Phagocytosis Exhibited by Neonatal Hepatocytes at the end of Murine Liver Hematopoiesis - An Ultrastructural Study

Ichiro KUMANO, Yuji SONODA and Kazunobu SASAKI

Department of Anatomy, Kawasaki Medical School
577 Matsushima, Kurashiki, Okayama, 701-0192 Japan

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ABSTRACT. To clarify hepatocyte phagocytosis of hematopoietic cells and their disposal processes in livers at the terminal point of liver hematopoiesis, neonatal mouse livers were examined by transmission electron microscopy. From two to ten days after birth, hepatocytes containing large inclusions appeared, and the inclusions were secondary phagolysosomes derived from hematopoietic cell elements. The heterophagosomes contained erythroblasts, nuclei expelled from erythroblasts, neutrophils and eosinophils, and, neutrophils, in particular, are the most frequently engulfed. These hematopoietic cells are degradated in the phagosomes. In the early stage, they are characterized by nuclear chromatin condensation, and then collapse of nuclei and cytoplasmic structures was followed. In the final stage of digestion, the phagosomes included residual bodies, such as dense bodies and myelin bodies. In the early postnatal period, in addition to sinusoidal macrophages, hepatocytes played a temporary role in removal of blood cell elements within liver lobules, and residual bodies appeared to be excreted into bile canaliculi.

Key words ① **phagocytosis** ② **neonatal hepatocyte** ③ **liver hematopoiesis** ④ **mouse**

Fetal liver is recognized as a major site of intraembryonic hematopoiesis in mice. The liver hematopoiesis declined from late gestational stages[1),2)], then blood cell production shifted to bone marrow after birth[3)]. In murine livers, numerous neutrophils underwent apoptosis during late gestation, and scavenger macrophages in sinusoidal and interlobular connective tissue played a main role for removal of those dying cells[4)]. In neonatal mouse livers, hepatocytes as well as macrophages often contained large heterophagosomes. Not only rat hepatocytes *in vivo* but mouse cultured hepatocytes were reported to engulf apoptotic cells due to intoxication with lead nitrate[5)]. Furthermore, in case of melioidosis, mouse hepatocytes are capable of killing pathogenic bacteria, the saprophytic gram-negative rod *Burkholderia pseudomallei*, by phagocytosis[6)]. In pathological conditions, therefore, hepatocytes are known to display obvious phagocytotic activity. However little information is available about the phagocytotic activity during physiological involution of fetal hematopoiesis. The purpose of this study was to morphologically clarify hepatocyte phagocytosis of hematopoietic cells and their disposal processes in livers during early postnatal period.

熊野一郎，園田祐治，佐々木和信
e-mail:kumano@med.kawasaki-m.ac.jp

MATERIALS AND METHODS

Thirty neonatal ICR mice (CLEA Japan Inc., Tokyo) at 2, 4, 8, 9 and 10 days of age were used in this study. The mice were killed either by decapitation or deeply anesthesia with chloroform, and livers were rapidly dissected out under the stereomicroscope. The livers were cut into small blocks, 1 mm^3, and immediately immersed in 4% paraformaldehyde with 5% glutaraldehyde in 0.1M cacodylate buffer (Karnovsky's fluid) for 3h at 4 ℃. After fixation, the tissue blocks were postfixed in 2% osmium tetroxide for 2h at 4 ℃. Then they were dehydrated in graded ethanols and embedded in Epon 812. Serial semithin sections, 1μm thick, were cut with a diamond knife and stained with 1% toluidine blue. Under light microscopy, semithin sections which contained hepatocytes with phagocytotic inclusions were selected for re-embedding. From re-embedded semithin sections, ultrathin sections were cut with a Leica Ultracut S ultra microtome (Leica Microsystems AG, Wetzlar, Germany), then they were mounted on formvar coated one-pore copper meshes. Ultrathin sections were conventionally stained with uranyl acetate and lead citrate before observations in a JEM-2000 EXII electron microscope (JEOL Ltd., Tokyo, Japan) operating at 80 kV.

This study was approved by the Animal Research Committee of Kawasaki Medical School (No. 04-083) and conducted according to the "Guide for the care and use of laboratory animals" of Kawasaki Medical School.

RESULTS

Phagocytosis of hematopoietic cells by hepatocytes

In early neonates from two to four days after birth, liver parenchyma assumed the architecture of hepatic cell cords, in which cuboidal hepatocytes were associated side by side, making cell sheets. Perisinusoidal spaces between hepatic cords and sinusoidal lining cells were expanded, and there macrophages and hematopoietic cells could be recognized. The hematopoietic cells consisted of erythroblasts, granulocytes and megakaryocytes, and, although hematopoietic cell number was dramatically decreased after birth, a few hematopoietic cells could still be recognized in perisinusoidal spaces in liver parenchyma until 10 days after birth. In neonatal livers, until 10 days of age, cells holding phagocytotic inclusions from hematopoietic cell elements were mainly recognized as macrophages, but, in addition to the professional scavenger macrophages, there existed a few hepatocytes containing a variety of large heterogenous inclusions in their cytoplasm (Fig. 1). On two to ten days after birth, in particular, the number of hepatocytes containing phagosomes appeared to be increased. Their ultrastructures revealed that most of the inclusions were derived from either erythroblasts (Fig. 1a) or nuclei expelled from erythroblasts, but, neutrophils, eosinophils, lymphocytes and also cell fragments of macrophages were phagocytosed. Granulocytes, in particular, were the most frequently engulfed by hepatocytes (Fig. 1b-e).

Degradation of granulocytes and formation of residual bodies in hepatocytes

The majority of the phagocytosed leukocytes by hepatocytes were neutrophils, and they showed various degradation stages in the phagosomes (Fig. 1b). At the early stage of degradation, they were characterized by chromatin condensation at the nuclear periphery and then collapse of the cytoplasmic structures progressed

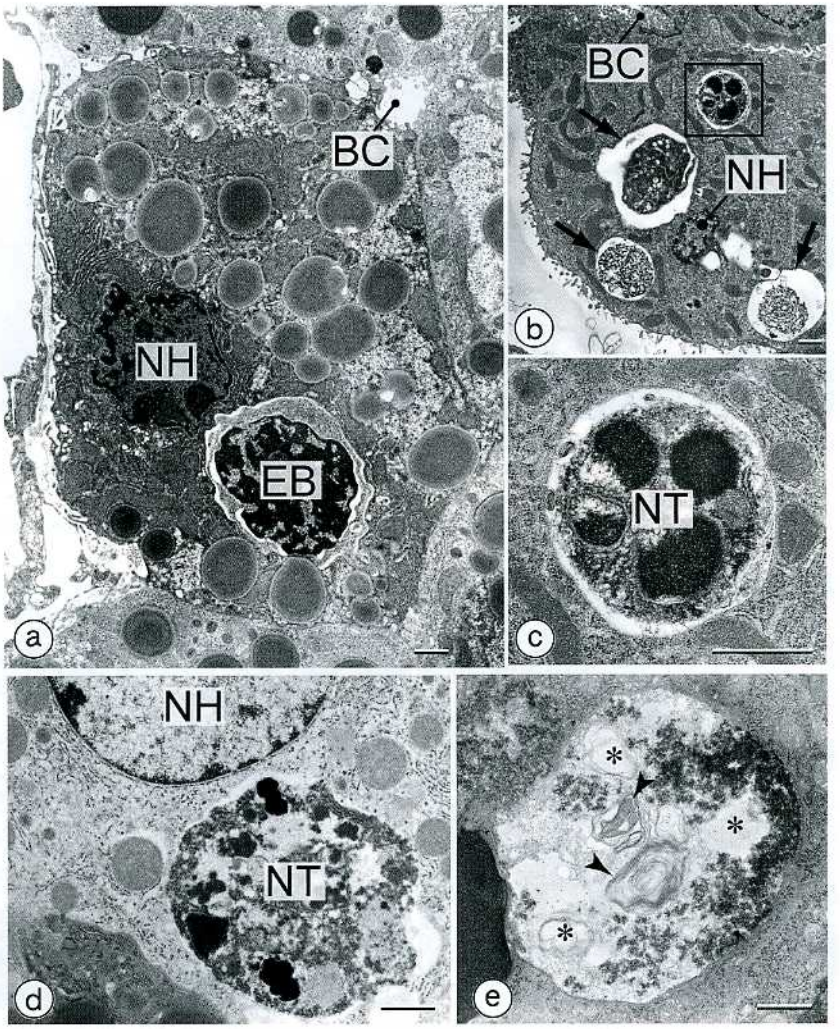

Fig. 1. Neonatal hepatocytes containing phagocytotic inclusions from hematopoietic cell elements. Scale bar = 1μm.
a) A hepatocyte showing erythrophagocytosis. Two days of age. BC = bile canalicus, EB = erythroblast, NH = nucleus of hepatocyte.
b) A hepatocyte containing numerous phagosomes. The phagosomes contain various degradation stages of neutrophils. Three days of age. BC = bile canalicus, NH = nucleus of hepatocyte.
c) A high-power micrograph of the framed area in b. The segmented nuclei of the neutrophil show typical apoptotic chromatin condensation.
d) Advanced degradation stage of neutrophils. In the phagosome, collapse of cytoplasmic structures progress. 10 days of age. NT = neutrophil.
e) Secondary lysosomes containing clear vacuoles (*) and small scrolls (arrowheads). Most of cell structures are broken down. 10 days of age.

(Fig. 1c, d). The nuclear configuration could be identified as a small and electron-dense remnant, and the cytoplasm become condensed. The condensation was frequently accompanied by the appearance of clear vacuoles, and most of cell organelles were broken down (Fig. 1e). At the periphery of such large phagosomes, a few small round scrolls of parallel dense lines, 0.1-0.2μm in diameter, derived from nuclear envelopes, could be observed (Fig. 2a). Clear phagosomes in the advanced stages of the digestion contained a

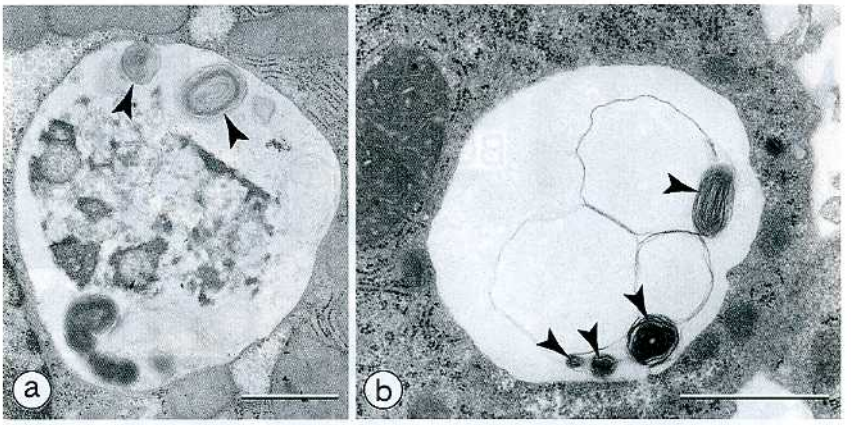

Fig. 2. Large clear phagosomes in neonatal hepatocytes. Scale bar = 1μm.
a) A clear phagosome. There remain small round scrolls of parallel dense lines (arrowheads). Three days of age.
b) Final stage of degradation of a clear phagosome. Round myelin figures (arrowheads) are recognized. Nine days of age.

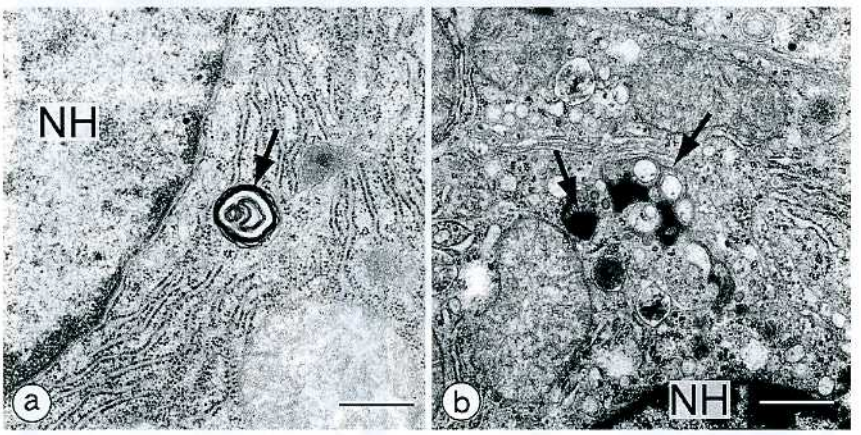

Fig. 3. Residual bodies in neonatal hepatocytes. Eight days of age. Scale bar = 0.5μm. NH = nucleus of hepatocyte.
a) A round myelin body (arrow) locating near the nucleus of hepatocyte.
b) Several dense bodies (arrows) in the cytoplasm of hepatocyte.

few myelin figures and clear vacuoles, and then myelin figures became much more electron-dense (Fig. 2b). Several dense myelin bodies could be observed in some of hepatocytes as residual bodies (Fig. 3a, b). In the neonatal livers, a few expanded canaliculi could be recognized, and these expanded canaliculi often contained not only myelin bodies, 0.3 to 1.5μm in diameter (Fig. 4a, b), but also dense bodies, approximately 1μm (Fig. 4c).

DISCUSSION

At the involution stage of the hematopoiesis, as is known, remaining neutrophils underwent apoptosis, to be phagocytosed and removed by macrophages[4]. In general, cells undergoing apoptosis were removed by professional scavengers, such as macrophages[7], and mouse fetal livers contained two functionally different

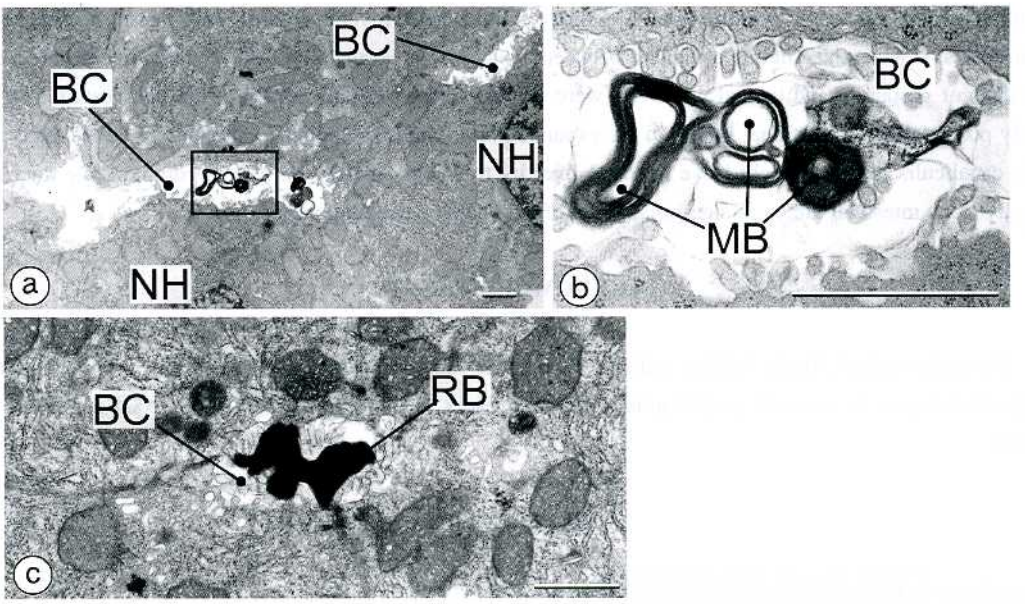

Fig. 4. Residual bodies in the lumen of the bile canalicus of neonatal livers. Eight days of age. BC = bile canalicus, NH = nucleus of hepatocyte. Scale bar = 1μm.
a) Cell residuals, mainly myelin figures, in a bile canalicus.
b) A high-power micrograph of the framed area in a. Myelin bodies (MB) could be recognized in a expanded bile canalicus.
c) A dense residual body (RB) in a bile canalicus.

macrophage subpopulations, namely sinusoidal macrophages as professional scavengers and hematopoietic macrophages as central cells of the erythroblastic island[8]. At the beginning of liver hematopoiesis, scavenger macrophages in liver sinusoids actively phagocytosed circulating primitive erythroblasts[9),10]. Then the macrophages moved into the primitive hepatic cell cords and located at the center of hematopoietic cells as central macrophages of erythroblastic islands[8]. The hematopoietic macrophages were characterized by engulfing nuclei expelled from erythroblasts, and, shortly after birth, they decreased in number and blood cell production in liver, then, ceased completely[2]. As mentioned earlier, the appearance of heterophagosomes derived from hematopoietic elements is typical of neonatal hepatocytes, and, macrophages in subcapsular connective tissue of neonatal livers were characterized by phagocytosing dying neutrophils[4]. Therefore, in the neonatal period, both macrophages and hepatocytes are involved in elimination of dying blood cells in different hepatic areas; macrophages mainly in connective tissues of subcapsular area and hepatocytes within liver lobules. At the terminal point of liver hematopoiesis, hepatocytes could be considered physiologically to play a temporary role in removal of blood cell elements of fetal hematopoiesis from neonatal liver.

Phagocytosis and ingestion of hematopoietic cell elements resulted in formation of residual bodies in hepatocytes. Neonatal hepatocytes contained various kinds of phagolysosomes, and residual bodies, i.e., dense bodies and myelin figures, were finally formed. Most of them could be regarded as degradation products from dying neutrophils and nuclei expelled from erythroblasts. At the beginning of splenic hematopoiesis in mouse, macrophages also engulfed and digested the various hematopoietic elements in the red pulp, and, in case of splenic macrophages, most of myelin figures were observed in phagosomes derived

from expelled nuclei of erythroblasts[11]. Interestingly, both myelin figures and dense bodies could sometimes be recognized in bile canaliculi between hepatocytes. Formation of bile canaliculi was initiated at 14 days of gestation, and typical microvilli were already formed in their lumen at the late gestation[12]. In early postnatal period, residual bodies in the expanded bile canaliculi could be estimated to be excreted into bile canaliculi, and they might leave neonatal liver by traveling through the bile in common bile duct and finally reach intestinal lumen as a terminal waste of fetal hepatic hematopoiesis.

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