Inflammatory response in the central nervous system following perinatal asphyxia

Respuesta inflamatoria en el sistema nervioso central secundaria a asfixia perinatal

MARTÍN-ANCEL, A.; GARCÍA-ALIX, A.; PASCUAL-SALCEDO, D.; VALCARCE, M., and QUERO, J.

Department of Pediatrics. Division of Neonatology (A. M. -A., M. V., J. Q.), and the Department of Biochemistry (D. P. -S.). La Paz Children's Hospital. Autonoma University of Madrid, Spain, and the Division of Neonatology, Hospital Materno-Infantil. Las Palmas de Gran Canaria. Spain (A. G. -A.).

ABSTRACT

Objectives: To examine the relation of IL-6 levels in the CSF to the severity of hypoxic-ischemic encephalopathy (HIE) and to the outcome. *Methods:* Asphyxiated term neonates were included. Cerebrospinal fluid (CSF) IL-6 was measured by a sensitive ELISA. *Results:* 20 neonates were studied: 3 had no HIE, 5 had stage 1, 6 had stage 2, and 6 had stage 3. CSF IL-6 levels (8 to 90 hours of life) were higher in neonates with HIE stage 3 (range 65 to 2250 pg/ml) when compared to neonates with HIE stage 0 to 2 (<2 pg/ml in 12 neonates, 10 pg/ml in 1)($p<0.01$). 5 neonates had adverse outcome: 4 died, and 1 had cerebral palsy. 13 had normal outcome. CSF IL-6 levels (10.8±6.1 months) were higher in neonates with adverse outcome (range 65 to 2250 pg/ml) compared to neonates with favorable outcome ($p<0.05$).

Conclusion: IL-6 levels in the CSF are related to neonatal neurological manifestations and to the outcome. Our results suggest that IL-6 is implicated in the pathogenesis of neonatal hypoxic-ischemic brain damage.

Key words: Perinatal asphyxia. Cerebral ischemia. Interleukin 6. Tumor necrosis factor.

RESUMEN

Objetivos: Examinar la relación entre los niveles de IL-6 en líquido cefalorraquídeo (LCR) con el grado de encefalopatía hipóxico-isquémica (EHI) y la evolución. *Métodos:* Se estudiaron recién nacidos a término asfixiados. Se determinaron los niveles de IL-6 en LCR mediante ELISA. *Resulados:* Se incluyeron 20 neonatos: 3 sin EHI, 5 con grado 1, 6 con grado 2, y 6 con grado 3. La concentración de IL-6 en LCR (8 a 90 horas de vida) fue mayor en neonatos con EHI grado 3 (rango 65 a 2250 pg/ml) que en neonatos con EHI grados 0 a 2 (<2 pg/ml en 12 neonatos, 10 pg/ml en 1) ($p<0.01$). 5 neonatos tuvieron una evolución adversa: 4 murieron, y 1 tuvo parálisis cerebral. 13 presentaron una evolución favorable. Los niveles de IL-6 en LCR (10.8±6.1 meses) fueron mayores en neonatos con evolución adversa (rango 65 a 2250 pg/ml) comparados con neonatos con evolución favorable ($p<0.05$).

Conclusión: Los niveles de IL-6 en LCR se relacionan con el grado de EHI y con la evolución. Nuestros resultados sugieren que la IL-6 está implicada en la patogenia del daño cerebral hipóxico-isquémico neonatal.
Palabras clave: Asfixia perinatal. Isquemia cerebral. Interleucina-6. Factor necrosis tumoral.

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INTRODUCTION

There is increasing evidence that inflammation is involved in the pathogenesis of ischemic brain injury. The inflammatory reaction consists of a large influx of leukocytes into the injured zone during the postischemic period. Polymorphonuclear cell infiltration peaks at 24-48 hours, T cell (suppressor/cytotoxic T cells and probably natural killer cells) at day 3, and the accumulation of monocytes at 2 to 14 days (1-3). In addition, microglia rapidly proliferate, are recruited to the site of lesion, and transform into phagocytes (4-5).

The migration of leukocytes into the injured tissue requires the expression of endothelial specific adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and the endothelial-leukocyte adhesion molecule-1 (ELAM-1) (6-7), which are probably driven by cytokines produced by the ischemic brain, mainly tumor necrosis factor a (TNF-a) and interleukin 1b (IL-1b) (8-9). Chemoattractant cytokines, such as cytokine-induced neutrophil chemoattractant (CINC, a member of the interleukin 8 family), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 (JE/MIP-1), induced in part by TNF-a and IL-1b, are involved in leukocyte recruitment to the injured brain (10-12).

Although there is ample evidence that interleukin 6 (IL-6) is involved in the pathogenesis of ischemic myocardial damage (13-15), few studies have examined in animal models the role of IL-6 in hypoxic-ischemic brain injury (16,17), and no data have been reported regarding IL-6 response in human central nervous system (CNS) following ischemic injury.

The objectives of this study were: (1) to determine cerebrospinal fluid (CSF) levels of IL-6 following perinatal asphyxia, and (2) to examine the relation of IL-6 levels in the CSF to the severity of hypoxic-ischemic encephalopathy (HIE), and to the neurological outcome.

PATIENTS AND METHODS

Patients

The study population comprised asphyxiated term neonates admitted to the Neonatal Unit at La Paz Children's Hospital, Autonoma University of Madrid, from January 1990 to December 1995. The infants were identified to have experienced perinatal asphyxia when at least three of the following criteria were present: (1) fetal scalp blood pH <7.20, (2) umbilical artery blood pH at birth < 7.20, (3) Apgar scores under 4 at 1 minute and/or under 7 at 5 minutes, (4) requirement of more than 1 minute of positive pressure ventilation before sustained respiration occurred. The criteria for exclusion were congenital malformations, metabolic disorders, congenital or adquired infections, maternal drug addiction, and absence of parental consent. Ten of the neonates had been enrolled in a study conducted at La Paz Children's Hospital from 1990 to 1992 (18,19).

Complete obstetrical histories were obtained and examinations performed at the time of admission. The neonatal clinical course was followed prospectively and data were recorded on predetermined proform sheets. Pathological examinations were performed in each infant who died.

Clinical Assessment

A detailed structured neurological examination (20) was performed at approximately 12, 36, 72 hours of age, and then at 7 days of life by a single investigator (A. G.-A.). The stage of encephalopathy was assessed according to a simplified Amiel-Tison and Ellison staging system (21). Briefly, Stage 1 was diagnosed when hyperexcitability and/or hypotonia persisted for at least 72 hours after birth. Stage 2 was diagnosed in the presence of lethargy, hypotonia, and weak or partially absent primitive reflexes with or without seizures. Finally, Stage 3 was considered when, in addition to severe tonus anomaly and frequent seizures, there was coma or stupor.

In all surviving infants, neurologic outcome was assessed at 3-month intervals by means of neurologic examinations based on the method of Amiel-Tison and Grenier (22) and the Denver Developmental Screening Test (23). Based on the outcome, children were classified as: (1) normal outcome, (2) mild motor impairment (slight abnormality in muscular tone or an abnormal pattern of motor development), or (3) adverse outcome, when the child developed cerebral palsy (diplegia, hemiplegia, or tetraplegia) or died in the early neonatal period.

Biochemical Determinations

CSF samples were obtained at approximately 12 (13.8±4.2) and/or 72 (67.8±24.5) hours of life by lumbar puncture. Aliquots of 0.4 ml were distributed in plastic tubes, which were immediately frozen and stored at -50°C until analized. Aliquots grossly contaminated with blood of hemolized were discarded. IL-6 was determined by means of a highly sensitive and specific enzyme-linked immunosorbent assay (24) by one investigator (D. P.-S.) unaware of the infant's neurologic status. The sensitivity of the method was 2 pg/ml. Intrassay and interassay variations were 4% and 15%, respectively.

Statistical Analysis

The data are expressed as mean ± standard deviation, or range (median), for descriptive purposses unless otherwise stated. The Kruskal-Wallis test was used to analyze differences among groups. When samples from the same infant at 12 and 72 horus were available, only the first one was considered for statistical analysis.

RESULTS

Twenty asphyxiated term neonates were studied; 13 were boys. Three infants had no HIE, 5 had HIE stage 1, 6 had stage 2, and 6 stage 3. Four infants with stage 3 died within the first week of life. No differences were found between groups in gestational age, birth weight, and umbilical artery pH at birth; Apgar scores at 1 and 5 minutes were lower in infants with HIE stage 3 ($p < 0.05$ and $p < 0.005$, respectively) (Table 1).

Table 1.—Main perinatal data of the neonates according to the stage of HIE. * $p<0.05$ vs no HIE and HIE stage1. # $p < 0.005$ vs no HIE and stages 1 and 2.

	No HIE (n = 3)	Stage 1 (n = 5)	Stage 2 (n = 6)	Stage 3 (n = 6)
GA (weeks)	41.0±1.7	40.2±0.4	39.7±1.6	40.0±0.9
Birth weignt (g)	3317±382	3082±386	2947±272	3372±218
Apgar 1 min	4±1.7	2.6±1.1	2.1±1.3	1±0.9*
Apgar 5 min	7.7±0.6	5.8±1.6	5.7±1.6	2.1±1.3#
Umbilical a pH	7.02±0.11	6.98±0.14	6.98±0.2	6.88±0.15

CSF concentration of IL-6 and stage of encephalopathy

Twenty six CSF samples from the 20 infants were available for IL-6 determinations; 15 samples were obtained at 12 hours of life, and 11 at 72 hours. All neonates with no encephalopathy and neonates with HIE stage 1 had undetectable levels of IL-6 in the CSF (lower than 2 pg/ml). Most infants with stage 2 also had undetectable levels, although two of them had IL-6 levels of 10 pg/ml. All neonates with HIE stage 3 showed IL-6 levels in the CSF higher than 60 pg/ml; the concentration of IL-6 in two infants with stage 3 who had both CSF samples markedly decreased form 12 to 72 hours of life. CSF levels of IL-6 were significantly higher in neonates with HIE stage 3 compared to neonates with stage 1, 2 or without encephalopathy ($p < 0.01$) (Fig. 1).

CSF concentration of IL-6 and outcome

Two neonates had mild motor impairment, one developed cerebral palsy, and four died within the first week of life. IL-6 concentration in the CSF was significantly higher in neonates with adverse outcome (cerebral palsy or death) than in neonates with mild motor impairment or normal outcome ($p < 0.05$) (Fig. 2).

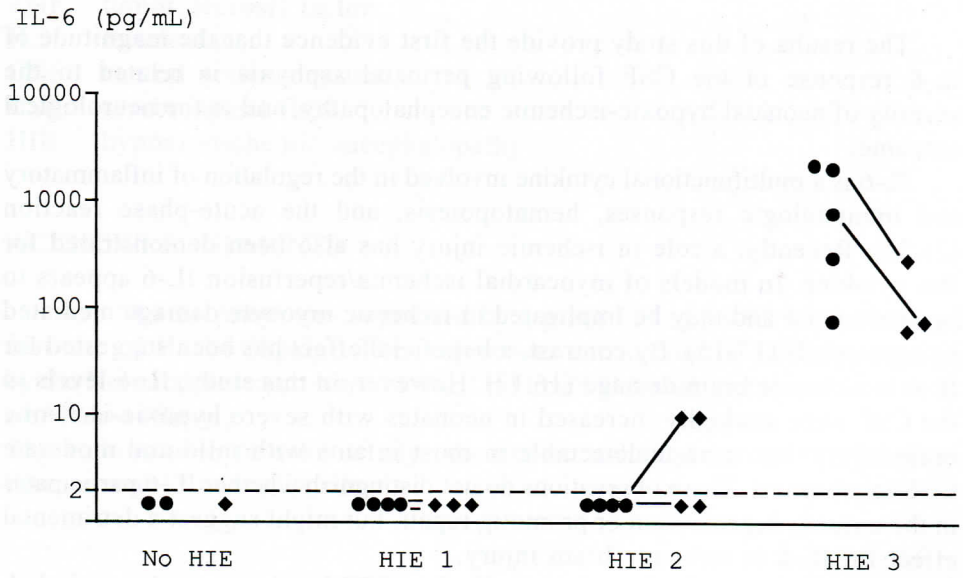

Fig. 1.—IL-6 levels in the CSF according to the severity of HIE at 12 (●) and 72 hours (◆) of age. Samples from the same infant at 12 and 72 hours are linked by a line.

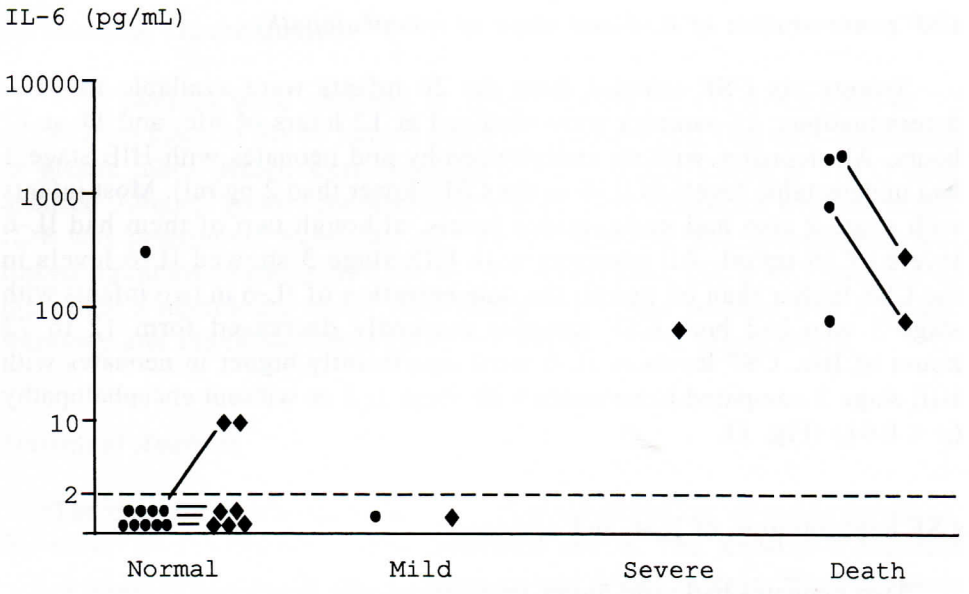

Fig. 2.—IL-6 levels in the CSF according to the neurological outcome at 12 (●) and 72 hours (◆) of age. Samples from the same infant at 12 and 72 hours are linked by a line.

DICUSSION

The results of this study provide the first evidence that the magnitude of IL-6 response in the CSF following perinatal asphyxia is related to the severity of neonatal hypoxic-ischemic encephalopathy, and to the neurological outcome.

IL-6 is a multifunctional cytokine involved in the regulation of inflammatory and immunologic responses, hematopoiesis, and the acute-phase reaction (25,26). Recently, a role in ischemic injury has also been demonstrated for this cytokine. In models of myocardial ischemia/reperfusion IL-6 appears to be detrimental and may be implicated in ischemic myocyte damage mediated by neutrophils (13-15). By contrast, a beneficial effect has been suggested for IL-6 in ischemic brain damage (16,17). However, in this study, IL-6 levels in the CSF were markedly increased in neonates with severe hypoxic-ischemic brain injury, but were undetectable in most infants with mild and moderate CNS involvement. These obsevations do not distinguish whether IL-6 participates in the ensuing degeneration or promotig repair, but might suggest a detrimental effect for IL-6 in ischemic brain injury.

A possible contribution of serum IL-6 in CSF levels cannot be excluded. Elevated IL-6 levels in serum have been detected in patients with acute

stroke, showing a maximum concentration of serum IL-6 of 12.65±3.63 (mean±SEM) pg/ml in 19 patients studied by Fassbender et al. (28), and lower than 20 pg/ml in 41 patients evaluated by Beamer et al. (29). Markedly higher levels were consistently found in the CSF in all the neonates with severe brain damage in this study. Therefore, it seems unlikely that serum levels are responsible for the presence of IL-6 in the CSF. However, perinatal asphyxia produces a more global insult compared to acute stroke, and no data regarding serum levels in asphyxiated neonates have been reported. In addition, IL-6 mRNA has been shown to be induced in rat ischemic cortex (17), which suggests that CSF IL-6 is, at least in part, produced by ischemic brain. But even if IL-6 is mainly produced in the brain, this may not preclude a role for peripheral IL-6 in hypoxic-ischemic encephalopathy.

In conclusion, our results indicate that IL-6 levels in the CSF relate to early and long-term neurological manifestations following perinatal asphyxia. Studies performed in experimental animal models during the last few years have implicated IL-6 in ischemic brain damage (16,17). Our data support the hypothesis that IL-6 plays a role in the pathogenesis of neonatal hypoxic-ischemic brain damage, and therefore, extend previous experimental findings to human CNS.

ABBREVIATIONS

TNF: tumor necrosis factor
IL: interleukin
CNS: central nervous system
CSF: cerebrospinal fluid
HIE: hypoxic-ischemic encephalopathy

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