during the time course of observation in both patients. Cardio-toxicity as possible side effect of DL sodium β -hydroxybutyrate is thus unlikely. Vomiting or loss of "orality" was preexisting to the administration of DL sodium β -hydroxybutyrate and did not deteriorate upon treatment.

In our observational study, DL sodium β -hydroxybutyrate was administered as an ultima ratio in two infants with PHHI who suffered from recurrent hypoglycemic events despite extensive conventional treatment. Results are preliminary and further experimental and clinical studies are needed prior to general clinical application. In the light of the fact that short chain fatty acid oxidation defects such as SCAD and SCHAD deficiency may be associated with PHHI, we fully agree with Hussain *et al.* that patients who might potentially benefit from this treatment have to be selected carefully. Administration of DL sodium β -hydroxybutyrate in PHHI is complementary and is not a substitute of conventional therapy.

Barbara Plecko

Department of Pediatrics
University of Graz, Austria

Edith Schober

Department of Pediatrics
University of Vienna, Austria

Sylvia Stöckler-Ipsiroglu

Department of Pediatrics
University Hospital of Vienna
Währinger Gürtel 18-20
A-1090 Vienna, Austria
Tel: 43 1 40400 3210
Fax: +43 1 4063484
e-mail: stoeckler@metabolic-screening.at

REFERENCE

1. Plecko B, Stöckler-Ipsiroglu S, Schober E, Harrer G, Mlynarik V, Gruber S, Moser E, Moeslinger D, Silgoner H, Ipsiroglu O 2002 Oral β -hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: monitoring of β -hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by *in vivo* magnetic resonance spectroscopy. *Pediatr Res* 52:301–306

DOI: 10.1203/01.PDR.0000063371.16142.66

To the Editor: We read with interest the article by Schultz *et al.* (1), who present data on the neonatal inflammatory response.

As detection of intracellular cytokines may not necessarily correlate with extracellular cytokine concentrations, we do not totally agree with the authors' conclusions on the neonatal immune system extracted from these data.

As well, there may be concern about some methodical issues:

1. The authors stored blood samples for up to 24 hours at room temperature. Reinsberg *et al.* (2) showed that IL-8 concentrations in whole blood lysate increased significantly after 3 hours of storage at room temperature. The authors do not show data on the influences of sample storage.

2. Differences in storage time between the groups may have affected the results.

3. Van Langevelde *et al.* (3) reported a maximum endotoxin concentration of 18 pg/ml in adult febrile patients. In this study a maximal LPS stimulus of 30,000 pg/ml has been used. The effect on intracellular cytokine production may not reflect *in vivo* conditions.

4. It has been shown that labor induces cytokine production in term and preterm infants (4). Modification of cytokine synthesis by labor may differ in neonatal cells from preterm and term infants. The diminished spontaneous production of intracellular cytokines as well as the production after *ex vivo* stimulation in preterm infants <32 weeks gestational age could well have resulted from the 100% cesarean section rate in this group.

5. The authors demonstrate a dose related inhibition of *ex vivo* monocyte cytokine expression by incubation with dexamethasone. They do not comment on the possible effects of the different rates of corticosteroid pretreatment between the study groups (50%, 96%, 100%). There is no information on the number of doses and the type of steroid used for prophylaxis of RDS.

6. The diagnosis of neonatal infection has been based on single parameters. This is not a commonly used definition.

7. Anti-inflammatory activity has not been investigated. Data on IL-10 derived from different study designs are compared in the discussion.

On the background of these questions, the hypothesis of an enhanced neonatal inflammatory response remains at least debatable. Evaluation of imbalances of pro- and antiinflammatory components in neonates may be more effective by investigation of a larger cytokine panel.

Jörg Dembinski

Daniela Behrendt

P. Bartmann

Department of Neonatology
University Bonn, Germany
Adenauerallee 199
D-53113 Bonn
Germany
jdembinski@uni-bonn.de

REFERENCES

1. Schultz C, Rott C, Temming P, Schlenke P, Moller JC, Bucszy P 2002 Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. *Pediatr Res* 51:317–322
2. Van Langevelde P, Joop K, van Loon J, Frölich M, Groeneveld PHP, Westendorp RGJ, van Dissel JT 2000 Endotoxin, cytokines and procalcitonin in febrile patients admitted to the hospital: identification of subjects at high risk of mortality. *Clin Infect Dis* 31:1343–1348
3. Dembinski J, Behrendt D, Heep A, Dorn C, Reinsberg J, Bartmann P 2002 Cell-associated interleukin-8 in cord blood of term and preterm infants. *Clin Diagn Lab Immunol* 9:320–323
4. Reinsberg J, Dembinski J, Dorn C, Behrendt D, Bartmann P, van Der Ven H 2000 Determination of total interleukin-8 in whole blood after cell lysis. *Clin Chem* 46:1387–1394

DOI: 10.1203/01.pdr.0000063368.16142.F9

Response

We would like to thank Dembinski and colleagues for the interest they have taken in our work. They raised concern about the method used and the conclusions drawn from our data. Until recently the neonatal inflammatory response was consid-

ered to be intrinsically hyporesponsive. However, both the high incidence of neonatal sepsis as well as elevated proinflammatory cytokines in neonatal blood during infection support an already well established inflammatory response in neonates. In our study we could demonstrate for the first time not only a well developed but also an enhanced inflammatory response in term and preterm infants directly at the cell level. We preferred the intracytoplasmic detection of cytokines by flow cytometry for several reasons. First, this method enables a direct relation between cytokine production and cell phenotype without performing cell-separation procedures. This is of special concern because cell-separation procedures require higher amounts of blood and strongly influence cell activity status. Second, a dissociation between plasma and cell-associated cytokines during sepsis favors cytokine analysis directly at the cell level (1).

Dembinski and colleagues argue that intracellular cytokine detection does not necessarily correlate with extracellular cytokine concentrations. To check this we analyzed cytokine production in culture supernatant by enzyme-linked immunosorbent assay (ELISA) in a smaller number of term infants and adults before we initiated the study. We found a comparable amount of IL-6 (median, interquartile range: 4084 pg/ml; 3962-4351 pg/ml versus 4991 pg/ml; 4253-5977 pg/ml; $p = 0.26$) but a strikingly higher amount of IL-8 in term infants than adults (11306 pg/ml; 8574-12553 pg/ml versus 1759 pg/ml; 1422-1821 pg/ml; $p < 0.01$). These data support an already established inflammatory response in neonates, as well.

Dembinski and colleagues raise concern about some methodical issues:

- 1, 2: They found an increase of IL-8 after 3 hours of storage at room temperature and argue that differences in storage time between groups may have affected the results. It is well known that the amount of cytokines in blood samples varies strikingly after storage at room temperature if analyzed by ELISA. However, in our study another method was used. To clarify this issue for this new method, we analyzed the influence of specimen age on intracytoplasmic cytokine levels after whole-blood culture assay (2). After 20 hours of storage, the amount of IL-6-positive and IL-8-positive monocytes shows only a mean deviation of 6.2% (SEM $\pm$ 9.3%) and 2% (SEM $\pm$ 7.8 %), respectively. After 48 hours of storage, the mean deviation increases to 10.9% for IL-6 (SEM $\pm$ 18.2%) but remains relatively stable for IL-8 (Mean 1.2%, SEM $\pm$ 4.4%). Therefore, it was acceptable to store blood samples up to 24 hours before flow cytometric analysis at the cell level. The argument that differences in storage time between groups may have affected the results is not plausible because the deviation was both-sided and considerably smaller than the differences between groups.
3. Dembinski and colleagues refer to a study in which maximum endotoxin concentrations of 18 pg/ml (= 0.018 ng/ml) in adult febrile patients were reported. However, the median age was 61 years, and only 31 out of 464 investigated patients had a septic shock in this study. In contrast, we investigated neonates and wanted to simulate a situation of septic shock. Therefore, higher endotoxin concentrations are needed. In really septic patients endotoxin levels above

2.7 ng/ml were reported (3). Furthermore, the used concentration of 30 ng/ml was necessary to induce detectable amounts of cytokines in several studies (4, 5). Amounts of 0.03 ng/ml, which are favored by Dembinski and colleagues, were defined as free of LPS (5).

4. To analyze the effect of labor on cytokine production, we compared the amount of cytokine positive cells in preterm infants delivered spontaneously with preterm infants delivered by elective cesarean section (> 32 weeks of gestational age). We found no correlation between the percentage of cytokine positive cells and delivery mode (6). However, in the group of preterm infants below 32 weeks of gestational age, the comparison could not be done because all of them were delivered by cesarean section. To circumvent the influence of labor, we also analyzed fetal blood samples obtained by cordocentesis from pregnant women undergoing prenatal diagnosis (7). These data were in accordance with the data obtained from preterm infants below 32 weeks of gestational age (6) and argue against the assumption of Dembinski and colleagues that the diminished cytokine production in preterm infants below 32 weeks of gestational age have resulted from the 100% cesarean section rate in this group. Furthermore, the amount of IL-8 positive cells was strikingly higher in fetuses below 27 weeks of gestational age than in adults (Mean (SD): 89% (3.6) versus 68.9% (8.1); $p < 0.0001$) (7), contradicting the commonly held view that the immature immune system is intrinsically hyporesponsive.
5. In our study betamethasone was used to accelerate fetal lung maturity. The number of doses and the time between last administration and delivery had no effect on the number of cytokine positive cells. The possible effect of different rates of corticosteroid pretreatment on cytokine production is discussed in detail in our article on page 320 (6). In brief, the percentage of IL-6 positive cells after stimulation with LPS was lower in preterm infants treated antenatally with corticosteroids ($n = 44$) compared with preterm infants without corticosteroid pretreatment ($n = 19$) (median, interquartile range: 48.3%, 39.9–54.4% versus 63.4%, 53.6–72.4%, $p < 0.0001$). However, most preterm infants without corticosteroid pretreatment were above 32 weeks (18 out of 19; 95%) whereas treated preterm infants were primarily below 32 weeks of gestational age (26 out of 44; 59%). For ethical reasons both groups could not be matched according to gestational age. Therefore, the lower percentage of IL-6 positive monocytes after stimulation with LPS in the group of pretreated preterm infants could also be the result of the lower gestational age. However, it is even more amazing that these very low birth weight infants display a higher percentage of IL-8 positive monocytes than adults despite pretreatment with corticosteroids and despite the 100% cesarean section rate (6).
6. We agree with Dembinski and colleagues that clinical diagnosis of neonatal infection should be based on more than one criterion. However, in our study we wanted to differentiate exactly between preterm infants with and without an initiated inflammatory response *in vivo*. Because

already one of these criteria (6) is indicative for inflammation, these infants were analyzed separately.

7. Meanwhile, we could demonstrate a reduced capacity of term and preterm infants to produce the main anti-inflammatory cytokines IL-10 and TGF- β . These data support our hypothesis of an imbalance between pro- and anti-inflammatory cytokines in neonates (8) (submitted for publication), which especially makes preterm infants with incomplete organ development susceptible to harmful effects of proinflammatory cytokines.

Christian Schultz

Department of Pediatrics
Medical University of Lübeck
Ratzeburger Allee 160
23538 Lübeck
Germany

E-mail: ChrSchultz@aol.com

Or Schultz@paedia.ukl.mu-luebeck.de

REFERENCES

1. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM 1991 Dissociation between plasma and monocyte-associated cytokines during sepsis. *Eur J Immunol* 21:2177–2184
2. Schultz C, Rott C, Temming P, von Puttkamer J, Bucskey P 2002 Influence of specimen age and use of different negative controls in determination of intracytoplasmic cytokine levels after whole-blood culture assay. *Clin Diagn Lab Immunol* 9:295–298
3. Hamilton G, Hofbauer S, Hamilton B 1992 Endotoxin, TNF-alpha, interleukin-6 and parameters of the cellular immune system in patients with intraabdominal sepsis. *Scand J Infect Dis* 24:361–368
4. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F 2000 Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol* 1:311–316
5. Kitchens RL, Thompson PA, Viriyakosol S, O'Keefe GE, Munford RS 2001 Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. *J Clin Invest* 108:485–493
6. Schultz C, Rott C, Temming P, Schlenke P, Möller JC, Bucskey P 2002 Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. *Pediatr Res* 51:317–322
7. Schultz C, Temming P, Gembruch U, Strunk T, Bucskey P 2002 Polyclonal intravenous immunoglobulin to prevent brain injury in preterm infants. *Lancet* 359:1522–1524
8. Schultz C, Temming P, Rott C, Göpel W, Möller JC, Bucskey P 2001 Imbalance of inflammatory cytokines in term and preterm infants. *Z Geburtsh Neonatol* 205 (Suppl 1):S21

DOI: 10.1203/01.PDR.0000063369.16142.B0

Erratum

In the article “3-Cholorotyrosine as a Marker of Protein Damage by Myeloperoxidase in Traceal Aspirates from Preterm Infants: Association with Adverse Respiratory Outcome” (*Pediatr Res* 53:455–462), the units for chlorotyrosine in Tables 2 and 3 should be millimoles instead of micromoles per mole tyrosine. The values in the tables are 1000-fold less than those in the text and figures, which are in micromoles per mole tyrosine. The authors regret the error.