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Chapter XIII

The Functional Diversity of Kupffer Cells

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Abstract

Kupffer cells are resident macrophages in the adult liver, located in the lumen of hepatic sinusoids. They play an important role in homeostasis and the immune defense system. Kupffer cells engulf and breakdown a variety of substances, including erythrocytes, dead cells, microorganisms, endotoxins, immune complexes, and chemicals. Kupffer cells also generate various products, including cytokines, prostanooids, nitric oxide, and reactive oxygen intermediates, these factors regulate the phenotypes of other immune cells, such as natural killer T cells. We previously demonstrated the rapid accumulation of circulating platelets in the liver after lipopolysaccharide injection in mice. After the direct contact of platelets with developing cell processes of Kupffer cells, some platelets migrate into hepatocytes, preventing the development of acute hepatitis in mice. At the fetal stage, Kupffer cells are also involved in erythropoiesis in the liver. Nitrogen-containing bisphosphonates (NBPs) are potent inhibitors of osteoclastic bone resorption. The injection of NBP induces a shift in erythropoiesis from the bone marrow to the liver of splenectomized mice by the depletion of bone marrow resident macrophages. Kupffer cells express several adhesion molecules to support erythropoiesis in such an emergency. This evidence demonstrates the functional diversity of Kupffer cells and may help to clarify the physiological and pathological role of Kupffer cells in humans.

Introduction

Macrophages are a heterogenous population of cells by their morphology, function, and metabolism. At least 2 types of macrophages are distributed in the body, namely resident and exudative macrophages (Daems and Brederoo 1972). Whereas exudative macrophages migrate into the inflammation loci in response to several chemokines secreted in these loci, resident macrophages are ubiquitously distributed throughout the body at a normal steady condition (Daems and Brederoo 1973; Daems et al. 1976; Soranzo et al. 1978; Daems et al. 1979; Daems et al. 1980). Based on the concept of the mononuclear phagocyte system (van Furth et al. 1972), both types of macrophages are derived from blood monocytes that originate in the bone marrow. It has been generally accepted that macrophages are relatively short-lived cells that do not exhibit proliferation activity (van Furth 1975, 1980, 1989, 1992). However, several recent studies have demonstrated the *in situ* proliferation of resident macrophages in various tissues in response to several cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) secreted in the proliferating microenvironment (Naito et al. 1996; Sawa et al. 2003; Douglass et al. 2008).

Kupffer cells are resident macrophages found in the liver (Figure 1). They are located in hepatic sinusoids and play a fundamental role in liver homeostasis and disease protection and progression via the synthesis of various bioactive substances such as eicosanoids, free radicals, cytokines, chemokines, and lysosomal enzymes (Weisse et al. 1996; Decker 1990). Kupffer cells actively endocytose particles, cells, and toxic and infective substances from the bloodstream, some of which are of intestinal origin.

The functional and morphological heterogeneity of Kupffer cells has been ascribed to their location.

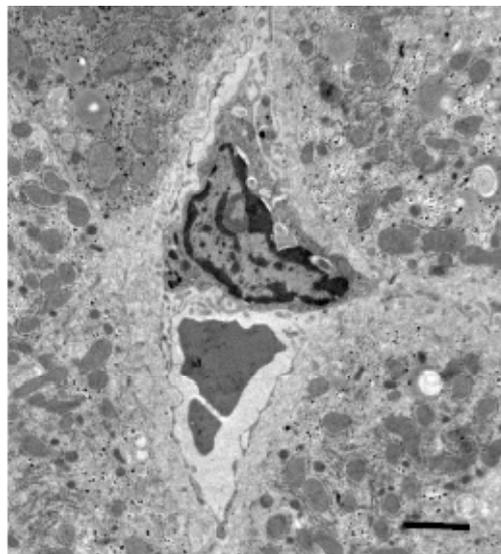


Figure 1. An electron micrograph of the Kupffer cell in the normal condition. Bar 5 μ m.

Kupffer cells located in the periportal zone are larger, contain more lysosomes and show more active endocytosis than cells located in the centrilobular zone, which are smaller and show more active cytokine production and cytotoxicity (Rømer et al. 1993; Laskin. 1996; Laskin et al. 2001).

In this chapter, we describe the functional heterogeneity of Kupffer cells during the 2 phases of acute inflammation in mice and discuss the role of Kupffer cells in healthy and disease conditions.

Interaction of Kupffer Cells with Circulating Platelets for the Prevention of Hepatitis

Increasing evidence suggests that, in addition to their role in various inflammatory diseases, platelets are important for defending the host against invasion by foreign organisms (Nakamura and Endo 1993; Herd and Page 1994; Mannel and Grau 1997; Weyrich and Zimmerman 2004). Platelets in mice, as well as in humans, contain a large amount of 5-hydroxytryptamine (5HT: histamine) in their granules. A low dose of lipopolysaccharide (LPS) induces hepatic platelet accumulation in mice (Endo 1983, 1984). The accompanying increase in 5HT occurs about 1 h after LPS injection, and reaches a maximum level within 4–5 h, with the elevated level maintained for 24–48 h. In this inducing response, LPS is very potent (about a 2-fold increase in 5HT is induced by as little as 0.1 mg/kg of LPS), with a maximum effect induced by a dose of 10 mg/kg (Endo 1983; Endo and Nakamura 1993). Interleukin-1 (IL-1; both α and β) and tumor necrosis factor α (TNF α) also induce this 5HT response (Endo et al. 1985; Endo 1991).

The Role of Kupffer Cells for the Migration of Platelets to the Liver

A decrease in 5HT concentration and the number of platelets in the blood occurs with the hepatic increase in 5HT. These effects correlate well in terms of both the time-course after injection of LPS and their dependency on the dose of LPS used (Endo and Nakamura 1992). In fact, the increase in 5HT reflects the translocation of circulating platelets to the liver. Ultrastructural studies have revealed some unexpected behavior by such platelets, marked accumulation of platelets was observed in sinusoidal and perisinusoidal spaces between hepatocytes and endothelial cells, i.e., in the Disse spaces (Figure 2a).

Many platelets in these spaces have shown to develop pseudopods. By extending these pseudopods, the platelets migrate into the Disse spaces, resulting in a direct interaction between platelets and hepatocytes (Endo and Nakamura 1992). Platelets in the sinusoidal spaces of LPS-treated mice were found to be mostly surrounded by the well-developed cell processes of Kupffer cells, with one or more sites of cell-to-cell contact between platelets and Kupffer cells (Figure 2b). This interaction has not been observed in the normal liver. Kupffer cells in the liver of LPS-treated mice were found to be Mac-2-positive, indicating that they had been activated (Endo and Nakamura 1992, 1993).

Dichloromethylene bisphosphonate (Cl₂MBP)-liposome is a well-known agent for the complete depletion of Kupffer cells that has been used to examine the functional role of Kupffer cells (Biewenga et al. 1996; Claassen et al. 1990; van Rooijen and Sanders 1996). Indeed, the injection of Cl₂MBP-liposome was found to completely deplete Kupffer cells, with no hepatic platelet accumulation observed in the liver (Nakamura et al. 1998). These results indicate that Kupffer cells may play an important role in platelet behavior.

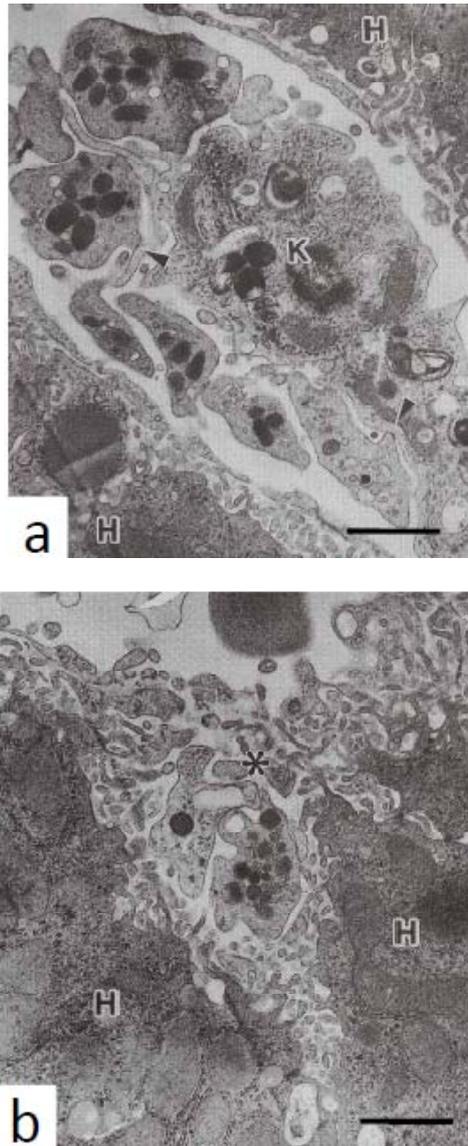


Figure 2. Electron micrographs of hepatic sinusoidal space in mice at 4.5 hr after the intraperitoneal injection of LPS (0.1 mg/kg i.p.). (a) Two platelets in the space of Disse (*) are located in the intercellular space between hepatocytes (H). (b) Kupffer cells (K) extended the well-developed cell processes and surrounded platelets. Arrow heads indicate the direct contact between Kupffer cell and platelets. Bars 2 μm.

Among the various cytokines, only IL-1 and TNF induce marked hepatic accumulation of 5HT (i.e., platelets), such that the ability to induce hepatic accumulation of platelets is probably specific to IL-1 and TNF (Nakamura et al. 1998). Macrophages, including Kupffer cells, are known to produce these cytokines, and LPS has been shown to be a potent in stimulator of macrophage cytokine to production (Dinarello 1989). LPS-induced accumulation of platelets in the liver is mediated by both IL-1 and TNF.

No anti-platelet agents, including heparin, aspirin and other inhibitors of thromboxane A₂ synthetase or cyclooxygenase, have been shown to effectively in prevent the accumulation of platelets in the liver (Nakamura et al. 1998). In addition, WEB2170 (Henriques et al 1990), an antagonist of platelet-activating factor, and N-acetylcysteine (Roederer et al. 1990), an oxidant scavenger, were also found to be ineffective, which indicates that the hepatic accumulation of platelets induced by LPS, IL-1, or TNF and the aggregation of platelets constitute 2 quite different phenomena (Endo and Nakamura 1992, 1993).

Although the primary role of Kupffer cells may be expected to be the production of IL-1 and TNF α , the accumulation of platelets induced by the combined injection of these 2 cytokines was prevented by the depletion of macrophages (Kupffer cells). Therefore, the role of these cells is not solely the production of IL-1 and TNF α . Instead, the cell-to-cell contact observed between Kupffer cells and platelets (Endo and Nakamura 1992) may also be important for inducing the accumulation of platelets.

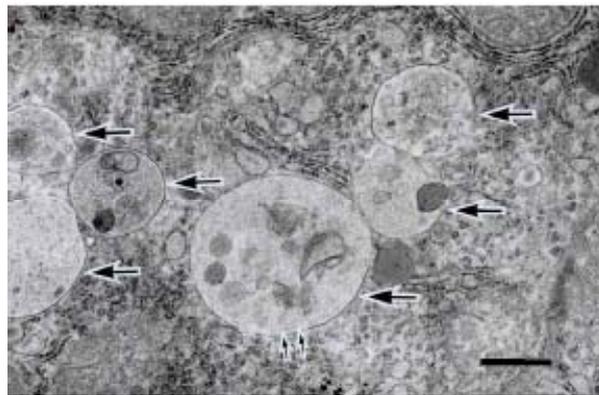


Figure 3. An electron micrograph of platelets inside the cytoplasm of hepatocyte at 4.5 hr after the intraperitoneal injection of LPS (0.1 mg/kg i.p.). Single arrows indicate platelets which most granules are not lost. Small double arrows indicate indicate microtubules beneath the cell membrane of platelets. Bar 1 μ m.

Although it is possible that the cell-to-cell contact is a step that occurs before phagocytosis by Kupffer cells, many platelets are surrounded by Kupffer cells, and they are all intact. No active phagocytosis of platelets by Kupffer cells is detected. It is just conceivable that the degradation of platelets in Kupffer cells is so rapid that it cannot be easily observed. However, this possibility seems unlikely, because the LPS-induced hepatic increase in 5HT would be transient, or at least not last for a period as long as several hours or more (Endo 1984; Endo and Nakamura 1992).

Many platelets migrate into the Disse spaces during inflammation; in fact, 10–20% of platelets have been shown to accumulate in the liver at 4.5 h after LPS injection (0.1 mg/kg).

Furthermore, several platelets are located in the cytoplasm of hepatocytes of mice given LPS (Figure 3). The volume ratio of platelets to hepatocytes is in the order of 1/1000 to 1/10 000. The detection of microtubules confirmed that the entities found in hepatocytes are morphologically identical to platelets, even though they had been, to a large extent, degranulated. No membrane structure derived from hepatocyte was observed outside the platelet membranes, suggesting that the platelets were not brought into the hepatocytes as a result of a phagocytic process. The inference is, therefore, that the platelets had actively invaded the hepatocytes.

Hepatocytes that contained platelets are not damaged. On the contrary, they seem to be activated, because many polysomes have been observed around the platelets, implying enhanced protein synthesis (Figure 3). In fact, in response to IL-1 and TNF, hepatocytes have been shown to synthesize a variety of proteins during the acute phase of inflammation (Steel and Whitehead 1994).

Normally, the sinusoidal endothelium inhibits the entry of platelets into the Disse spaces. However, injection of LPS, IL-1, or TNF clearly induces such a translocation of platelets. At present, the mechanisms underlying platelet penetration into hepatocytes remain unknown. Moreover, the importance of this translocation of platelets is not yet clear. However, some results suggest that platelets may be involved in a number of effects induced by LPS, IL-1, and TNF, including hypoglycemia, disseminated intravascular coagulation, septic shock, hepatitis, Shwartzman-type reactions, and self-defense reactions (Endo and Nakamura 1993).

The Role of Platelet Accumulation for the Prevention of Hepatitis

Several murine hepatitis models have been developed, including the induction of hepatitis by the injection of LPS after *Propionibacterium acnes* treatment, co-injection of LPS with D-galactosamine (GalN), or injection of Concanavalin A (ConA) alone. It is generally recognized that ConA-induced hepatitis is T-cell mediated, because nude mice (mostly deficient in mature T-cells) are resistant to ConA-induced hepatitis (Tiegs et al. 1992). On the other hand, both the hepatitis induced by *P. acnes*+LPS and that induced by GalN+LPS are mediated by macrophages (via the production of inflammatory cytokines) (Freudenberg et al. 1986; Seki et al. 2000; Silverstein 2004; Tsutsui et al. 2000). It has been repeatedly reported that the depletion of Kupffer cells markedly reduces ConA-induced hepatitis (Morita et al. 2003; Okamoto et al. 1998; Schumann et al. 2000), suggesting that in addition to T-cells, Kupffer cells are also causally involved in ConA-induced hepatitis.

Platelet-depletion reportedly affords significant protection against the mortality induced by GalN+LPS injection (Piguet et al. 1993), and indeed, activation of platelets occurs in GalN+TNF - and ConA-induced hepatitis (Miyazawa et al. 1998; Van Molle et al. 2000). GalN+LPS injection has been shown to induce marked hepatic platelet accumulation, which precedes the development of severe congestion (Shibazaki et al. 1996). Cumulatively, these results suggest that platelets play a causal role in these hepatitis models, irrespective of whether the hepatitis is T cell-mediated or macrophage-mediated. However, Ohtaki et al. (2009) reported that platelets were protective in Fas-mediated hepatitis, when the hepatitis was local or not severe. A very interesting observation is that, although LPS induces hepatic

platelet accumulation, LPS-pretreatment protects mice against the hepatitis induced by either ConA or GalN+LPS injection (Endo et al. 1992; Endo et al. 1999a; Freudenberg and Galanos 1988; Nishikage et al. 1999).

Platelet-depletion has been shown to significantly exacerbate ConA-induced hepatitis, and anti-P-selectin antibody and P-selectin receptor blockade have been found to reduce both ConA-induced hepatic platelet accumulation and hepatitis. Prior induction of hepatic platelet accumulation by pretreatment with low-dose LPS powerfully reduced ConA-induced hepatitis (Endo et al. 1992; Ohtaki et al. 2009). Such protection by LPS-pretreatment was not effective in mice depleted of Kupffer cells. In platelet-depleted mice, LPS-pretreatment severely exacerbated ConA-induced hepatitis (Yu et al. 2011). In mice depleted of both Kupffer cells and platelets, neither ConA nor LPS-pretreatment+ConA injection induced hepatitis. In mice deficient in IL-1 α and IL-1 β (but not TNF α), ConA-induced hepatitis was mild, and a protective effect of LPS was not detected.

Taken together, the above results suggest that (i) hepatic platelet accumulation may be causal or protective, (ii) causal accumulation involves hepatic aggregation of platelets, which may be induced by platelet stimulants leaked from injured hepatocytes, (iii) protective accumulation is inducible by administration of prior low-dose LPS in a manner dependent on Kupffer cells, and (iv) IL-1 is involved in both causal and protective hepatic platelet accumulation.

Kupffer Cells Support Extramedullary Erythropoiesis in the Liver

In adult mice, erythropoiesis occurs in the bone marrow and the spleen under normal conditions throughout life. The spleen is the main site of extramedullary hematopoiesis in response to acute anemia to maintain hematopoietic homeostasis. The liver has been also reported that erythropoiesis in response to anemia occurs in the splenectomized mice (Bringman et al. 2007). In certain pathological conditions in human, extramedullary erythropoiesis is also detected in several organs, including the liver (Horwood et al. 2003; Jelali et al. 2006; Rajiah et al. 2011). Therefore, the liver may act as a site of extramedullary erythropoiesis in adult mice, especially in the absence of the spleen.

Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption that are used as therapeutic agents in bone-resorptive disorders, such as osteoporosis and metastatic bone diseases (Soni et al. 2006, 2007). Bisphosphonates are classified into 2 types by the presence or absence of nitrogen in side chains, namely, nitrogen-containing bisphosphonates (NBPs) and non nitrogen-containing bisphosphonates (non-NBPs). NBPs have strong anti-bone resorptive effects that are much more powerful than those of non-NBPs, however, NBPs are associated with inflammatory side effects such as fever, jaw osteomyelitis, osteonecrosis and extramedullary erythropoiesis (Sadahira and Mori, 1999; Hanspal, and Hanspal 1994; Body 2003; Cremers et al. 2003; Escudero et al. 2009; Yamaguchi et al. 2010). The previous study reported that a single injection of a relatively large dose of one of NBP, 4-amino-1-hydroxybutylidene-1,1-bisphosphonate (AHBuBP) into mice inhibits bone resorption by osteoclasts, even though it increases the number of osteoclasts (Endo et al. 1999b). Furthermore, AHBuBP injection induced extramedullary erythropoiesis in the spleen by the

depletion of bone marrow-resident macrophages and increased the number of granulocytes in peripheral blood, no such effects are reported in mice treated with non-NBP (Endo et al. 1999b; Nakamura et al. 1999).

We have focused on the liver as the site of extramedullary hematopoiesis in splenectomized mice treated with an NBP and describe the role of Kupffer cells in hepatic erythropoiesis (Otsuka et al. 2011).

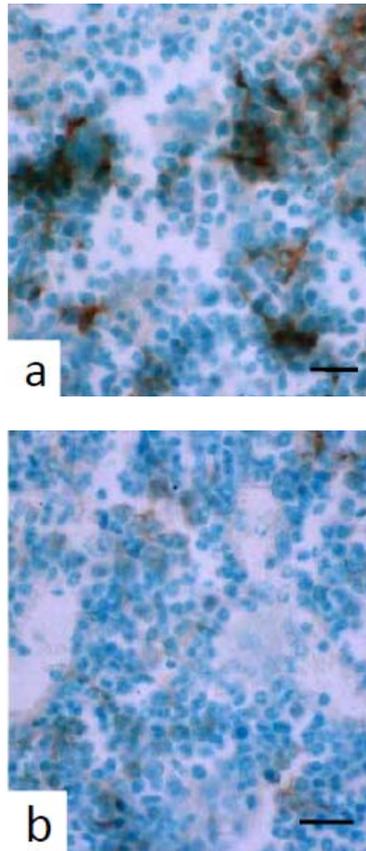


Figure 4. Immunohistochemical detection of F/80-positive bone marrow resident macrophages in normal (a) and NBP-treated (b) mice. (a) Normal bone marrow resident macrophages develop cell processes and form erythroblastic islands. (b) No detectable macrophages in the bone marrow by NBP injection. Bars 20 μ m.

Effects of NBPs on Hematopoiesis in the Bone Marrow

The bone marrow of mice treated with an NBP became obviously whitish in color compared to that of control mice. Hematocrit values of NBP-treated mice were not changed throughout the study period, indicating that no anemia was present in NBP-treated mice. A significant increase in erythropoietin (EPO) concentration in the serum was observed in anemia mice, whereas no significant increase in EPO was detected in the sera of NBP-treated

mice. In the bone marrow, the number of resident macrophages was drastically decreased and no apparent erythroblastic islands could be detected following NBP injection (Figure 4).

The Onset of Extramedullary Erythropoiesis in the Liver

At 4 days after NBP treatment, aggregations of mononuclear cells and megakaryocytes were detected within the liver. At 7 days after NBP treatment, clusters of mononuclear cells were detected in the parenchyma of the liver and beneath the capsule. Immunohistochemical analysis indicated cluster formation of TER-119-positive nucleated cells (erythroblast lineage cells) (Figure 5a). In addition, CD34 positive mononuclear cells, which may be hematopoietic precursor cells, were often observed in the livers of NBP-treated mice (Figure 5b). Double immunostaining of the liver at 7 days after NBP treatment indicated that F4/80-positive macrophages developed cell processes and surrounded TER-119-positive erythroblasts. Ultrastructural assessment showed that erythroblastic islands were detected in the sinusoid lumen. Erythroblasts were surrounded by Kupffer cells which extended their processes toward the erythroblasts, forming erythroblastic islands. Intravenous injection of Cl₂MBP-liposome disappeared erythroblastic islands by the depletion of Kupffer cells, confirming the role of Kupffer cells for erythropoiesis (Figure 6).

The Functional Change of Kupffer Cells for Extramedullary Hematopoiesis

Vascular cell adhesion molecule-1 (VCAM-1) plays an essential role in binding erythroblasts in the bone marrow and regulating erythropoiesis via hypoxia-inducible factor-2 α expression on erythroblasts (Paris 2009; Manwani, and Bieker 2008; Chasis, and Mohandas 2008; Yamashita et al. 2008). Under normal conditions, sinusoidal endothelial cells express VCAM-1, whereas Kupffer cells do not express VCAM-1. However, in the liver at 7 days after NBP treatment, Kupffer cells were found to express VCAM-1. These results strongly suggest that Kupffer cells may change their phenotype from the static phase to functioning as stromal cells for erythropoiesis following NBP treatment (Figure 7).

Other interesting findings include the down-regulation of EPO receptor expression in mononuclear cells in the liver, no changes in the expression of other mRNAs related to hematopoietic cells, and the disappearance of hematopoietic cells in the liver following Cl₂MBP-liposome treatment (Otsuka et al. 2011). These results indicate that Cl₂MBP-liposome treatment causes the arrest of proliferation and differentiation of hematopoietic precursor cells migrated to the liver, as well as impairment of the microenvironment for hematopoiesis. Kupffer cells have been implicated in EPO production (Eckardt et al. 1994; Paulet et al. 1984). EPO is now known to stimulate not only erythroid cells but also other hematopoietic cells such as myeloid cells, lymphocytes and megakaryocytes (Huang et al. 2010; Lisowska et al. 2010). Recent studies have also indicated an effect of EPO on non-hematopoietic cells such as endothelial cells, neurons and trophoblast cells (Benyo and Conrad 1999; Santhanamet et al. 2010; Sugawara et al. 2002). These results suggest that hematopoietic precursor cells migrated to the liver may not proliferate and differentiate in the presence of insufficient EPO concentrations because of the depletion of Kupffer cells.

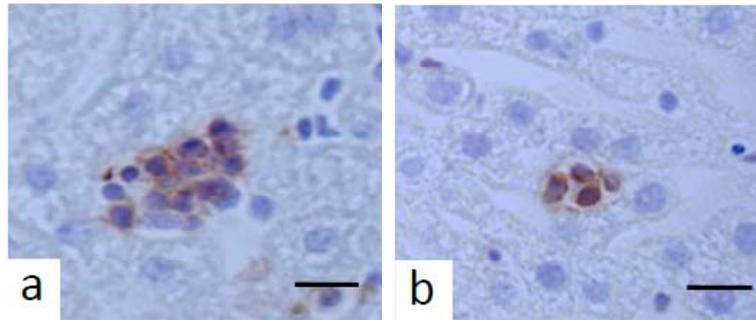


Figure 5. Immunohistochemical detection of hematopoietic cell clusters in the liver of splenectomized mice treated with NBP (AHBuBP 40 μ mol/kg i.p.). (a) TER-119 positive erythroblastic cluster. (b) CD34 positive hematopoietic cell cluster. Bars 25 μ m.

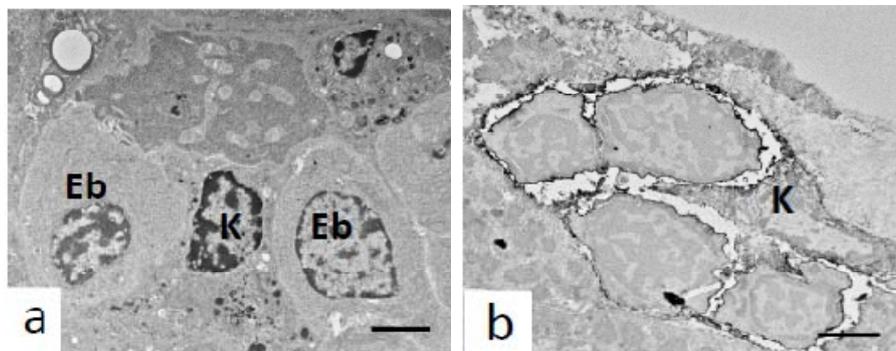


Figure 6. Erythroblastic islands in the liver of splenectomized mice treated with NBP (AHBuBP 40 μ mol/kg i.p.). (a) An electron micrograph of the erythroblastic island in the sinusoidal lumen. (b) Immunocytochemical detection of TER-119 positive erythroblastic cells contacted with Kupffer cells. K: Kupffer cell, Eb: erythroblast. Bars 2 μ m.

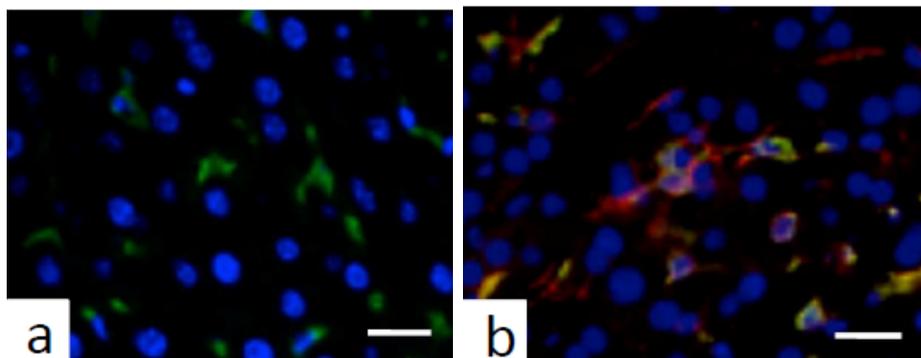


Figure 7. Double immunostaining of Kupffer cells (F4/80: green, VCAM-1: red). in normal (a) and splenectomized mice treated with NBP (b). Kupffer cells express VCAM-1 after NBP treatment.

Conclusion

The liver is composed of many types of cells, including hepatocytes and Kupffer cells and has a variety of functions, including plasma protein synthesis, detoxification, glycogen storage, and erythrocyte decomposition. In this chapter, we introduced 2 types of functions of Kupffer cells. To understand precisely the function of Kupffer cells may lead to clarify the cause and prevention of hepatitis.

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