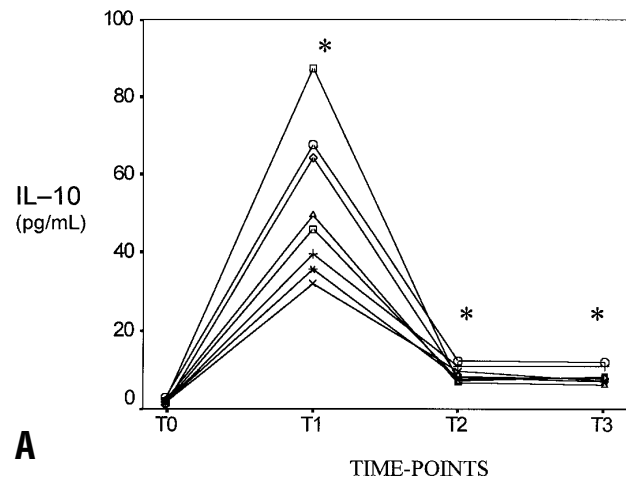
Open Cardiac Surgery Induces Different Patterns of IL-10 Response in Newborns and Other Pediatric Patients

IL-10 serum levels in the 2 groups of patients analyzed before sternotomy (T0), at admission to the PICU (T1), 24 hours after onset of the operation (T2), and 3 days after the operation (T3) are demonstrated in Figure 1 and Table 3. There were no significant differences in IL-10 levels between the series of subjects at T0. At admission to the PICU, IL-10 serum concentrations increased significantly and reached the peak level during the whole study period in the 2 groups of patients ($P < .001$). Interestingly, at this time (T1), the serum value of IL-10 was markedly higher in neonates than in the other group of subjects ($P = .001$). Subsequently, IL-10 serum levels declined at T2 and T3 in both groups, although newborns still demonstrated significantly higher levels of IL-10 than the other pediatric patients ($P < .001$). Although in neonates IL-10 serum concentrations remained significantly elevated from T0, in older children IL-10 serum levels dropped to the level found at T0.

Neonates Did Not Show Changes in IFN- γ Serum Levels as a Response to Cardiac Surgery

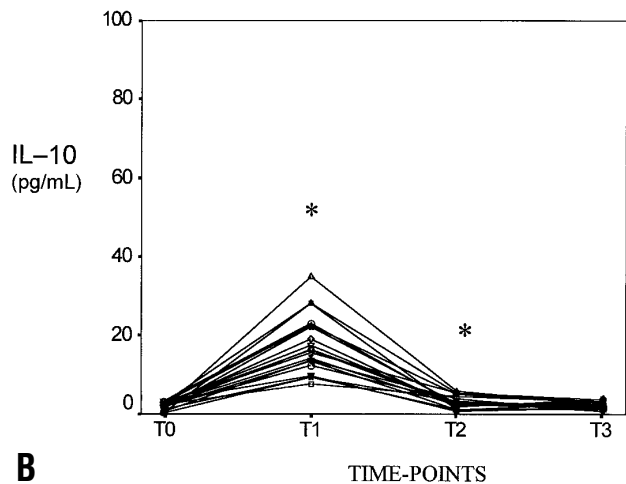
As shown in Table 3, no significant changes in IFN- γ serum levels were observed in neonates across the measurements. In contrast, nonneonate patients demonstrated a significant increase in IFN- γ serum levels at T1 compared with that at

NEONATES



A

NON-NEONATES



B

Figure 1. IL-10 serum levels in the 2 groups of pediatric patients. Lines and symbols show individual values. T0, Before sternotomy; T1, at PICU arrival; T2, 24 hours after onset of the operation; T3, 3 days after the operation. *Significant increase with respect to T0 IL-10 serum levels.

T0 ($P < .001$). It should be emphasized that at T1 IFN- γ serum concentrations in nonneonate children were significantly higher than those found in newborn patients ($P < .001$). Subsequently, at T2 and T3, IFN- γ serum levels in the nonneonate group decreased to values similar to those detected at T0.

Cortisol Secretion in Response to CPB Is Preserved in All Pediatric Patients

We were next interested in whether cortisol secretion could be associated with the distinct patterns of IL-10 and IFN- γ

TABLE 4. Clinical significance of peak IL-10 serum levels at T1

	Neonates	Nonneonates
CPB time	0.501 (.206)	0.253 (.297)
Crossclamp time	-0.448 (.266)	0.283 (.240)
Minimum temperature on bypass	0.526 (.180)	-0.194 (.426)
Post/pre BW (%)	0.739 (.036)	0.097 (.692)
Mechanical ventilation time	-0.295 (.477)	0.610 (.006)
Days of PICU stay	-0.206 (.625)	0.634 (.004)
Postoperative blood loss at first day	-0.287 (.490)	0.300 (.213)
PaO ₂ /Fio ₂ at third hour	-0.753 (.031)	-0.506 (.027)
PaO ₂ /Fio ₂ at 18 hours	-0.785 (.021)	-0.671 (.006)
Dopamine >10 µg · kg ⁻¹ · min ⁻¹ vs dopamine < 10 µg · kg ⁻¹ · min ⁻¹ *	63.0 ± 16.6 vs 35.8 ± 3.7 (.036)	21.9 ± 7.2 vs 14.9 ± 6.3 (.028)

Data represent Pearson correlation coefficients (*P* values). *T1*, At PICU arrival; *Post/pre BW*, postoperative/preoperative body weight percentage ratio at 6 hours after admission into the PICU.

*Statistical association: data represent means ± SD, with *P* value in parentheses.

serum concentrations observed in the different groups of pediatric patients. As Table 3 shows, the levels of cortisol before sternotomy (T0) were not significantly different between the 2 groups of patients. After CBP at T1, cortisol serum concentrations in the 2 groups show a clear increase with respect to values obtained at T0 (*P* < .001). Subsequently, at T2 and T3, cortisol levels markedly declined in all patients. Still, these values remained significantly higher than those found at T0 in both newborns (*P* = .001 and *P* < .001) and older children (*P* = .001 and *P* = .002). It is noteworthy that after CPB, there were no significant differences in serum cortisol concentrations between the 2 groups of subjects at the distinct moments analyzed (T1, T2, and T3; Table 3).

Clinical Significance of Cytokines and Cortisol Serum Levels

First, in the nonneonate group there were no significant differences between children with or without preoperative cyanosis with respect to serum concentrations at T1 of IL-10 (19.78 ± 7.17 pg/mL vs 16.65 ± 7.51 pg/mL, *P* = .298), IFN-γ (17.55 ± 4.09 pg/mL vs 20.75 ± 2.95 pg/mL, *P* = .087), and cortisol (34.86 ± 10.58 µg/dL vs 29.24 ± 7.10 µg/dL, *P* = .391).

The potential correlation in each group of patients between both surgical and postoperative clinical parameters and peak IL-10 serum levels at T1 is shown in Table 4. It should be emphasized that there were no correlations between surgical variables (CPB time, crossclamp time, and depth of intraoperative hypothermia) and peak IL-10 serum levels in either neonates or older children. Moreover, the

presence or absence of circulatory arrest in newborn patients during CBP was not related to serum concentrations of IL-10 at T1 (45.32 ± 16.89 pg/mL vs 57.28 ± 20.51 pg/mL, *P* = .393). Interestingly, in the group of neonates we found significant positive correlations between peak IL-10 serum levels and both PaO₂/Fio₂ ratio and postoperative/preoperative body weight percentage ratio, suggesting a possible relation of IL-10 response with pulmonary dysfunction associated with capillary leak syndrome. In nonneonate patients there were also correlations between peak IL-10 serum levels and PaO₂/Fio₂ ratio. However, in this group of children we found no correlations between this cytokine and body weight gain.

In addition to these remarkable correlations, there was also significant association between the need for high doses of vasoactive drugs (ie, dopamine >10 µg · kg⁻¹ · min⁻¹ or epinephrine in any dose) and peak IL-10 serum levels at T1 in both neonates (*P* = .036) and children (*P* = .028). On the other hand, only in nonnewborn children were peak IL-10 concentration levels correlated with both length of mechanical ventilation and stay in the PICU. The absence of correlations between these parameters in neonate patients could be ascribed to the fact that weaning of mechanical ventilation in these children is frequently conditioned by other problems not related to pulmonary dysfunction, such as decreased functional residual capacity, elevated compliance of the chest wall, and predisposition to bronchopulmonary collapse.

With regard to IFN-γ and cortisol serum concentrations, we found no correlations among these parameters at T1 and either surgical or postoperative clinical variables (data not shown). However, the 2 nonneonate patients with respiratory infections during the postoperative period showed low peak IFN-γ serum levels (14.92 and 15.37 pg/mL, respectively).

No correlations were observed between cortisol and IL-10 or IFN-γ serum levels in either of the 2 patient groups evaluated (data not shown).

Discussion

In this article we have demonstrated that tissue injury induced by cardiac surgery in pediatric patients is associated with profound modifications in IL-10 and IFN-γ serum levels that are mainly related to the age of the children.

Our results show that surgical cardiac stress promotes a significant increase of IL-10 serum levels in children, regardless of their age, nutritional status, preoperative clinical condition, or the magnitude of surgical injury. It should be emphasized that newborn patients undergoing cardiac surgery display greater amounts of serum IL-10 than older children at each postoperative time point analyzed. In contrast, IFN-γ serum levels detected in neonates did not show significant changes after cardiac surgery. Interestingly, in the other groups of pediatric patients, a clear increase of serum IFN-γ was observed in the immediate postoperative period.

In our group of nonneonate patients, there were no significant associations between IFN- γ , IL-10, or cortisol serum concentrations and previous presence or absence of congenital cyanotic heart disease. Importantly, the unusual pattern of IFN- γ and IL-10 serum release detected in neonates cannot be ascribed to changes in pituitary-adrenal response, as evaluated with serum cortisol levels.

The pattern of response of IL-10 to CPB procedures in newborns has not been previously analyzed. However, our findings in nonneonate children do agree with the results of the few published studies²⁸⁻³⁰ that address IL-10 production in response to CPB procedures in children. We have found no prior studies investigating the response of IFN- γ induced by cardiac surgery in pediatric patients. IFN- γ serum concentrations induced by CBP have been only reported in adults, whose patterns of IFN- γ response are similar to those observed in our series of nonneonate patients.³¹

There were no correlations between either peak IL-10 or IFN- γ serum levels and CPB time, crossclamping time, minimum temperature on CPB, and circulatory arrest in either neonates or the other pediatric patients. The distinctive pattern of IL-10 production seen in neonates compared with that in nonnewborn children could not be ascribed to group-related differences in the preoperative clinical condition or surgical parameters tested. In support of this evidence it can be argued that pediatric patients from the nonneonate group show similar serum concentrations of these cytokines in spite of striking surgical stress differences.

The potential clinical relevance of this distinctive pattern of IL-10 and IFN- γ responses to cardiac surgery in neonates has also been evaluated in our study. Interestingly, in neonates there was a marked correlation among peak IL-10 serum levels and both pulmonary dysfunction and postoperative weight gain, suggesting an intriguing possible implication of this cytokine in the physiopathology of capillary leak syndrome, which is associated with CBP in newborn patients. In line with this presumption, it should be pointed out that older children also showed significant correlation between IL-10 serum concentrations and pulmonary dysfunction. Nevertheless, taking into account that the group of neonates exhibited higher IL-10 serum levels and more complications related to capillary leak syndrome, it is conceivable that IL-10 response could play a critical role in the postoperative course of newborn patients. Moreover, in both groups of children, there was also a significant association between the need for high doses of vasoactive drugs and IL-10 serum concentrations, providing additional proof of the potential involvement of IL-10 in the systemic inflammatory response linked to CBP in pediatric patients.

Recent studies have provided evidence that in the active phase of other severe systemic inflammatory diseases, the production of IL-10 is also increased. In septic shock, for instance, high IL-10 serum levels are related with concomi-

tant increased release of proinflammatory cytokines and greater clinical severity and mortality.³²⁻³⁴ Given the biologic evidence that IL-10 acts as an inhibitory functional peptide on activated macrophages and T lymphocytes that secrete proinflammatory cytokines, it is reasonable to propose that increases in this cytokine reflect a mechanism of counterregulation to limit the systemic effects of an inappropriate and deleterious biologic response. The fact that neonates display more extensive and sustained IL-10 serum levels induced by cardiac surgery supports the view advocated by other investigators that, in this early period of the life, the biologic response is more severe.^{17-19,24} However, it cannot be excluded that IL-10 might directly mediate harmful effects, as has been indicated in some multiorgan diseases, such as systemic lupus erythematosus.³⁵

On the other hand, the clinical significance of the absence of IFN- γ release in newborn patients undergoing cardiac surgery could be relevant, conditioning the course of the systemic biologic response. In this regard it is possible to suggest that this lack of IFN- γ response could predispose to serious infectious diseases that can complicate the postoperative period in neonate patients. In support of this hypothesis, it can be argued that older children with infectious complications displayed low peak IFN- γ serum concentrations. Whether this deficient neonatal IFN- γ response to an aggressive surgical stimulus is intrinsic or the result of a suppressor hyperresponse of IL-10 on IFN- γ production is unknown. Nevertheless, it is noteworthy that in previous reports stimulated peripheral mononuclear cells from healthy neonates exhibited decreased capacity for IFN- γ secretion.²⁴

It is clear that other peptides that make up the cytokine and chemokine families could be implicated in the regulation of the biologic response associated with tissue injury.^{7,9} Nevertheless, the data are still fragmentary, and much more information will be needed before reaching any definitive conclusions.

An important question that has to be elucidated is the source for the striking IL-10 serum levels induced by CPB in neonates. The potential producer cells are not unique and include monocytes-macrophages and both T and B cells. Taking into account that IL-10 peak levels are achieved immediately after surgical intervention, it is likely that in this phase of the biologic response, activated monocytes-macrophages are the main producers of IL-10. However, our results show that newborn patients exhibit sustained elevated IL-10 serum concentrations for 72 hours after cardiac surgery. This finding is consistent with the presumption that T lymphocytes, and perhaps also B cells, activated later might be implicated in the secretion of IL-10. On the basis of this presumption, it is reasonable to postulate that major surgical stress could promote a predominant T_H2 functional response in neonates. This possibility is further supported

by the observation that these patients do not display measurable amounts of the IFN- γ T_H1 cytokine in serum.

In summary, this study expands current knowledge on the state of systemic biologic response to CPB in pediatric patients and demonstrates the presence of a distinctive pattern of IFN- γ and IL-10 release in neonates with potential prognostic implications. Future investigations will be necessary to identify the pathogenic and clinical significance of this cytokine imbalance, which may provide a rationale for new immunologic approaches to selected newborn patients with severe systemic inflammatory responses to cardiac surgery.

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