High expression of uncoupling protein 2 in foetal liver

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Abstract To assess the putative role of mitochondrial uncoupling protein 2 (UCP2) during perinatal development, its expression was analysed in mice and rats. Expression was detected in a large range of foetal tissues. A unique developmental pattern of UCP2 expression was found in liver, where the level of UCP2 mRNA was about 30-fold higher in foetuses than in adults (mice data), and started to decline immediately after birth. Neither UCP1 nor UCP3 mRNA was expressed in foetal liver. As in adult liver, immunohistochemical analysis suggested exclusive localisation of UCP2 in the monocyte/macrophage cells. Our results indicate a role of UCP2 in haematopoietic system development.

Key words: Fetus; Mitochondrion; Macrophage; Uncoupling protein; Liver; Rodent

1. Introduction

The efficiency of energy conversion in mammalian mitochondria may be controlled by at least three distinct uncoupling proteins (UCPs) belonging to the gene family of mitochondrial ion transporters [1]. UCP1, the first UCP to be identified, is exclusively located in brown adipocytes [2,3]. It increases proton conductivity of the inner mitochondrial membrane, and enables regulatory heat production at the expense of ATP synthesis [4]. UCP2 and UCP3, which have been described only recently [5–9], also collapse the mitochondrial membrane potential, suggesting that all UCPs function in a similar manner. UCP2 is expressed in many human and rodent [5–9] tissues, with the highest levels observed in white adipose tissue, spleen and thymus. In the latter two organs, UCP2 expression probably reflects mainly the contribution by tissue macrophages, as has been documented in liver of adult rats (where only non-parenchymal, mainly Kupffer cells, express UCP2; hence UCP2 expression in liver is very low [10]). UCP3 is preferentially expressed in skeletal muscle and brown fat [6,8].

All three UCPs are likely to be involved in the control of total energy balance. However, their contribution may differ depending on the type of diet [5], environmental temperature [11], differential expression of UCPs in various tissues (see above), and also complex hormonal control of their expression [2,3,9,12,13]. Thus, both UCP1 and UCP3 are likely to be primarily the mediators of adaptive, hormonally induced thermogenesis [8,9,11,14]. UCP2 might be involved in diet-induced thermogenesis [5] and also in the control of reactive oxygen species production [15].

In order to better assess the physiological roles of UCPs, their expression during perinatal development should also be characterised. Analysis of UCP1 in various animal species [16–19] clearly indicated a link to perinatal recruitment of brown fat thermogenesis. We now report that induction of UCP2 gene expression in various tissues can be detected prior to the appearance of UCP1 in brown fat. Expression of UCP2 was much stronger in foetal liver than after birth, and, as in adults, it was restricted to developing macrophages. Our data suggest that UCP2 may be important for differentiation and function of the cells of the haematopoietic system.

2. Materials and methods

2.1. Animals and tissues

Experiments were performed on the C57BL/6J mice and Wistar rats. The animals were kept in a controlled environment (20°C; 12 h light/dark cycle) with free access to water and standard chow diet. Tissues and organs (liver, brain, lung, kidney, heart, interscapular brown fat, colon, placenta, hind leg and spleen) were dissected from foetuses obtained by Caesarean section between 10 and 19 days of gestation (d 10–d 19), or from pups which remained with their mothers for 1 h–21 d after spontaneous delivery. Animals were killed by cervical dislocation under diethylether anaesthesia.

2.2. RNA analysis

Total RNA was isolated [20] from tissue samples stored in liquid nitrogen, and gene expression was analysed by Northern blots [21], using the full-length cDNA probes for the mouse UCP1 [22], the mouse UCP2 and the rat UCP3 genes (see Acknowledgements) and the 682-bp *Eco*RI fragment derived from the human liver subunit IV of mitochondrial cytochrome oxidase (COX IV) gene (ATCC, Rockville, MD, USA). Radioactivity was evaluated by PhosphorImager SF (Molecular Dynamics). The blots were rehybridised with the 1400-bp *Sal*I-*Mlu*I restriction fragment of the ribosomal 18S cDNA, and the signal was used to correct for small intersample variations.

2.3. Immunohistochemistry

Mouse liver samples were fixed (3 h) in 4% paraformaldehyde-PBS, dehydrated and paraffin embedded. Sections (5 mm) were incubated for 1 h at room temperature in medium containing first antibodies, diluted in Dako antibody diluent to reduce background. The batch of sheep anti-rat UCP1 IgG (1:400 dilution in single labelling, 1:200 in double labelling) used to detect UCP antigens was described previously [10]. Second antibodies coupled to alkaline phosphatase (goat anti-sheep, 1:100 dilution, Sigma Chemical Co.) were visualised using BCIP/NBT (Dako). Endogenous alkaline phosphatase activity was inhibited by levamisole (Dako) and endogenous peroxidase activities by hydrogen peroxide blocking reagent (Dako). With the double labelling method, UCP2 was revealed using peroxidase-conjugated antibodies (goat anti-sheep, 1:100, Sigma Chemical Co.) with AEC substrate chromogen (Dako). Antiserum against F4/80 was used as a marker for the monocyte/macrophage lineage [23] (Biosource Interna-

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Abbreviations: UCP, uncoupling protein; COX IV, subunit IV of mitochondrial cytochrome oxidase; d X, X days of gestation

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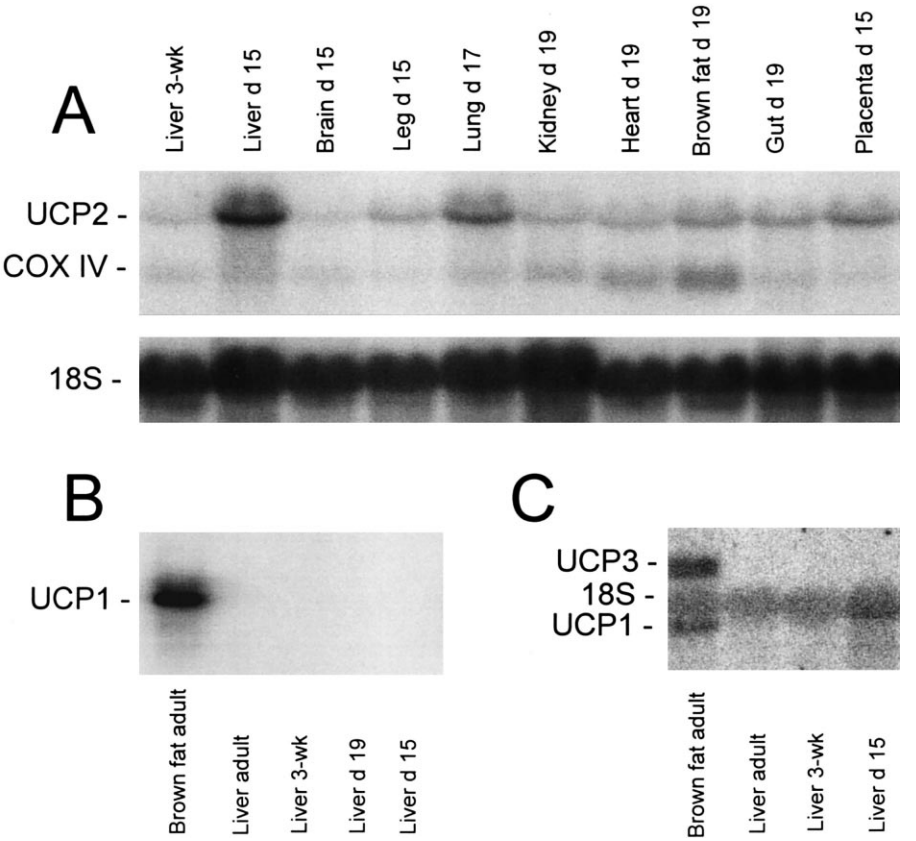

Fig. 1. Northern blot analysis of gene expression in mouse (20-μg sample of total RNA was loaded in each lane). A: After hybridisation with a mixture of cDNA probes for mouse UCP2 (1.7-kbp transcript), and human COX IV (0.9-kbp transcript), the blot was reprobed for 18S ribosomal RNA (18S). All the samples were isolated from foetal tissues at indicated days of gestation, except for the sample isolated from liver of a 3-week-old pup. B: Hybridisation of mouse UCP1 cDNA (1.4-kbp transcript) with RNA samples from brown fat of adult mouse, and liver during development (adult mice, 3-week-old pup, and fetuses at d 15–d 19). C: Rat UCP3 cDNA probe detected UCP3 (2.5-kbp) and UCP1 (1.4-kbp) gene transcripts in the samples as in B (the 18S ribosomal RNA probe was also used).

tional, 1:40) with rabbit anti-rat IgG (1:100) coupled to alkaline phosphatase used as second antibodies. Slides were counterstained with nuclear red or methyl green (single or double labelling, respectively). Control experiments were performed using purified IgG and yielded no staining.

2.4. Statistical analysis

Statistical analysis was performed using Student's *t*-test. All tests were judged to be significant at $P < 0.05$ (see legend to Fig. 3).

3. Results

3.1. The UCP2 gene is highly expressed in foetal liver

In order to characterise the tissue-specific pattern of UCP2 expression in mouse foetuses, Northern blot analysis was performed with total RNA isolated from different tissues dissected at the indicated age (Fig. 1). Foetal expression (d 15–d 19) of the UCP2 gene was found in all tissues analysed (Fig. 1A). Surprisingly, a much stronger signal was detected in foetal liver compared to the livers of 3-week-old (Fig. 1A) or adult mice [5,8]. Also in murine lung, the levels of UCP2 gene transcript were relatively high, but they did not change during development (not shown). In contrast to UCP2, expression of the UCP1 and UCP3 genes could not be detected in liver, either pre- or postnatally, while both transcripts were present in brown fat (Fig. 1B,C). The UCP3 probe also hybridised with UCP1 transcript in brown fat (Fig. 1C), and possibly with the UCP2 gene transcript in foetal liver (Fig. 1C; the faint band between 18S and UCP1 transcripts; see also [8]). As a marker of mitochondrial oxidative capacity in different tissues, expression of the gene for nuclearly encoded COX IV was also characterised. Expression of the COX IV gene did not correlate with that of UCP2, the former being highest in brown fat relative to other tissues (Fig. 1A).

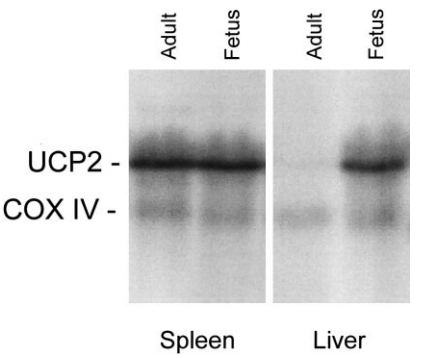

Fig. 2. Northern blot analysis of gene expression in rat. Total RNA was isolated from spleen and liver of foetal (d 17) and adult rats. Transcripts for UCP2 and COX IV were detected as in Fig. 1A. Two parts of the same blot are shown. This figure is representative of several independent experiments.

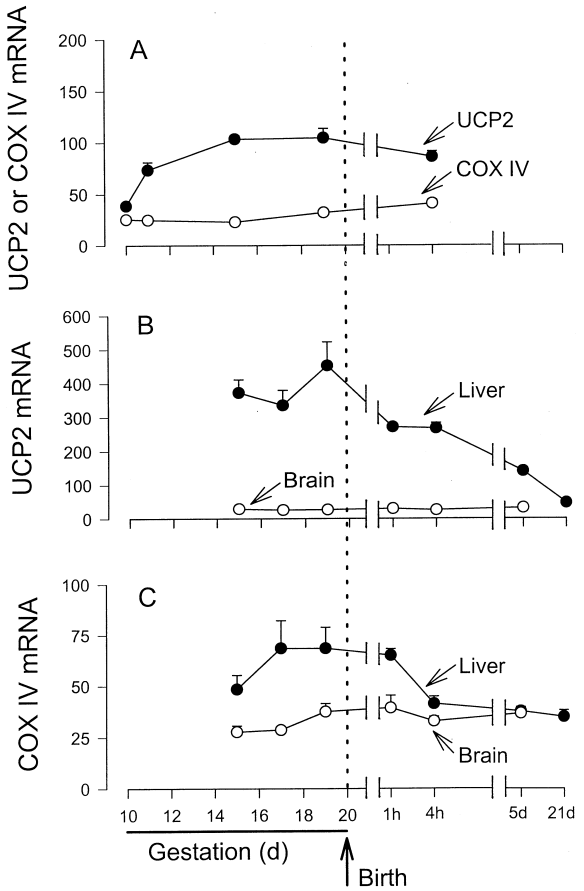

Fig. 3. Expression of UCP2 and COX IV mRNA during mouse perinatal development. A: Expression of UCP2 (●) and COX IV (○) in whole foetus or newborn body homogenate. B: Expression of UCP2 gene in liver (●) and brain (○). C: Expression of COX IV gene in liver (●) and brain (○). Samples from several foetuses or newborns ($n = 4$–5) were subjected to Northern blot analysis (see Fig. 1). Transcripts for UCP2 and COX IV (detected as in Fig. 1A) were expressed relative to that of 18S ribosomal RNA (see Section 2). Data are means ± S.E. All the differences in UCP2 mRNA levels between homogenate and liver, and homogenate and brain, respectively, were significant. Differences in COX IV mRNA levels between homogenate and liver (d 15–d 19 of gestation), and brain and liver (d 15 of gestation–1 h after birth) were also significant.

The levels of UCP2 transcripts in foetal rat livers (Fig. 2) were similar to those in spleen, the organ showing the strongest expression in adult mice and humans [8,9]. As in mice, the abundance of UCP2 transcripts in rat liver decreased considerably between the foetus and adult (see also [6,9,10] and [5,7–9] for expression in adult rats and humans, respectively). However, in contrast to liver, no differences could be observed in UCP2 expression in foetal and adult rat spleens (Fig. 2). The results indicate a specific developmental pattern of UCP2 expression in liver.

3.2. *Quantification of UCP2 and COX IV genes expression during development*

To characterise the changes in UCP2 and COX IV gene expression during perinatal development, samples of whole body homogenates (Fig. 3A), liver (Fig. 3B,C) and brain (Fig. 3B,C) were analysed starting from d 10 (whole body homogenate, up to 4 h after birth) or d 15 of gestation (liver and brain, until 3 weeks or 5 days after birth, respectively). In whole foetus homogenates, expression of both genes could be detected at the earliest gestational period studied (d 10). However, while approximately a threefold stimulation of UCP2 gene expression was observed between d 10 and d 15 of gestation (with no further changes following d 15 until birth), expression of the COX IV gene remained relatively stable during the entire period.

In the liver at d 15, UCP2 gene expression was about fourfold higher (Fig. 3B) than that measured in the whole body homogenate (Fig. 3A), and it remained relatively stable prenatally. Regarding its relatively large size, it is apparent that the liver is the major site of foetal UCP2 gene expression. UCP2 expression started to decline 1 h after birth, reaching the lowest levels in the 3-week-old pups (the levels about one order of magnitude lower compared to foetal levels, but still about threefold higher compared with adult mice; not shown). A postnatal decline in COX IV gene expression was also observed (Fig. 3C). However, this decline was relatively low (approximately twofold), and occurred faster (within the first postnatal hours) compared with that of UCP2. It is apparent that the changes of UCP2 expression and that of mitochondrial oxidative capacity (COX IV gene expression) occur independently during the perinatal development.

UCP2 gene expression in brain (Fig. 3B) was significantly lower than in whole body homogenate (Fig. 3A) and liver (Fig. 3B); and it remained stable during development. Also COX IV gene expression in the brain remained relatively constant (Fig. 3C).

3.3. *Immunohistochemical analysis*

As before [10], in the absence of either UCP1 or UCP3 transcripts (Fig. 1), the cell type expressing UCP2 in foetal liver could be identified using the anti-UCP1 antibodies which presumably cross-reacted with UCP2 [10]. Immunohistochemistry experiments performed on foetal liver (d 16) revealed highly positive UCP2 cells in non-parenchymal cells but not in hepatocytes (Fig. 4, part 1a, 1b). Most of the labelled cells had a sinusoid localisation. These cells were also labelled by F4/80 antibodies (Fig. 4, part 1bc). Taken together, these data suggest that the UCP immunoreactive cells belonged to the monocyte/macrophage lineage [23]. However, it was noteworthy that not all the F4/80-positive cells were labelled by anti-UCP1 antibodies. At 3 d after birth, UCP2-positive cells were not intensely stained and poorly contrasted with the pink counterstain (Fig. 4, part 2a). Closer examination (Fig. 4, part 2b) showed that the cells were less stained than in foetuses (Fig. 4, part 1b).

4. Discussion

The principal finding of this report is the strong expression of UCP2 gene in foetal liver, and its specific developmental pattern in this organ. Similarly to adults [10], cells expressing UCP2 in foetal liver belong mostly to the monocyte/macrophage lineage.

The liver is an organ where mitochondrial biogenesis and function change abruptly at parturition, resulting in a switch from foetal glycolysis to postnatal oxidative phosphorylation (for references see [24,25]). An alteration in mitochondrial function could result from changes in proton conductivity of the inner mitochondrial membrane [25]. Therefore, the developmental pattern of UCP2 expression in liver (and the ab-

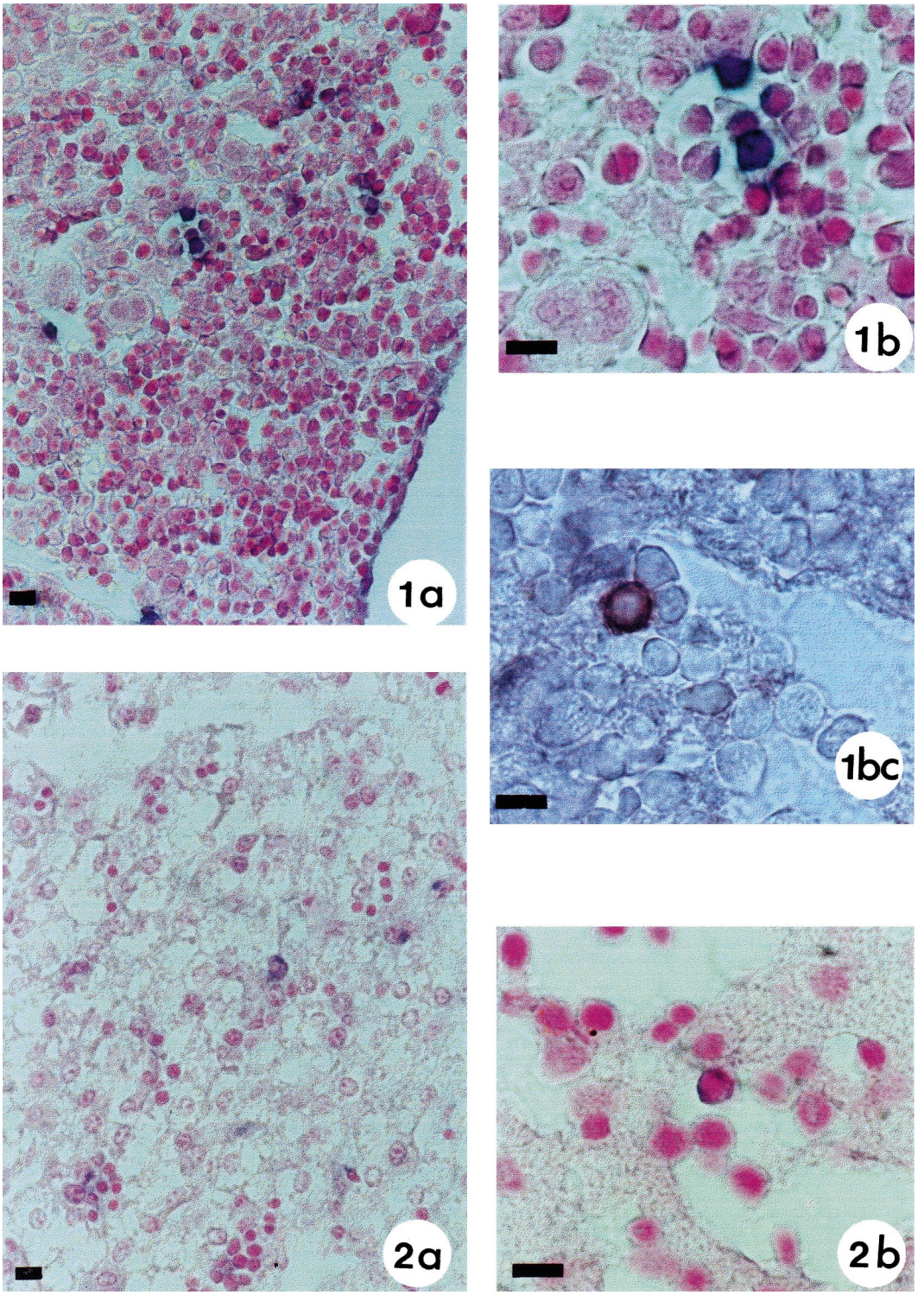

Fig. 4. Immunohistochemical detection of UCP antigen in slices of mouse foetal (d 16) liver (1a, 1b, 1bc) and 3-day-old mouse liver (2a, 2b). Immunostaining was performed using sheep anti-rat UCP1 IgG (1:400 and 1:200 dilution for parts 1 and 2 respectively). 1a, 1b, 2a, 2b: Detection of UCP-positive cells by single labelling. Dark blue precipitates indicate BCIP/NBT deposit following alkaline phosphatase reaction. Counterstain is nuclear red. 1bc: Double localisation of UCP2 and F4/80 in mouse foetal liver (d 16). Red cytoplasmic precipitates indicate UCP2 localisation by AEC deposit following immunoperoxidase reaction. Dark blue peripheral precipitates indicate in this experiment the presence of the membraneous F4/80 antigen by BCIP/NBT deposit following alkaline phosphatase reaction. Counterstain is methyl green. a: magnification $\times 320$, bar = 10 μm; b: magnification $\times 800$, bar = 10 μm.

sence of either UCP1 or UCP3; see Fig. 1) suggested that UCP2 could be involved. However, UCP2 was not expressed in hepatocytes, but only in cells contributing very little (see Fig. 4) to the hepatic cell pool (i.e. F4/80-positive cells). Therefore, it is unlikely that UCP2 is the key element controlling the changes of mitochondrial energetics in liver during perinatal development. Thus, the mechanism by which mitochondrial energetics is regulated in liver during perinatal development will require further clarification.

The physiological role of UCP2 is not known yet with certainty. Our results clearly demonstrate that UCP2 is expressed in various tissues of the foetus and that this expression occurs much earlier than that of UCP1 in brown fat (where UCP1 is the marker of the thermogenic function, and appears around d 19 in mice and rats; see [16]). Early recruitment of the UCP2 gene suggests that the main role of UCP2, at least during intrauterine development, is not related to thermogenesis (see also [26]). The differential expression of UCP2 during development in tissues rich in macrophages is especially striking: only in liver does strong expression occur in the foetus and declines postnatally, while in spleen and lung no changes could be detected during the perinatal period. This difference may be related to the role of foetal liver in haematopoiesis, and migration of this activity to the bone marrow just before birth (for references see [27,28]). It is inferred that UCP2 might have some role in differentiation and/or function of the cells of the myelopoietic lineage.

The control of UCP2 expression during liver development is likely to be quite complex. It might involve hormones which sharply change their circulating and tissue levels around birth, such as thyroid hormones [29,30] or insulin [31], or leptin [32,33] which is released both from adipocytes and from placenta [34]. If thyroid hormones [9] and insulin [35] have no effect on UCP2 mRNA levels, leptin induces UCP2 gene expression in lipid-containing cells [13]. The effect of this hormone on early differentiation of the cells of the myelopoietic lineage in foetal liver has also been demonstrated [28]. It is probably mediated by the receptor expressed in the haematopoietic cells [28,36,37]. Thus leptin may also represent a key hormone controlling expression of UCP2 in developing macrophages.

In summary, our results indicate that developing monocyte/macrophage cells in foetal liver represent the major site of UCP2 gene expression in the foetus. They suggest a role of UCP2 in differentiation of the myelopoietic lineage and thus in immune system development.

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