Fast Decline of Hematopoiesis and Uncoupling Protein 2 Content in Human Liver after Birth: Location of the Protein in Kupffer Cells

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ABSTRACT

Hepatic hematopoiesis is prominent during fetal life and ceases around birth. In rodent liver, the decline of the hepatic hematopoiesis starts abruptly at birth being accompanied by a decrease of mitochondrial uncoupling protein 2 (UCP2) expression in monocytes/macrophages, whereas hepatocytes may express UCP2 only under pathologic situations. The goals of this study were to characterize hepatic hematopoiesis in humans around birth, and to identify cells expressing UCP2. Hematopoiesis was evaluated histologically in the liver of 22 newborns (mostly very premature neonates), who died between 45 min and 140 d after birth, and one fetus. UCP2 expression was characterized by Northern blots, immunoblotting, immunohistochemistry, and by *in situ* hybridization. The number of hematopoietic cells started to decrease rapidly at birth, irrespectively of the gestational age (23–40 wk) of neonates. A similar decline was observed for UCP2 expression, which was relatively high in fetal

liver. UCP2 was detected only in myeloid cells (mainly in Kupffer cells), but not in hepatocytes, although sepsis or other pathologies occurred in the critically ill newborns. Kupffer cells represent the major site of mitochondrial UCP2 expression in the human newborn. UCP2 may be essential for the differentiation and function of macrophages and serve as a marker for these cells in human liver during the perinatal period. (*Pediatr Res* 49: 440–447, 2001)

Abbreviations

UCP2, uncoupling protein 2
PPAR γ , peroxisome proliferator-activated receptor γ
ROS, reactive oxygen species
SSC, standard saline citrate
BCIP/NBT, 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium

The liver is important for hematopoiesis in human embryonic and fetal life. This organ is colonized by yolk-sac-derived stem cells by 5–6 wk of gestation, and the liver is the primary source of red blood cells from the 9th week on. During the 24th week, bone marrow replaces the liver as the main site of hematopoiesis (1–4). Hematopoietic activity in human liver is thought to cease soon after birth. However, the relative importance of the gestational age of the fetus and the process of birth, respectively, on the postnatal decline of hepatic hematopoiesis is not well defined (1, 5–8).

In addition to erythroid differentiation, myelopoiesis, lymphopoiesis, and megakaryocyte production take place in the fetal liver (2, 8). The factors, that affect cell lineage preference of hematopoietic stem cells, as well as the interactions between cells of different lineages during their replication and differentiation, are now intensely characterized [for refs., see (4, 9, 10)]. New cell-type-specific markers to study the development of the hematopoietic system are required. Our recent study (11) has identified mitochondrial UCP2 as a specific marker of monocyte/macrophage cells developing in mouse and rat liver. The liver was the major site of UCP2 expression in the rodent fetus and the level of UCP2 mRNA declined soon after birth. Also in the liver of adult animals, UCP2 was found in monocytes/macrophages and not in hepatocytes (12). However, under pathologic situations, such as during inflammation (13), steatosis (14, 15), or neoplastic transformation (16), UCP2 gene expression could occur also in the hepatocytes of adult animals.

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UCP2 is a relatively new member (17, 18) of the family of mitochondrial UCPs (19). It is highly expressed in the spleen and the thymus (20, 21), probably reflecting contribution by tissue macrophages (11, 12), as well as in peripheral blood leukocytes (20) and in adipose tissue (17, 18). This protein may control the efficiency of oxidative phosphorylation, and mitochondrial synthesis of ATP, *via* its protonophoric short-circuiting activity in the inner mitochondrial membrane (17–19, 22–24). The function of UCP2 is probably tissue-specific. In macrophages, UCP2 may inhibit the production of ROS in mitochondria (21). The present research was designed to characterize hepatic hematopoiesis and UCP2 expression during perinatal development in humans. Very premature neonates, who died at different postnatal age, were mainly studied.

METHODS

Human material. Liver samples were obtained from 22 human newborns during autopsy 2–3 h after death. Most of the newborns had very low birth weight (<1000 g with gestational age at birth between 23 and 32 wk; 16 cases); they died between 45 min and 140 d after birth. There were also six children with a birth weight >1000 g (and gestational age >28 wk); two of these were full-term neonates (Table 1, cases A0 and A62). Some of the newborns were already enrolled in our

previous study (25) (Table 1). Infants born from mothers who suffered from endocrinological disorders or who abused drugs were not eligible for the study. One fetus, which originated from legal termination of pregnancy after 23 wk of gestation because of a hereditary disease, was also included (Table 1). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and it was *a priori* approved by the Committees of Medical Ethics at all the collaborating institutions. Informed consent was obtained from the parents. Samples were frozen and stored in liquid nitrogen for isolation of total RNA and for immunoblotting analysis. Tissues were also fixed for histopathological and immunohistochemical examinations (see below).

Mouse material. Adult male C57BL/6J mice maintained at 20°C and fed standard chow diet (ST-1, Velaz, Prague, Czech Republic) were killed by decapitation in diethyl ether anesthesia and interscapular brown fat was dissected and stored in liquid nitrogen. The study protocol was approved by the Animal Care and Use Committee at the Institute of Physiology.

RNA analysis. Total RNA was isolated and analyzed on Northern blots using a full-length cDNA probe for mouse UCP2 and human liver subunit IV of mitochondrial cytochrome oxidase (COX IV), as described previously (11). In addition, a 209-bp fragment corresponding to nucleotides 238–

Table 1. Characterization of the cases studied

Case no.	Sex	Gestational age at birth (wk)	Birth weight (g)	Survival* (d)	Hematopoiesis†	UCP2 expression‡	Clinical diagnosis and pathological anatomy characteristics
A0	M	39	3400	7	0	nt	IA, MOF
A11§	M	32	950	3	++	nt	CHT, DIC, H, S
A12§	M	28	1030	135	0	nt	CLD, P, RDS
A14§	M	27	940	7	++	nt	ICH, LH, PROM, RDS, RF
A16§	F	23	480	16	++	nt	G, PROM, RDS, RF, S
A27§	M	24	590	7 h	+++	nt	RDS, RF
A28§	F	26	835	3	+	nt	G, IA, ICH, NEC, RDS
A31§	F	24	600	140	+	0	IA, CLD, M, RDS
A36§	F	30	620	12	0	nt	H, PH, PDA, RDS, SGA
A44§	F	27	800	90	++	0	G, HIE, NEC, P
A48§	F	27	635	7	+	0	DIC, ICH, PDA, PH, RDS
A50§	M	23	550	8	+	0	G, ICH, RDS, S
A60	M	38	2620	45 min	++	nt	Osteogenesis imperfecta
A61	M	23	630	36	0	0	CLD, HY, NEC, PDA, RF, RDS, S
A62	M	40	3670	4 h	++	D	TGV
A63	F	26	750	38	0	0	CLD, HY, ICH, NEC, PDA, PP, RDS, S
A64	M	30	1240	9 h	+++	100	HS, IA, ICH, SGA
A65	F	22	450	9	+++	nt	HY, IA, S, SGA
A66	F	31	1450	5	+++	43	HIE, IA
A67	F	24	635	39	0	0	G, ICH, NEC, PP, RDS, S
A68	F	24	450	45	+	12	CHH, G, ICH, PP, RDS, S
AB1	F	23	450	0	+++	53	AB
D14§	F	25	730	11	+	0	G, IA, ICH, NEC, RDS

AB, abortion elicited for osteochondrodysplasia; CHH, cholestatic hepatopathy; CHT, chromosomal translocation 45/14q21q; CLD, chronic lung disease; DIC, disseminated intravascular coagulation; G, gemini; H, hypotrophy; HIE, hypoxic-ischemic encephalopathy; HS, hemorrhagic shock; HY, severe hypothermia (body temperature <31°C) during the early postnatal period; IA, intrauterine asphyxia; ICH, intracranial hemorrhage; LH, lung hypoplasia; M, meningitis; MOF, multiorgan failure; NEC, necrotizing enterocolitis; OS, osteochondrodysplasia; P, pneumonia; PP, peripheral pancytopenia; PDA, patent ductus arteriosus; PH, pulmonary hemorrhage; PROM, premature rupture of membranes; RDS, respiratory distress syndrome; RF, respiratory failure; S, sepsis; SGA, small for gestational age (43); TGV, transposition of the great vessels.

* In days, except where indicated otherwise (in bold).

† Hepatic hematopoiesis was scored according to the number of nonparenchymal cells (see "Methods"); nt, not tested.

‡ Evaluated by Northern blotting and hybridization with a 209-bp fragment of human UCP2 cDNA (arbitrary units; see Fig. 2); D, degraded RNA.

§ Cases examined in a previous study (25).

447 of human UCP2 mRNA was used. It was obtained by digestion with *Hind*III and *Pvu*II of the human UCP2 cDNA inserted into the *Eco*RI site of the pTRE plasmid (Clontech, Palo Alto, CA, U.S.A.). The presence of equivalent amounts of ribosomal RNA on the blots was checked by UV shadowing at 256 nm. The DECAprime II Random Priming DNA Labeling Kit (Ambion, Austin, TX, U.S.A.; cat. 1455) was used, and the radioactivity was evaluated by Bioimage Analyzing System (BAS-5000, Fuji, Tokyo, Japan).

Immunoblotting analysis. Crude cell membranes were isolated from tissue homogenates by centrifugation at 100,000 g and analyzed by immunoblotting (26). Proteins separated on 15% polyacrylamide gels were transferred on PVDF (Immobilon-P, Sigma Chemical Co., St. Louis, MO, U.S.A.; cat. P-2938) membrane by electroblotting. The blots were blocked overnight in PBS containing 0.05% Tween 20 and 5% nonfat milk. Incubation with the primary antibodies was performed for 12 h in the same buffer as above, except that the concentration of the nonfat milk was 1% and the solution also contained 0.1% sodium azide. The following primary antibodies were used: (a) whole rabbit serum (1:500; C4/98 serum) against UCP1 isolated (27) from mouse interscapular brown fat; (b) sheep anti-rat UCP1 IgG [1:2000; this was a different batch of the antiserum used in previous studies; see refs. (11, 12). and "Acknowledgments"]; (c) rabbit anti-mouse UCP2 IgG against amino acids 144–157 of the protein from Calbiochem (San Diego, CA, U.S.A.; cat. 662047; 1:500); and (d) rabbit anti-mouse UCP2 antiserum against a 14-amino acid peptide sequence at the C-terminus of the protein from Alpha Diagnostics Intl. (San Antonio, TX, U.S.A.; cat. UCP22-S; 1:2,000). Blots were washed in PBS containing 0.05% Tween 20 (five times, 5 min/wash) and incubation with the secondary antibodies was done in the same buffer for 40 min. Secondary antibodies were goat anti-rabbit IgG coupled to horseradish peroxidase (Bio-Rad, Hercules, CA, U.S.A.; cat. 170-6525; 1:100,000) or donkey anti-sheep/goat IgG coupled to horseradish peroxidase (The Binding Site Limited, Birmingham, U.K.; cat. AP360; 1:100,000). The reaction was visualized by chemiluminescence (ECL+Plus Western Blotting Detection System; kit RPN2132; Amersham, Uppsala, Sweden) and detected by Luminescence Analyzing System (LAS-1000, Fuji, Tokyo, Japan). The protein concentration was measured using the bicinchoninic acid procedure (28) and BSA as standard.

Histologic and immunohistochemical evaluations. Human liver samples were fixed (3 h) in 3.7% formaldehyde-PBS, dehydrated, and embedded in paraffin. Sections (5 μ m) were stained with hematoxylin and eosin (Fig. 1, A and B), and also with trichrome in parallel (not shown). They were then evaluated by a board-certified pathologist (I.V.). Hematopoiesis in the liver was scored on a four-tier scale (0, +, ++, or +++), based on the number of nonparenchymal cells. Immunohistochemical labeling (Fig. 1, C–H) was performed similarly as before (11). Rabbit anti-mouse UCP1 C4/98 antiserum was used to detect UCP2 (see "Results"). In the single labeling protocol, a 1:1000 dilution of the antiserum was used, and the secondary antibodies (anti-rabbit immunoglobulin conjugated with alkaline phosphatase; 1:100 dilution; Dako, Glostrup, Denmark; cat. D0487) were visualized using BCIP/NBT

(Dako). With the double-labeling method, C4/98 antiserum was used at a 1:500 dilution, and UCP2 was revealed using goat anti-rabbit IgG conjugated to Texas red (Jackson, Baltimore, MD, U.S.A.; cat. 111-076-045; 1:100). Monoclonal mouse anti-human CD68 antiserum (Dako, clone PG-M1; cat. M0876; 1:50) was used as a marker specific for monocytes/macrophages. Secondary antibodies were horse anti-mouse IgG labeled with fluorescein (Vector Laboratories, Inc., Burlingame, CA, U.S.A.; cat. FI 2000; 1:2000). Fluorescence was visualized using a Leica microscope (model DMRB; Leica Microsystems AG, Wetzlar, Germany) and a CCD video camera (model DXC-930; Sony Corporation, Tokyo, Japan). Negative controls were performed by omitting the first antibody.

In situ hybridization. Tissues were fixed in 3.7% formaldehyde in PBS, dehydrated, and embedded in paraffin. Sections (5 μ m) were dewaxed and rehydrated through successive baths of Bioclear (a biodegradable xylene substitute; Microstain, Toulouse, France), ethanol (10% and 95%), and PBS containing 0.1% fresh diethylpyrocarbonate. After equilibration in $5 \times$ SSC, the sections were prehybridized for 1 h at 42°C in a solution containing 50% formamide, $5 \times$ SSC, and salmon sperm DNA (250 μ g/mL). Hybridization was performed overnight at 42°C in a solution containing 50% formamide, $5 \times$ SSC, salmon sperm DNA (40 μ g/mL), and antisense UCP2 DIG-labeled RNA probe (0.5 μ g/mL). The probe was prepared using the DIG RNA Labeling Kit (SP6/T7; Roche Molecular Biochemicals, Mannheim, Germany) by *in vitro* transcription from *Sal*I-linearized plasmid containing mouse UCP2 cDNA. Samples were washed in $2 \times$ SSC for 30 min at room temperature, treated with RNAase A (20 μ g/mL) for 20 min at 37°C, and washed in $2 \times$ SSC, and in $0.2 \times$ SSC (each wash for 30 min, twice, at 50°C). UCP2 transcripts were detected using the DIG and the Block Buffer Set, following the manufacturer's instructions, and visualized using a chromogenic substrate BCIP/NBT (Dako). Sections were counterstained with nuclear red.

RESULTS

Decline of hepatic hematopoiesis after birth. The highest number of hematopoietic cells (+++) was found in four infants (A27, A64, A65, and A66) who died within 9 d after birth, and two of whom (A27 and A64) died within 9 h postnatally (Table 1 and Fig. 1, A and B). Very high hematopoiesis was also detected in the liver of the aborted fetus (AB1). Relatively high hematopoiesis (++) was observed in six children (A11, A14, A16, A44, A60, and A62) who died within 16 d of postnatal life, except for infant A44 (who died at 90 d postnatally). On the other hand, a negligible hematopoietic activity (0) was observed in six infants (A0, A12, A36, A61, A63, and A67) who survived for at least 7 d after birth (the case A0), but mostly much longer than 7 d (Table 1). Other infants (cases A28, A31, A48, A50, A68, and D14) had low but detectable hematopoiesis (+) in the liver and died between 3 and 140 d after birth.

The results suggest an acceleration of the decline in the number of hematopoietic cells in the liver following birth. In general, no link between the postnatal decline of hematopoiesis

Figure 1. Microphotographs of histologic and immunohistochemical analysis of newborn liver. *A* and *B*: Sections stained with hematoxylin and eosin. *C*–*F*: Single immunohistochemical detection of UCP2 using anti-mouse UCP1 antiserum. *G* and *H*: Double immunohistochemical labeling of UCP2 (Texas red; micrograph *G*) and CD68 (fluorescein; micrograph *H*). *A*, *C*, *E*, *G*, and *H*: The newborn A64 who died 9 h after birth at 30 wk of gestation. *B* and *D*: The infant A63 who died 38 d after birth at 26 wk of gestation. *F*: the newborn A66 who died 5 d after birth at 31 wk of gestation (see Table 1 for further details). In panel *A*, there is evidence of considerable hematopoiesis (+++), whereas in panel *B*, no hematopoiesis is seen (0). In panels *C*–*F*, dark blue precipitates indicate BCIP/NBT deposit in UCP-positive cells (arrows). Counterstain is rouge nuclear. In panel *D*, one UCP-positive cell is seen, but such cells were rare and the section was graded as 0. In panels *E* and *F*, the following structures are labeled: Erythroblasts (*e*), hepatic cord (*hc*), nucleus in a hepatocyte (*nh*), hemolyzed erythrocytes (*he*), granulocyte (*g*), sinusoid (*s*), and macrophages (arrow) located in sinusoid (panel *E*) or placed centrally in an erythroblastic island (micrograph *F*). Bar = 60 μm (*A*–*D*), bar = 20 μm (*E* and *F*), and bar = 5 μm (*G* and *H*).

and the gestational age, the clinical diagnosis, or the pathologic anatomy characteristics could be found (Table 1). The only exception could be prenatal or postnatal hypoxia (Table 1, neonates with intrauterine asphyxia and respiratory distress syndrome, respectively), which was frequently present in the infants with high hematopoiesis.

Decline of UCP2 expression in neonatal liver. To characterize the expression of UCP2 mRNA in human liver, total RNA isolated from 14 out of 23 cases in our cohort (Table 1) was analyzed using Northern blots (Fig. 2). Total RNA was also isolated from mouse interscapular brown fat, which contained UCP2 mRNA (17, 18) and was used as a standard for identification of UCP2 transcript in human samples (Fig. 2). In spite of the fact that the liver samples have been collected 2–3 h after death, only one sample (case A62) contained RNA that was highly degraded, as revealed by UV shadowing of the blots. RNA in the other liver samples was more than 70% intact (not shown, see also below). When hybridized using a full-length cDNA for mouse UCP2, significant levels of UCP2 transcript in the liver were detected (Fig. 2 and Table 1) only in the aborted fetus (case AB1) and in two infants (cases A64 and A66) who died within 5 d after birth. A marginal expression was detected in one more infant (case A68; not shown). The size of the transcript (1.7 kbp) was similar in both liver and brown fat samples, indicating its identity with UCP2 mRNA. Rehybridization with a 209-bp human UCP2 probe (corresponding to a divergent region of human and mouse UCP2 cDNA sequence) confirmed the results. No reactivity was observed in mouse brown fat, indicating high specificity of the 209-bp probe for the human UCP2 mRNA. Final rehybridization with a cDNA probe for subunit IV of mitochondrial cytochrome oxidase from human liver indicated the presence of the transcript of the subunit in all human liver samples (Fig. 2). The signal was very weak in brown fat, apparently reflecting the differences in the sequence of cytochrome oxidase

subunit IV between human and mouse (Fig. 2). These results documented that the absence of detectable UCP2 mRNA in the majority of human liver samples did not reflect degradation of RNA. Besides the liver, UCP2 was expressed also in the heart, skeletal muscle, spleen, lung, and adipose tissue. However, the levels of the transcript detected in various organs were always lower compared with that in the liver of the fetus or newborns who died soon after birth (not shown).

Expression of UCP2 in myeloid cells. Further experiments were focused on the immunohistochemical identification of UCP2-producing cells. Because there is almost no convincing published description of antibodies specifically reacting with human UCP2 [see ref (19).], various antibodies were first characterized using immunoblotting: Two types of antibodies against whole UCP1 isolated from brown adipose tissue of rodents and two types of the anti-UCP2 antibodies raised against 14 amino acid peptide sequences from different parts of mouse protein (see "Methods") were compared. Membranous fractions isolated from human liver differing in the level of UCP2 mRNA (cases A63 and A64, with a negligible or high UCP2 transcript level, respectively; see Fig. 2), as well as mitochondria isolated from interscapular brown fat of the mouse, were used (Fig. 3). In brown fat [containing UCP1, UCP2, and UCP3 (17, 18, 20)], all except one of the antibodies recognized the antigen with an apparent molecular mass close to 33 kD (Fig. 3, A–D). Regarding the similarity in molecular mass and a high sequence homology of all three UCP (19), the

Figure 2. Northern blot analysis of gene expression in human fetal and newborn liver. Total RNA was isolated from the liver of human fetus and newborns (see Table 1) and mouse brown fat (BF). Hybridization was performed using a full-length cDNA probe for mouse UCP2 (mUCP2, 1.7-kbp transcript); the blot was rehybridized using a 209-bp fragment of human UCP2 cDNA (huUCP2, 1.7-kbp transcript) and finally rehybridized using a cDNA probe for human liver subunit IV of mitochondrial cytochrome oxidase (huCOX IV, 0.9-kbp transcript). Two different parts of the same blots are shown.

Figure 3. Immunoblotting analysis of gene expression in human newborn liver. Membranous fractions isolated from the liver (30 μ g protein) of the infant with undetectable (case A63) or high (case A64) level of UCP2 transcript, respectively (see Fig. 2), and mouse brown fat (BF; 2 μ g protein) were analyzed using (A) the rabbit anti-mouse UCP1 antiserum; (B) sheep anti-rat UCP1 IgG; (C) rabbit anti-mouse UCP2 IgG against amino acids 144–157 of the protein from Calbiochem (San Diego, CA, U.S.A.); and (D) rabbit anti-mouse UCP2 antiserum against a 14 amino acid peptide sequence at the C-terminus of the protein from Alpha Diagnostics (San Antonio, TX, U.S.A.). Arrow, position of the 33-kD antigen (UCP1) in brown fat.

33-kD antigen in brown fat could correspond to any of these proteins. In the liver, only the anti-mouse UCP1 antiserum recognized a single band in the 33-kD region (Fig. 3A), in the infant with high UCP2 mRNA (case A64) and not in the other infant (case A63). In the absence of either UCP1 or UCP3 in the liver (11, 12, 29, 30) the 33-kD antigen recognized by anti-UCP1 antiserum should represent UCP2 (11, 12). This antiserum, apparently cross-reacting with human UCP2, also revealed a major band at approximately 15 kD in all the liver samples. However, this band did not correlate with the levels of UCP2 transcript and could represent a nonspecific interaction of the antiserum with proteins loaded in relatively large quantities on the polyacrylamide gel [not shown; see also ref (31)]. The anti-rat UCP1 antiserum reacted with the 33-kD antigen in mouse brown fat (Fig. 3B) and with UCP2 in mouse liver (11, 12); however, it did not cross-react with human UCP2 (Fig. 3B). One of the antisera raised against the peptide sequences of UCP2 did not show any significant reactivity with the human liver samples in the 33-kD region (Fig. 3C), whereas the other antiserum revealed several faint bands (Fig. 3D).

Based on the above experiments, the anti-mouse UCP1 antiserum cross-reacting with human UCP2 (see above) was selected for the immunohistochemical staining of the human liver samples. The single-labeling experiment revealed strongly UCP-positive cells in nonparenchymal cells in the liver of the newborn who survived for only 9 h and had intense hepatic hematopoiesis and high UCP2 expression (Table 1, case A64). However, neither erythroid cells nor hepatocytes were stained (Fig. 1, C and E). Most of the labeled cells had sinusoid localization and showed morphology typical of Kupffer cells. Some monocytes and other myeloid cells (presumably granulocytes) were also labeled, but these cells were stained less than the Kupffer cells (Fig. 1E). In the other neonate with high hematopoietic activity and UCP2 expression in the liver (Table 1, case A66), strongly UCP-positive cells were also detected in the liver parenchyma. These cells were placed centrally in the erythroblastic islands and surrounded by erythroid cells (Fig. 1F). Morphology of these cells and their position in the erythroblastic islands were typical of the Kupffer cells (8, 32). Examination of the liver of the infant with negligible hepatic hematopoiesis and undetectable UCP2 expression (Table 1, case A63) revealed very few UCP-positive

cells (Fig. 1D). These results, indicating UCP2 expression in the nonparenchymal cells, and not in hepatocytes, were confirmed by the single-labeling analysis with the anti-mouse UCP1 antiserum in all the infants from our cohort (not shown).

Monocyte/macrophage cells can be characterized by expression of specific antigenic markers including cytoplasmic CD68. A double-labeling experiment indicated that antigens recognized by the anti-mouse UCP1 antiserum (Fig. 1G) and by the anti-human CD68 antibody (Fig. 1H) were located in the same cells. However, not all the UCP-positive cells were stained with the anti-CD68 antibody and *vice versa* (Fig. 1H). These experiments supported the view that the majority of the UCP-positive cells were identical with Kupffer cells. To confirm this notion, *in situ* hybridization experiment was performed (Fig. 4). Analysis of the liver of the neonates with high hematopoietic activity (cases A64 and A66) clearly revealed the presence of UCP2 transcript in cells with the morphology and localization typical of Kupffer cells (Fig. 4, A and B; compare with Fig. 1, E and F).

DISCUSSION

Results of this study document that the process of birth accelerates the decrease in the content of hematopoietic cells in the liver. The gestational age affects the number of hematopoietic cells in the liver at birth but not the rate of their disappearance from the liver (Table 1). Hepatic hematopoiesis was largely suppressed within 7 d of postnatal life in a majority of the newborns, in spite of the difference in their gestational age at birth (between 23 and 40 wk, see Table 1). A recent study on mice indicated an abrupt postnatal decrease of hematopoietic cells in the liver and suggested that the elevation of blood oxygen tension at birth was the precipitating factor of the decrease (6). This mechanism was probably also responsible for the destruction and steatosis observed postpartum in the mouse liver, as well as for the neonatal granulocytosis (6). Oxygen stress at birth may also contribute to the termination of fetal hepatic hematopoiesis in humans, whereas intrauterine or postnatal hypoxia seem to promote liver hematopoiesis (Table 1). However, no association between liver steatosis and early postnatal death of human newborns was detected, whereas

Figure 4. Microphotographs of *in situ* hybridization analysis of UCP2 transcript in newborn liver. The mRNA UCP2 was detected as described in "Methods" and visualized using BCIP/NBT. In panel A, the newborn A64, in panel B, the newborn A66 (for details, see Fig. 1 and Table 1). The following structures are labeled: UCP2 transcript-positive cells (black arrow), sinusoid (s), nucleolus in a hepatocyte (nh), and erythroblast (e). Bar = 20 μ m.

steatosis was observed in the majority of infants with sepsis (not shown).

Concerning the UCP2 expression, high levels of UCP2 transcript were only detected in two neonates (Table 1, cases A64 and A66) who died within 5 d after birth, and in the fetus (Table 1, case AB1). In all these cases, hepatic hematopoiesis was also very intense (+++). On the other hand, no infant with undetectable UCP2 transcript by Northern blot analysis survived less than 7 d (Table 1). These results indicate that UCP2 expression in human liver ceases soon after birth, similarly as in rodents. The correlation between the number of hematopoietic cells and the level of UCP2 transcript strongly indicated that nonparenchymal cells represented the major site of UCP2 expression in the fetal and neonatal liver. Immunohistochemical analysis and *in situ* hybridization documented UCP2 expression in monocyte/macrophage cells, similarly as found in mice and rats (11). Macrophages in human liver showed a much higher UCP2 content compared with the other cell types, but the anti-UCP1 serum also reacted significantly with some other myeloid cells. Recent studies indicated that UCP2 might be induced in hepatocytes in adult rodents under various pathologic situations (see "Introduction"). However, no UCP-positive hepatocytes were observed in any infant (or fetus) included in our experimental cohort, in spite of sepsis and other pathologies (Table 1). Also important, the levels of UCP2 transcript in the liver correlated best with the length of postnatal life (and the activity of hepatic hematopoiesis) and not with the clinical diagnosis or the pathologic anatomy characteristics. Therefore, UCP2 seems to be absent in hepatocytes of human fetuses and critically ill neonates.

The liver appeared to be the major site of UCP2 expression in the fetus and the newborn, both in rodents (11) and in humans (this study) and Kupffer cells, representing (33) the largest population of the body's tissue macrophages (80%–90%), are the main source of UCP2 in the liver. The expression of UCP2 in these cells must be very high, because Kupffer cells represent less than 1% of nonparenchymal cells in the liver following the 8th week of gestation (8). Whether the postnatal decline of UCP2 expression only reflects the decrease in the number of hematopoietic cells expressing UCP2 or the differential expression in cells before and after birth is not clear (11). This question is related to the putative function of UCP2.

In nonparenchymal (presumably in Kupffer) cells, but not in hepatocytes isolated from rat liver, UCP2 was shown to decrease mitochondrial ROS production (21). A special subpopulation (34) of macrophages supports erythropoiesis in fetal liver (34), and their concomitant activation may increase ROS production (2). UCP2 may be induced in Kupffer cells activated during erythropoiesis and participate in the self-defense of these cells against oxidative stress. It has also been shown that tumor necrosis factor α could increase ROS production and induce expression of UCP2 in regenerating rat liver. However, the cellular distribution of UCP2 in these experiments has not been assessed (35). Some studies indicate transcriptional control of UCP2 expression by PPAR γ in adipocytes (36–38). PPAR γ , the target for thiazolidinediones used as insulin sensitizers in diabetic patients (39), also promotes monocyte/macrophage differentiation (40) and inhibits macro-

phage activation (41). Whether PPAR γ stimulates transcription of UCP2 gene in monocytes/macrophages remains to be elucidated. Our previous studies documented phagocytotic activity of preadipocytes and UCP2 expression in these cells (42). It was shown very recently that mice with genetically disrupted UCP2 gene were resistant against infection by *Toxoplasma gondii* and their macrophages showed higher functional activities, compared with control animals (44). Moreover, high UCP2 content was demonstrated in spleen using reliable antibodies (44, 45). Therefore, it is apparent that UCP2 may have a role common to cells engaged in phagocytosis and immunity.

In summary, the present results indicate the rapid postnatal decline of hematopoiesis in human liver, associated with the diminution of mitochondrial UCP2 expression. They document a specific expression of UCP2 in myeloid cells, particularly the Kupffer cells, and suggest that UCP2 is absent from hepatocytes of human neonates.

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