In: Handbook of Macrophages
Editors: Rikiya Takahashi and Hibiki Kai
ISBN: 978-1-62081-162-7

Chapter X

Hepatic Macrophages and Macrophages with Different Functions in Hepatic Fibrosis

Hossain Md. Golbar and Jyoji Yamate
Graduate School of Life and Environmental Sciences,
Osaka Prefecture University, Izumisano City, Japan

Abstract

Macrophages are important cells with crucial roles in the homeostasis and regulation of immune response. They are heterogenous cells of mononuclear phagocytic system and display a wide range of patho-biological properties. Macrophages differ in ontogeny, morphology, immunophenotypes and functions. Three major types have been recognized: exudate macrophages, resident macrophages and dendritic cells. Exudate macrophages are in the monocytic lineage of monocytes, promonoblasts, monoblasts, and macrophage-colony forming unit (M-CFU) originating from hematopoietic stem cells in the bone marrow. Resident macrophages such as microglia, Kupffer cells, and alveolar macrophages also develop from hematopoietic stem cells; however, the precursors migrate from the bone marrow into connective tissues during ontogeny, and then differentiate into resident macrophages. Dendritic cells are composed of the interstitial dendritic cells, the interdigitating follicular cells of spleen and lymph nodes, and the Langerhans cells of the epidermis; the differentiation pathway is distinct from those of exudate or resident macrophages. Macrophages are scavengers or antigen presenting cells (APCs); they pick up, ingest and process foreign materials, and occasionally act as APCs by stimulating lymphocytes through the complicated immune system. Dendritic cells are more efficient in antigen presentation than other macrophages. Liver-specific macrophages during prenatal hepatogenesis until adulthood in rats, detectable by the immunohistochemistry with different antibodies, exhibit divergent properties including phagocytosis, proinflammatory cytokine productions, lipid metabolism and antigen presentation. Macrophages with phagocytic activity, which are predominant in prenatal period, maintain homeostasis by clearing apoptotic hematopoietic cells in the liver; additionally, hepatic macrophages play roles in homeostasis or host defense mechanisms through the production of proinflammatory cytokines, lipid metabolism and antigen

presentation in postnatal life. Hepatocyte injury, induced in perivenular areas by hepatotoxicants, is followed mainly by infiltration of exudate macrophages and activated resident macrophages, and APCs appear later. Chemically-induced cholangiocyte injury and subsequent biliary fibrosis in the Glisson's sheath are accompanied by consistent appearance of APCs and infiltration of exudate macrophages and activated Kupffer cells is later for APCs. Among these diverse properties, functions relating to fibrosis have recently been focused on hepatic macrophages, because the setting is emerging as a worldwide public health problem; in this light, macrophages secrete soluble factors such as TGF-β1 capable of inducing myofibroblasts which can excessively produce extracellular matrices, leading to fibrogenesis. Based on macrophage properties which are different in early, mid and late stages of fibrosis, the concepts of the classically activated M1 (pro-inflammatory) macrophages and the alternatively activated M2 (reparative) macrophages have currently been proposed. Macrophages undergo functional changes from M1 to M2 (or vice versa) under microenvironmental conditions evoked by cell-cell interactions. In this chapter, the general notions of genesis, distribution and functions of macrophages, and the unique properties of hepatic macrophages, particularly in hepatic fibrosis, are addressed.

Background

Macrophages are the mononuclear phagocytes that exist virtually in all tissues in the human and animal body. They are considered one of the most active secretory cell types in the body. They can release a multitude of mediators that regulate all aspects of homeostasis, inflammation and host defense. Beutler BA, Hoffma JA, and Steinman RM received Nobel Prize in 2011 for the novel insights into the activation and regulation of immune system relating to macrophage functions; Beutler BA and Hoffman JA discovered macrophage receptor proteins that can recognize microorganism and activate innate immunity, whereas Steinman RM discovered dendritic cells (in 1973) whose unique capacity is to activate and regulate adaptive immunity. Macrophages are heterogenous cells; they show variability in cell morphology, distribution and function depending on the requirements and microenvironmental conditions of the tissues in which they reside.

The term 'macrophage' was first used by Metchnikoff in 1892 to indicate large phagocytic cells [1]. Because of intense debate on the precise origin and differentiation of macrophages and their related cells, Aschoff introduced the concept of reticuloendothelial system (RES) in 1924 and included macrophages (histiocytes) as a major cell in this system together with reticulum cells and reticuloendothelial cells (phagocytic endothelial cells) [2,3]. Contrasting to this theory, van Forth and his colleagues proposed the concept of mononuclear phagocyte system (MPS) in 1972. According to this theory, all macrophages, regardless of those that appear in the inflammatory lesion or reside in tissues under normal steady-state conditions, derive from monocytes which differentiate via promonocytes from monoblasts originating in the bone marrow. The monocytes always circulate in the blood and migrate into tissues to replenish the long-lived tissue specific macrophages in organs and sites such as the bone (osteoclasts), alveoli (alveolar macrophages), central nervous system (microglial cells), connective tissues (histiocytes), skin (Langerhans cells), liver (Kupffer cells), spleen (interdigitating follicular cells) and peritoneum. The MPS theory states that blood monocytes have no proliferating capacity and macrophages are considered to be short-lived, non-dividing terminal cells of the MPS. However, during evolutionary processes in animals, macrophages

develop before monocytes emerge, which conflicts with the basic concepts of the MPS theory saying that all macrophages are derived from monocytes. Additionally, the differentiation pathway of macrophages from hematopoietic stem cells varies; some macrophage-related cells, particularly osteoclasts and dendritic cells, derive without the passage through the developmental stages of monocytic cells, and there are macrophage populations that develop from hematopoietic stem cells via common lymphoid progenitors. Current notion is that macrophages, reticulum cells and endothelial cells differ from each other in the origin, differentiation pathway, morphology and function.

In response to tissue injury, activated resident macrophages together with infiltrating macrophages produce increased amount of cytotoxic and proinflammatory mediators resulting in tissue damage and resultant inflammation. Macrophages in inflammation play an essential role in the promotion at early stages and in the suppression at late stages (wound repair), suggestive of biphasic properties of macrophages. The aberration of these activities may lead to exaggerated responses to etiology or the development of fibrosis (abnormal healing such as scleroderma and cirrhosis). It appears that macrophages can function as agents of defense or destruction, either protecting the host from etiology or promoting tissue injury and chronic disease condition. Such specific responses depend on the degrees of causes, the exposure level/time, and the nature of the inflammatory mediators.

Life Cycle of Macrophages

At prenatal stages before the development of bone marrow, macrophages generate from the yolk sac and are known as primitive or fetal macrophages (Figure 1) [4, 5]. After birth, macrophages differentiate from circulating peripheral blood monocytes which migrate into tissues in steady state condition or in inflammation. These monocytes develop from a common myeloid progenitor in the bone marrow that is also the precursor of neutrophils, eosinophils, basophils and mast cells [6]. The differentiation of progenitors towards macrophage lineage and the growth and survival of macrophages are strictly regulated under colony stimulating factor-1 (CSF-1) [7].

Liver specific macrophages in normal and pathological settings are categorized into exudate macrophages (infiltrating macrophages), resident (fixed) macrophages (Kupffer cells) and dendritic cells as APCs. Exudate macrophages are included in the monocytic lineage of macrophage-colony forming unit (M-CFU), monoblasts, promonoblasts and monocytes, originating from hematopoietic stem cells; the derivation and differentiation coincide with the notion of the MPS theory (Figure 1) [8]. By contrast, resident macrophages develop through differentiation pathway different from that of blood monocytic-derived exudate macrophages [9, 10]. Although the resident macrophages also derive from hematopoietic stem cells, the precursors are generated mainly from granulocyte/macrophage-colony forming unit (GM-CFU); ontogenetically, the precursor migrates from bone marrow into connective tissues, and then differentiates into resident macrophages [9]. The dendritic cells are composed of the interstitial dendritic cells, interdigitating follicular cells of the afferent lymphoid tissues such as spleen, lymph nodes and Peyer's patches, and the Langerhans cells of the epidermis [11, 12]. The interstitial dendritic cells are widely distributed in the connective tissues of the most non-lymphoid organs. The interstitial dendritic cells develop from hematopoietic stem cells

including GM-CFU, and ontogenetically, the precursor cells spread over the connective tissues [9, 13]. Interstitial dendritic cells migrate into the lymphoid organs and epidermis, and adapt their phenotypes based on *in situ* tissue requirements and immune conditions; they serve as interdigitating follicular cells and Langerhans cells as APCs, respectively. The differentiation pathway of these dendritic cells is distinct from that of the exudate or resident macrophages. During the maturation process, the dendritic cells originally develop from MHC class II-negative precursors, and then give rise to low MHC class II-positive cells (immature type). Finally, the immature type of dendritic cells can further up regulate the expression of MHC class II molecule under immunologically stimulated conditions [9, 12, 14].

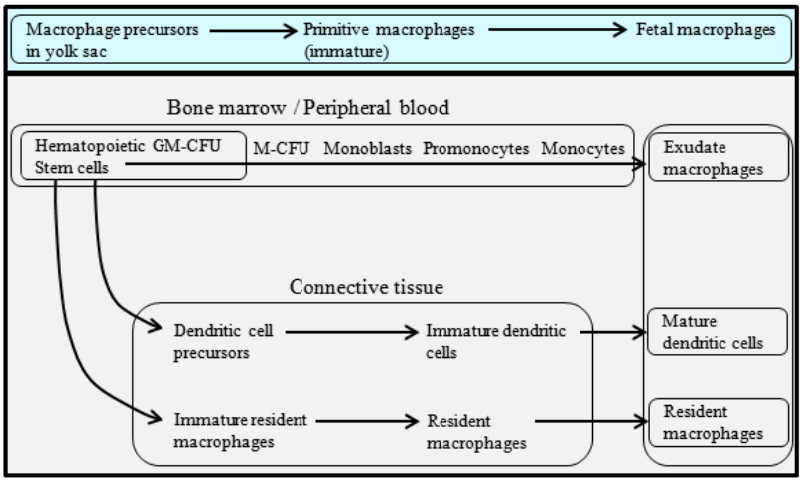

Figure 1. The ontogeny and differentiation of macrophages; details are in the text.

As mentioned above, macrophage populations may be divided mainly into three types; exudate macrophages, resident macrophages and dendritic cells. In pathological settings, however, some macrophages can exhibit phenotypes transitional/intermediate between exudate and resident macrophages [9]. In addition, some types of dendritic cells may be formed from blood monocytes [15]. These studies indicate that the different types of macrophages can be interchangeable in their functions and morphology which may be dependent on microenvironmental conditions, as mentioned below.

Peripheral blood monocytes in humans and mice show variability in expressions of cell surface antigens. Based on differential expression of CD14 and CD16 (FcγRIII), human monocytes are subdivided into two subsets: one subtype ($CD14^{high}CD16^{-}$ cells) is often called classical monocytes, because this phenotype resembles the original description of monocytes, whereas other subtype ($CD14^{+}CD16^{+}$ cells) is called non-classical monocytes [16]. The $CD14^{+}CD16^{+}$ cells possess features of resident macrophages and express higher amount of MHC class II molecule, suggesting that they might be the precursor of dendritic cells [17]. Additionally, both subsets of blood monocytes can differentiate into dendritic cells in culture in the presence of granulocyte/macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) [18, 19]. Chemokine-receptors also differentially expressed in blood monocyte subsets; $CD14^{+}CD16^{+}$ monocytes express CC chemokine receptor 5 (CCR5),

whereas $CD14^{high}CD16^{-}$ monocytes express CCR2 [20]. Mouse blood monocytes are divided into two subsets according to their expression of CCR2, CD62L (also known as L-selectin) and CX3C-chemokine receptor 1 (CX3CR1). One subset of mouse blood monocytes expresses CCR2, CD62L and only moderate amount of CX3CR1, whereas the other does not express CCR2 or CD62L but expresses higher amount of CX3CR1. The $CCR2^{+}$ blood monocyte subset migrates towards CCR2 ligand CC-chemokine ligand 2 (CCL2; also known as MCP-1) and are termed as inflammatory monocytes (exudative macrophages) [21, 22]. Ly6C, which is a part of the epitope of Gr1, is expressed in inflammatory monocytes and serves as an additional marker of $CCR2^{+}$ blood monocytes [23]. $CCR2^{+}CD62L^{+}CX3CR1^{low}Ly6C^{+}$ mouse blood monocytes corresponds to $CD14^{high}CD16^{-}$ (classic) human blood monocytes (which are also $CCR2^{+}CX3CR1^{low}$), whereas $CCR2^{-}CD62L^{-}CX3CR1^{high}Ly6C^{-}$ mouse blood monocytes are corresponding to $CD14^{+}CD16^{+}$ human blood monocytes (which also express large amount of CX3CR1). The heterogeneity of blood monocytes in rats is characterized by using CD43 as a differential marker [24]; $CD43^{low}$ blood monocytes express higher amount of CCR2 and CD62L, showing their similarity to $CCR2^{+}Ly6C^{-}$ (inflammatory) mouse blood monocytes; $CD43^{high}$ blood monocytes express higher amount of CX3CR1, indicating that this population is analogous to $Ly6C^{-}$ (resident) macrophage population in mice [6]. The blood monocyte subset expressing $CCR2^{+}Ly6C^{+}(Gr1^{high})$ infiltrate into tissues at early phases of inflammation and become exudate macrophages and dendritic cells, whereas blood monocyte subset expressing $CCR2^{-}Ly6C^{-}(Gr1^{low})$ infiltrate into tissues at steady state condition and become resident macrophages [25].

Blood monocyte-derived exudate macrophages have no proliferating potential and are short lived, whereas resident macrophages are long lived in inactive steady-state condition in tissues and can sustain by self-renewal [26]. Kupffer cells, a type of resident macrophages in the liver, are continuously replenished by blood monocytes in steady state condition, and show proliferating activity after partial hepatectomy [27-29]. After completing life cycle, macrophages follow lymphatic drainage system or die through apoptosis. The mechanisms of disappearance of macrophages after completion of their functions remain to be investigated.

Activation of Macrophages

Microenvironmental conditions influence the properties of macrophages, and based on appropriate conditions, macrophages participate in homeostatic process such as tissue remodeling and wound healing, as well as host defense. Recently, macrophages appearing in pathological lesions are divided into “classically activated macrophages (M1 macrophages)” and “alternatively activated macrophages (M2 macrophages)” (Table 1). Several *in vivo* studies have showed that the phenotypes of macrophages can change over time i.e. from M1 to M2 or vice versa. However, it is not clear whether this phenotypic alteration is the result of de-differentiation of the original macrophages back to the resting state or of the migration of a new population of macrophages into tissue sites where they replace the original cells. A number of microenvironmental signals can stimulate activation of M1 or M2 macrophages, which produce a variety of inflammatory cytokines and mediators (Table 1).

The M1 macrophages are the effecters that are generated during cell-mediated immunity in combined response to interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). The M1 macrophages possess enhanced microbicidal and tumoricidal capacity, and secrete high levels of pro-inflammatory cytokines and mediators. Pro-inflammatory cytokines produced by the M1 macrophages are an important component of host defense, but they can cause extensive damage to host tissues.

Table 1. Mediators that are released by classically (M1) and alternatively (M2) activated macrophages in liver injury

M1 Mediators	Toxicants
Proinflammatory cytokines (TNF-α, IL-1β, chemokines)	Endotoxin [30] Acetaminophen [31-33] Carbon tetrachloride [34] Galactosamine [35] Cadmium [36]
Reactive oxygen species	Endotoxin [37] Acetaminophen [38] Carbon tetrachloride [39] Phenobarbital [40] 1, 2 Dichlorobengene [41]
Reactive nitrogen species	Endotoxin [30] Acetaminophen [42] Carbon tetrachloride [43] Ethanol [44]
Bioactive lipids	Endotoxin [30] Acetaminophen [33] Carbon tetrachloride [45, 46]
Proteases	Endotoxin [47] Acetaminophen [48] Carbon tetrachloride [46] Thioacetamide [49]
M2 mediators	
TGF- β	Endotoxin [50] Acetaminophen [32] Carbon tetrachloride [34, 51] Thioacetamide [52]
IL-4	Acetaminophen [32] Concanavalin-A [53]
IL-10	Endotoxin [30] Acetaminophen [32, 54] Carbon tetrachloride [55]
IL-13	Endotoxin [56] Acetaminophen [57]

For example, IL-1β, IL-6, and IL-23, which are produced by the M1 macrophages, induce the development of T_H17 cells; the T_H17 cells produce IL-17, a cytokine which is

associated with high level of polymorphnuclear cell recruitment into inflammatory tissues; these processes contribute to autoimmune pathologies [58]. By contrast, the M2 macrophages can develop in response to innate or adaptive signals. IL-4, produced mainly by macrophages themselves and mast cells at early phases of tissue injury, is considered the first innate signal for the induction and activation of the M2 macrophages. IL-4 stimulates arginase activity in macrophages, allowing them to convert arginine to urea and ornithine; the later ornithine is metabolized to proline, a component of collagens, thereby contributing to deposition of extracellular matrices as seen in wound healing via reparative fibrosis [59]. Therefore, the M2 macrophages are regarded as wound healing macrophages. Similar to the dysregulated activity of the M1 macrophages in autoimmunity, the M2 macrophages may be detrimental to the host when extracellular matrix enhancing activity is dysregulated in pathological conditions such as cirrhosis and scleroderma. Additionally, the M2 macrophages are not always beneficial for the host, because high levels of IL-10 produced by the activated M2 macrophages can predispose the host to infection [60].

Immunohistochemical Detection of Rat Macrophages

The authors have investigated the heterogeneity and plasticity of macrophages in rat tissues of hepatic fibrosis after hepatocyte or cholangiocyte damage, which was induced by hepatotoxicants [52, 61, 62]. Monoclonal antibodies (ED1, ED2, OX6 and SRA-E5) useful for the detection of different macrophages have been generated [63-65]. ED1 (for CD68) is commonly used for detection of blood monocytes and exudate macrophages in rat pathological lesions [63, 64]; ED1 recognizes the antigen (CD68) located on the membrane of lysosomes, especially phagolysosome, of macrophages; thus the amount of ED1 expression implies the extent of phagocytic activity [66]. ED2 (for CD163) recognizes cell surface antigen of resident macrophages [63]; CD163 is a glycoprotein, which belongs to scavenger receptor cysteine-rich group B family and functions as the scavenger receptor (SR) for hemoglobin-haptoglobin complexes [67]; ED2 labels with Kupffer cells in normal liver [68]. SRA-E5 (for CD204) is an antibody generated against human type 1 SR protein and its expression is related to metabolism of oxidized low density lipoprotein (LDL) [65]; in normal rat liver, CD204 is expressed in Kupffer cells. OX6 recognizes MHC class II molecule (rat Ia) of the activated macrophages and dendritic cells [64, 69]. Interestingly, it has been demonstrated that the kinetics and distribution of macrophages expressing different immunophenotypes differ in developing livers and hepatic lesions.

Macrophage Phenotypes in Normal Liver of Rats

The majority of macrophage populations in developing liver at prenatal stages are of CD68-positive, indicative of prodigious phagocytic activity [68, 70]; such macrophages are regarded as fetal or primitive macrophages [4, 5]. During prenatal development of the liver, Kupffer cells ($CD163^+$, $CD204^+$) and dendritic cells (MHC class II^+) are fewer. After birth, however, the number of macrophages expressing CD68 gradually decreases, whereas those

expressing CD163, CD204 and MHC class II increase in the number [68]. Liver macrophages with phagocytic activity at prenatal stages have to represent the principal function to meet the requirement of recycling large amount of hemoglobin from old erythrocytes to reuse by the host, because fetal hematopoiesis occurs in the liver. This clearance is vital metabolic contribution without which the host can not survive. Macrophages have to also remove cellular debris and cells undergoing apoptosis that may be generated during tissue modeling (for constructing tissue architecture). These processes are independent of immune-cell signaling, and the removal seems to result in little or no production of immune mediators by macrophages phagocytizing cell debris and apoptotic cells [71]. The receptors that mediate these homeostatic clearance processes include the scavenger receptors, phosphatidyl serine receptors, thrombospondin receptor, integrins and complement receptors [72]. The emergence of Kupffer cells and dendritic cells after birth is related to maturation of immune system and host defense, because Kupffer cells secrete pro-inflammatory cytokines, and dendritic cells act as professional APCs [73, 74]. Although both Kupffer cells and dendritic cells can phagocytize foreign particles, process and present antigens to T-cells, the dendritic cells are more efficient for the specialty than Kupffer cells. Interestingly, $CD163^+$ and $CD204^+$ Kupffer cells reside along the sinusoids of the hepatic cords, whereas dendritic cells are localized in the Glisson's sheath as an interstitial type [61, 68]. The employment of the Kupffer cells and dendritic cells in maintaining homeostasis and immune response are different from each other.

Macrophage Phenotypes in Hepatic Fibrogenesis in Rats

Fibrosis is the consequence of wound healing responses characterized by excessive deposition of extracellular matrices. The extracellular matrices include mainly collagens (type I, III, IV), proteoglycans (biglycan, decorin) and glycoproteins (laminin, fibronectin, tenascin, glycosaminoglycan); the composition varies depending on the cells involved in its production. Hepatic parenchymal fibrosis is provoked following hepatocyte injury and necrosis, which may be induced by etiologies such as hepatitis virus infection, alcohol abuse, excess intake of hepatotoxicants and metabolic diseases (obesity, diabetes) [75-78]. Biliary fibrosis, a hepatic fibrosis with pathological process observed in cholangiopathies including primary sclerosing cholangitis, bile duct stenosis/obstruction and drug-induced bile duct damage, is evoked due to the abnormal interplay between injured cholangiocytes, mesenchymal cells (hepatic stellate cells (HSCs), portal fibroblasts, and vascular smooth muscle cells), and inflammatory cells (neutrophils, macrophages, and lymphocytes) [79, 80]. Although the exact cause of cholangiocyte injury remains undetermined, enteric bacterial toxins and autoimmunity are believed to be contributing factors for the progressive biliary fibrosis [81]. Hepatic fibrosis after hepatocyte and cholangiocyte injury may advance towards cirrhosis, an end stage condition, depending on degrees of injury or type of injurious agents.

Macrophages and myofibroblasts are the two crucial cells in fibrogenic process (Figure 2). Macrophages produce fibrogenic growth factors; the most potential factor is transforming growth factor-β1 (TGF-β1) which can stimulate the induction of myofibroblasts from HSCs for parenchymal fibrosis and portal fibroblasts for biliary fibrosis. The myofibroblasts are the

key source of extracellular matrices. The initial event of fibrosis is started with injury/necrosis of hepatocytes and cholangiocytes; generated cellular debris must be cleared up by macrophages expressing CD68. Because the debris may be loaded with endogenous danger signals such as heat-shock proteins, nuclear proteins (high mobility group box 1 protein), histone, and DNA, the phagocytosis by macrophages leads to dramatic changes in their properties, including productions of cytokines and pro-inflammatory mediators [82]. The production of these factors influencing inflammation may be due to $CD163^+$ macrophages, suggesting that $CD68^+$ macrophages may come to express CD163 in injured areas [61, 62].

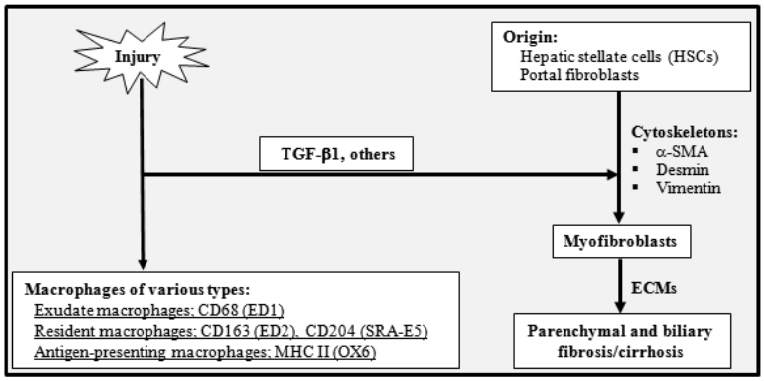

Figure 2. The possible pathogenesis of hepatic fibrosis. Briefly, injury to hepatic cells or cholangiocytes is followed by activation/infiltration of macrophages ($CD68^+$ exudate macrophages, $CD163^+$ and $CD204^+$ resident macrophages (Kupffer cells), and MHC class II^+ dendritic cells); these macrophages produce fibrogenic factors (e.g. TGF-β1) and induce myofibroblasts from hepatic stellate cells (HSCs) for parenchymal fibrosis and portal fibroblasts for biliary fibrosis. Myofibroblasts produce extracellular matrices (ECMs) leading to fibrosis and resultant cirrhosis (end stage of hepatic fibrosis). The myofibroblasts can express α-smooth muscle actin (α-SMA), desmin and vimentin in varying degrees during the development [52, 62, 89].

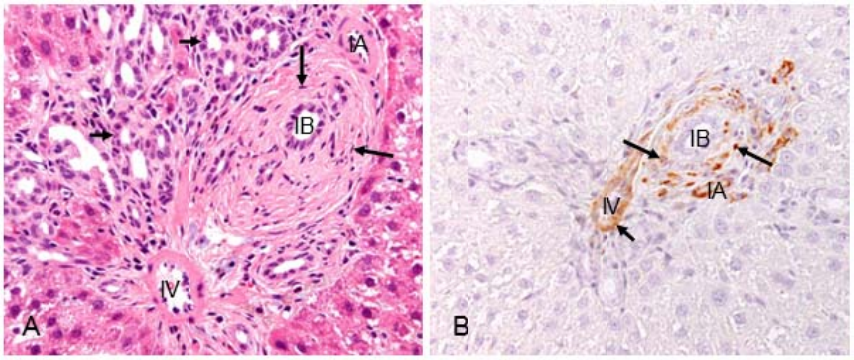

Figure 3. Histopathological sections showing biliary fibrosis induced in rats by α-naphthylisothiocyanate (ANIT). Proliferation of spindle-shaped cells (myofibroblasts; large arrows) and deposition of extracellular matrices are seen in the affected Glisson's sheath with biliary fibrosis; there are also proliferating interlobular bile ducts (small arrows) (A). The myofibroblasts (large arrows) express α-smooth muscle actin (α-SMA); small arrow indicates vascular smooth muscles reacting to α-SMA different from the myofibroblasts (B). HE (A); immunohistochemistry counterstained with hematoxylin (B).

Macrophages can detect the endogenous danger signals through Toll-like receptors (TLRs), intracellular pattern recognition receptors and IL-1R [83]. Macrophages are mainly recruited by MCP-1 (a member of CC chemokine family) secreted by injured hepatocytes and cholangiocytes, as well as HSCs [61, 81, 84, 85]. Activated resident macrophages ($CD163^+$ or $CD204^+$ Kupffer cells) together with infiltrated $CD68^+$ macrophages become the dominant source of TGF-β1, leading to progressive fibrogenesis through the induction of myofibroblasts. In rat hepatic fibrosis models, increased expressions of MCP-1 and TGF-β1 are clearly related to initial increment of macrophages and development of myofibroblasts (which can mainly express α-smooth muscle actin (α-SMA), a marker of myofibroblasts), respectively (Figures 3-4) [61, 62].

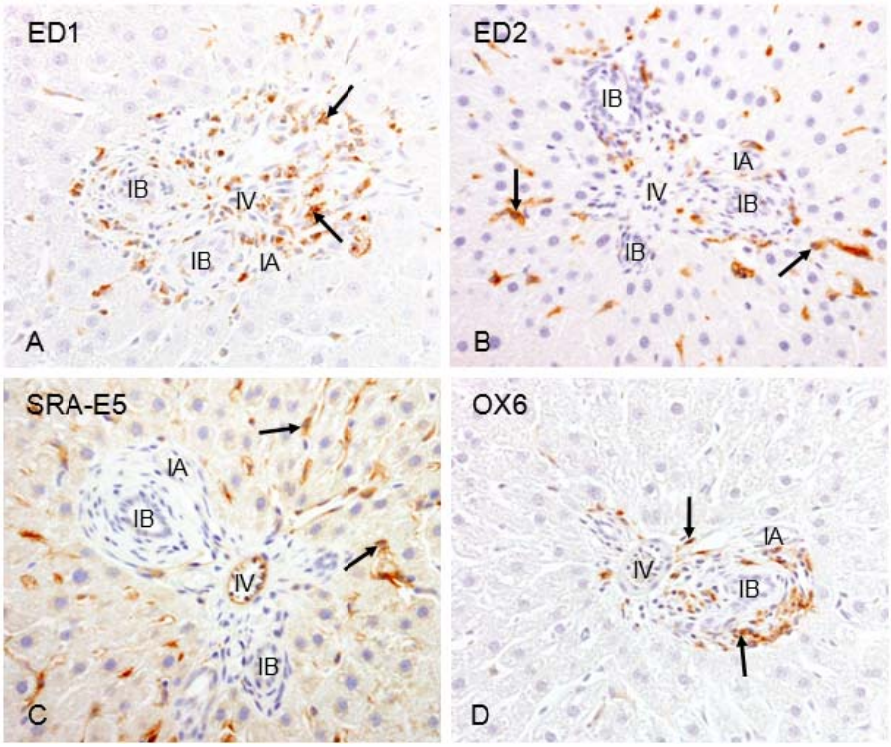

Figure 4. Histopathological sections showing ANIT-induced rat biliary fibrosis. There are exudate macrophages reacting to ED1 (for CD68) (A), resident macrophages reacting to ED2 (for CD163) (B) and SRA-E5 (for CD204) (C), and dendritic cells reacting to OX6 (for MHC class II) (D) in the affected Glisson's sheath and in the vicinity with progressive biliary fibrosis. Arrows indicate representative cells. IA: interlobular arteriole, IB: interlobular bile duct, IV: interlobular vein. Immunohistochemistry, counterstained with hematoxylin.

According to the immunophenotypical analyses, increased macrophages display divergent functions such as phagocytosis (by CD68 immunohistochemistry), pro-inflammatory cytokine production (by CD163), lipid metabolism (by CD204) and antigen presentation (by MHC class II). Interestingly, there are macrophages co-expressing these antigens to each other in injured areas; e. g. $CD68^+/CD163^+$ macrophages, $CD68^+$/MHC class II^+ macrophages, $CD163^+$/MHC class II^+ macrophages (Figure 5).

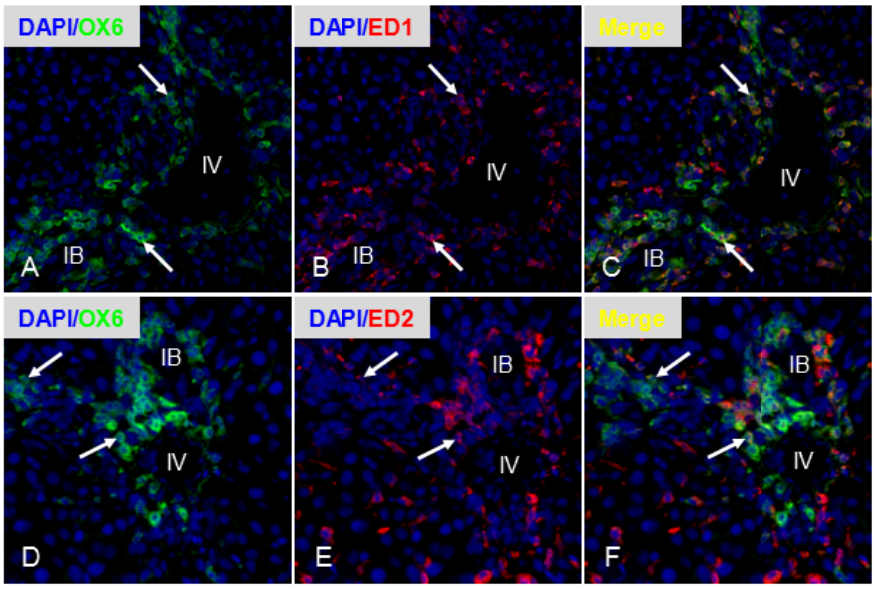

Figure 5. Fresh frozen tissue sections showing ANIT-induced rat biliary fibrosis. In the affected Glisson's sheath, antigen presenting dendritic cells (A, D; $OX6^+$ (for MHC class II); green color) displaying immunophenotypes for ED1 (for CD68) (C; yellow; merged) and ED2 (for CD163) (F; yellow; merged) are shown. Red color indicates macrophages with immunophenotypes for ED1 (B) and ED2 (E). Arrows indicate representative double positive macrophages. Immunofluorescence, nuclei are stained blue with 4' 6 diamidino-2-phenylindole (DAPI).

The M1 response may be due to macrophages expressing CD68, whereas the M2 macrophages contain cells expressing CD163 and CD204 [86-88]. MHC class II^+ macrophages/dendritic cells may participate both in the M1 and M2 responses in fibrogenesis. Based on these findings in the double immunofluorescence, it seems to be difficult to distinguish the M1 (pro-inflammatory) macrophages from the M2 (reparative) macrophages. The M1 and M2 macrophages may be interchangeable to each other under microenvironmental conditions which have not yet been decided.

In comparisons between post-hepatocyte injury-fibrosis (perivenular fibrosis induced by thioacetamide) and post-bile duct injury-fibrosis in the Glisson's sheath (biliary fibrosis induced by α-naphthylisothiocyanate (ANIT)), there are differences in the macrophage properties; hepatocyte injury and resultant fibrosis in perivenular areas are followed by quick infiltration of $CD68^+$ macrophages and $CD163^+$ Kupffer cells, and MHC class$^+$ APCs appear later; on the contrary, cholangiocyte injury and subsequent biliary fibrosis in the Glisson's sheath are accompanied by consistently increased number of MHC class II^+ APCs, and infiltration of $CD68^+$ macrophages and activated $CD163^+$ Kupffer cells is later for APCs with less number. This is partly because MHC class II^+ macrophages are more predominant in the Glisson's sheath than in perivenular areas where Kupffer cells exist as the main resident macrophages [68]. However, the reasons why macrophages with different immunophenotypes between the perivenular fibrosis and biliary fibrosis emerge remain to be clarified, which should lead to the clarification of the pathogenesis of cirrhosis. At least, it should be noticed that macrophages with divergent functions participate in hepatic fibrosis (Figures 4-5) [62].

Conclusion

Because of pivotal roles of macrophages in homeostasis, tissue modeling and immunity, macrophage-based therapy may open a new avenue to modulate disease process, particularly in chronic inflammation and cirrhosis (persistent fibrosis). Additionally, the heterogeneity of macrophages may lead to ideas for establishment of biomarkers specific of liver diseases. However, macrophages can change their properties depending on microenvironmental conditions in normal and pathological settings. Therefore, the detail mechanisms including cellular signals and factors affecting the properties of macrophages should be investigated, in addition to the origin and ontogeny.

References

[1] Metchnikoff, E. (1892). Leçns sur la pathologie comparée de l'inflammation. Paris, Masson.

[2] Aschoff, L. (1924). Das reticuloendotheliale System. *Ergeb. Inn. Med. Kinderheilkd*, 26, 1-118.

[3] Akazaki, K.; Watanuki, T. and Teshima, T. (1964). A further consideration on the reticuloendothelial system. In Organizing Committee of the 6th International RES Symposium (eds): The Reticuloendothelial System, Morphology, Immunology, and Regulation. Proceedings of the 6th International Symposium on RES, May 29 -June 1, 1964, in Otsu and Kyoto, Japan. pp 3-16.

[4] Takahashi, K.; Yamamura, F. and Naito, M. (1989). Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a lightmicroscopic, enzyme-histochemical, and ultrastructural study. *Journal of Leukocyte Biology*, 45, 87-96.

[5] Takahashi, K. and Naito, M. (1993). Development, differentiation, and proliferation of macrophages in the rat yolk sac. *Tissue and Cell*, 25, 534-539.

[6] Gordon, S. and Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. *Nature Reviews Immunology*, 5, 953-964.

[7] Alikhan, M. A.; Jones, C. V.; Williams, T. M.; Beckhouse, A. G.; Fletcher, A. L.; Kett, M. M.; Sakkal, S.; Samuel, C. S.; Ramsay, R. G.; Deane, J. A.; Wells, C. A.; Little, M. H.; Hume, D. A. and Ricardo, S. D. (2011). Colony-stimulating factor-1 promotes kidney growth and repair via alteration of macrophage responses. *The American Journal of Pathology*, 179, 1243-1256.

[8] van Furth, R. and Thompson, J. (1971). Review of the origin and kinetics of the promonocytes, monocytes, and macrophages and a brief discussion of the mononuclear phagocyte system. *Ann. Inst. Pasteur (Paris),* 120, 337-355.

[9] Takahashi, K.; Naito, M. and Takeya, M. (1996). Development and heterogeneity of macrophages and their related cells through their differentiation pathway. *Pathology International*, 46, 473-485.

[10] Valledore, A. F.; Borrs, F. E.; Cullell-Young, M. and Celada, A. (1998). Transcription factors that regulate monocyte-macrophage differentiation. *Journal of Leukocyte Biology*, 63, 405-417.

[11] Valladeau, J. and Saeland, S. (2005). Cutaneous dendritic cells. *Seminars in Immunology*, 17, 273-283.

[12] Zimmerli, S. C. and Hauser, C. (2007). Langerhans cells and lymph node dendritic cells express the tight junction component Claudin-1. *Journal of Investigative Dermatology*, 127, 2381-2390.

[13] Banchereau, J.; Paczesny, S.; Blanco, P.; Bennett, L.; Pascual, V.; Fay, J. and Palucka, A. K. (2003). Dendritic cells: controllers of the immune system and a new promise for immunotherapy. *Annals of the New York Academy of Sciences*, 987, 180-187.

[14] Fischer, U. B.; Jacovetty, E. L.; Medeiros, R. B.; Goudy, B. D; Zell, T.; Swanson, J. B.; Lorenz, E.; Shimizu, Y.; Miller, M. J.; Khoruts, A. and Ingulli, E. (2007). MHC class II deprivation impairs CD4 T cell motility and responsiveness to antigen-bearing dendritic cells in vivo. *Proceedings of the National Academy of Sciences, USA*, 104, 7181-7186.

[15] Gao, D.; Mondal, T. K. and Lawrence, D. A. (2007). Lead effects on development and function of bone marrow-derived dendritic cells promote Th2 immune responses. *Toxicology and Applied Pharmacology*, 222, 69-79.

[16] Passlick, B.; Flieger, D. and Ziegler-Heitbrock, H. W. (1989). Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood*, 74, 2527–2534.

[17] Ziegler-Heitbrock, H. W.; Fingerle, G.; Ströbel, M.; Schraut, W.; Stelter, F.; Schütt, C.; Passlick, B. and Pforte, A. (1993). The novel subset of CD14+/ CD16+ blood monocytes exhibits features of tissue macrophages. *European Journal of Immunology*, 23, 2053–2058.

[18] Sallusto, F. and Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *Journal of Experimental Medicine*, 179, 1109–1118.

[19] Sanchez-Torres, C.; Garcia-Romo, G. S.; Cornejo-Cortes, M. A.; Rivas-Carvalho, A. and Sanchez-Schmitz, G. (2001). CD16+ and CD16– human blood monocyte subsets differentiate in vitro to dendritic cells with different abilities to stimulate CD4+ T cells. *International Immunology*, 13, 1571–1581.

[20] Weber, C.; Belge, K. U.; von Hundelshausen, P.; Draude, G.; Steppich, B.; Mack, M.; Frankenberger, M.; Weber, K. S. and Ziegler-Heitbrock, H. W. (2000). Differential chemokine receptor expression and function in human monocyte subpopulations. *Journal of Leukocyte Biology*, 67, 699–704.

[21] Kurihara, T.; Warr, G.; Loy, J. and Bravo, R. (1997). Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *Journal of Experimental Medicine*, 186, 1757–1762.

[22] Lu, B.; Rutledge, B.J.; Gu, L.; Fiorillo, J.; Lukacs, N. W.; Kunkel, S. L.; North, R.; Gerard, C. and Rollins, B. J. (1998). Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *Journal of Experimental Medicine*, 187, 601–608.

[23] Geissmann, F.; Jung, S. and Littman, D. R. (2003). Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*, 19, 71–82.

[24] Ahuja, V.; Miller, S. E. and Howell, D. N. (1995). Identification of two subpopulations of rat monocytes expressing disparate molecular forms and quantities of CD43. *Cellular Immunology*, 163, 59–69.

[25] Tacke, F.; Alvarez, D.; Kaplan, T. J.; Jakubzick, C.; Spanbroek, R.; Llodra, J.; Garin, A.; Liu, J.; Mack, M.; van Rooijen, N.; Lira, S. A.; Habenicht, A. J. and Randolph, G. J. *(2007).* Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *Journal of Clinical Investigation,* **117,** 185–194.

[26] Takahashi, K. (2001). Development and differentiation of macrophages and related cells: historical review and current concepts. *Journal of Clinical and Experimental Hematopathology*, 41, 1-33.

[27] Yang, L.; Magness, S. T.; Bataller, R.; Rippe, R. A. and Brenner, D. A. (2005). NF-kappaB activation in Kupffer cells after partial hepatectomy. *American Journal of Physiology Gastrointestinal and Liver Physiology*, 289, G530-538.

[28] Klein, I.; Cornejo, J. C.; Polakos, N. K.; John, B.; Wuensch, S. A.; Topham, D. J.; Pierce, R. H. and Crispe, I. N. (2007). Kupffer cell heterogeneity: functional properties of bone marrow derived and sessile hepatic macrophages. *Blood*, 110, 4077-4085.

[29] Heymann, F.; Trautwein, C. and Tacke, F. (2009). Monocytes and macrophages as cellular targets in liver fibrosis. *Inflammation and Allergy-Drug Targets*, 8, 307-318.

[30] Laskin, D. L.; Chen, L.; Hankey, P. A. and Laskin, J. D. (2010). Role of STK in mouse liver macrophage and endothelial cell responsiveness during acute endotoxemia. *Journal of Leukocyte Biology*, 88, 373–382.

[31] Holt, M. P.; Cheng, L. and Ju, C. (2008). Identification and characterization of infiltrating macrophages in acetaminophen-induced liver injury. *Journal of Leukocyte Biology*, 84, 1410–1421.

[32] Dambach, D. M.; Durham, S. K.; Laskin, J. D. and Laskin, D. L. (2006). Distinct roles of NF-κB p50 in the regulation of acetaminophen-induced inflammatory mediator production and hepatotoxicity. *Toxicology and Applied Pharmacology*, 211, 157–165.

[33] Gardner, C. R.; Gray, J. P.; Joseph, L. B.; Cervelli, J.; Bremer, N.; Kim, Y.; Mishin, V.; Laskin, J. D. and Laskin, D. L. (2010). Potential role of caveolin-1 in acetaminophen-induced hepatotoxicity. *Toxicology and Applied Pharmacology*, 245, 36–46.

[34] Karlmark, K. R.; Weiskirchen, R.; Zimmermann, H. W.; Gassler, N.; Ginhoux, F.; Weber, C.; Merad, M.; Luedde, T.; Trautwein, C. and Tacke, F. (2009). Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology*, 50, 261–274.

[35] Hishinuma, I.; Nagakawa, J.; Hirota, K.; Miyamoto, K.; Tsukidate, K.; Yamanaka, T.; Katayama, K. and Yamatsu, I. (1990). Involvement of tumor necrosis factor-αin development of hepatic injury in galactosamine-sensitizedmice. *Hepatology*, 12, 1187–1191.

[36] Kayama, F.; Yoshida, T.; Elwell, M. R. and Luster, M. I. (1995). Role of tumor necrosis factor-α in cadmium-induced hepatotoxicity. *Toxicology and Applied Pharmacology*, 131, 224–234.

[37] McCloskey, T. W.; Todaro, J. A. and Laskin, D. L. (1992). Lipopolysaccharide treatment of rats alters antigen expression and oxidative metabolism in hepatic macrophages and endothelial cells. *Hepatology*, 16, 191–203.

[38] Dambach, D. M.; Watson, L. M.; Gray, K. R.; Durham, S. K. and Laskin, D. L. (2002). Role of CCR2 in macrophage migration into the liver during acetaminophen-induced hepatotoxicity in the mouse. *Hepatology*, 35, 1093–1103.

[39] Alric, L.; Orfila, C.; Carrere, N.; Beraud, M.; Carrera, G.; Lepert, J. C.; Duffaut, M.; Pipy, B. and Vinel, J. P. (2000). Reactive oxygen intermediates and eicosanoid production by Kupffer cells and infiltrated macrophages in acute and chronic liver injury induced in rats by CCl4. *Inflammation Research*, 49, 700–707.

[40] Shaik, I. H. and Mehvar, R. (2010). Cytochrome P450 induction by phenobarbital exacerbates warm hepatic ischemia-reperfusion injury in rat livers. Free Radical Research, 44, 441-453.

[41] Guanawardhana, L.; Mobley, S. A. and Sipes, I. G. (1993). Modulation of 1, 2-dichlorobenzene hepatotoxicity in the Fischer-344 rat by a scavenger of superoxide anions and an inhibitor of Kupffer cells. *Toxicology and Applied Pharmacology*, 119, 205–213.

[42] Abdel-Zaher, A. O.; Abdel-Rahman, M. M.; Hafez, M. M. and Omran, F. M. (2007). Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. *Toxicology*, 234, 124–134.

[43] Morio, L. A.; Chiu, H.; Sprowles, K. A.; Zhou, P.; Heck, D. E.; Gordon, M. K. and Laskin, D. L. (2001). Distinct roles of tumor necrosis factor-α and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. *Toxicology and Applied Pharmacology*, 172, 44–51.

[44] Venkatraman, A.; Shiva, S.; Wigley, A.; Ulasova, E.; Chhieng, D.; Bailey, S. M.; Darley-Usmar, V. M. (2004). The role of iNOS in alcoholdependent hepatotoxicity and mitochondrial dysfunction in mice. *Hepatology*, 40, 565–573.

[45] Horrillo, R.; Planaguma, A.; Gonzalez-Periz, A.; Ferre, N.; Titos, E.; Miquel, R.; López-Parra, M.; Masferrer, J. L.; Arroyo, V. and Clària, J. (2007). Comparative protection against liver inflammation and fibrosis by a selective cyclooxygenase-2 inhibitor and a nonredox-type 5-lipoxygenase inhibitor. *Journal of Pharmacology and Experimental Therapeutics*, 323, 778–786.

[46] Titos, E.; Claria, J.; Planaguma, A., Lopez-Parra, M.; Gonzalez-Periz, A.; Gaya, J.; Miquel, R.; Arroyo, V. and Rodés, J. (2005). Inhibition of 5-lipoxygenase-activating protein abrogates experimental liver injury: role of Kupffer cells. *Journal of Leukocyte Biology*, 78, 871–878.

[47] Tanner, A.; Keyhani, A.; Reiner, R.; Holdstock, G. and Wright, R. (1981). Proteolytic enzymes released by liver macrophages may promote injury in a rat model of hepatic damage. *Gastroenterology*, 80, 647–654.

[48] Gardner, C. R.; Laskin, J. D.; Dambach, D. M.; Chiu, H.; Durham, S. K.; Zhou, P.; Bruno, M.; Gerecke, D. R.; Gordon, M. K. and Laskin, D. L. (2003). Exaggerated hepatotoxicity of acetaminophen in mice lacking tumor necrosis factor receptor-1: potential role of inflammatory mediators. *Toxicology and Applied Pharmacology*, 192, 119–130.

[49] Park, S. Y.; Shin, H. W.; Lee, K. B.; Lee, M. J. and Jang, J. J. (2010). Differential expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in thioacetamide-induced chronic liver injury. *Journal of Korean Medical Science*, 25, 570–576.

[50] Zhang, X.; Yu, W. P.; Gao, L.; Wei, K. B.; Ju, J. L. and Xu, J. Z. (2004). Effects of lipopolysaccharides stimulated Kupffer cells on activation of rat hepatic stellate cells. *World Journal of Gastroenterology*, 10, 610–613.

[51] Rodriguez-Rivera, A.; Galicia-Moreno, M.; Reyes-Gordillo, K.; Segovia, J.; Vergara P.; Moreno, M. G.; Shibayama, M.; Tsutsumi, V. and Muriel, P. (2008). Methyl palmitate prevents CCl4-induced liver fibrosis. *Journal of Applied Toxicology*, 28, 1021–1026.

[52] Ide, M.; Kuwamura, M.; Kotani, T.; Sawamoto, O. and Yamate, J. (2005). Effects of gadolinium chloride (GdCl3) on the appearance of macrophage populations and fibrogenesis in thioacetamide-induced rat hepatic lesions. *Journal of Comparative Pathology*, 133, 92–102.

[53] Hatano, M.; Sasaki, S.; Ohata, S.; Shiratsuchi, Y.; Yamazaki, T.; Nagata, K. and Kobayashi, Y. (2008). Effects of Kupffer cell-depletion on concanavalin A-induced hepatitis. *Cellular Immunology*, 251, 25–30.

[54] Bourdi, M.; Masubuchi, Y.; Reilly, T. P.; Amouzadeh, H. R.; Martin, J. L.; George, J. W.; Shah, A. G. and Pohl, L. R. (2002). Protection against acetaminophen-induced liver injury and lethality by interleukin 10: role of inducible nitric oxide synthase. *Hepatology*, 35, 289–298.

[55] Louis, H.; Van Laethem, J. L.; Wu, W.; Quertinmont, E.; Degraef, C.; Van den Berg, K.; Demols, A.; Goldman, M.; Le Moine, O.; Geerts, A. and Devière, J. (1998). Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. *Hepatology*, 28, 1607–1615.

[56] Matsukawa, A.; Hogaboam, C. M.; Lukacs, N. W.; Lincoln, P. M.; Evanoff, H. L.; Strieter, R. M. and Kunkel, S. L. (2000). Expression and contribution of endogenous IL-13 in an experimental model of sepsis. *The Journal of Immunology*, 164, 2738–2744.

[57] Yee, S. B.; Bourdi, M.; Masson, M. J. and Pohl, L. R. (2007). Hepatoprotective role of endogenous interleukin-13 in a murine model of acetaminophen-induced liver disease. *Chemical Research in Toxicology*, 20, 734–744.

[58] Kolls, J. K. and Linden, A. (2004). Interleukin-17 family members and inflammation. *Immunity*, 21, 467–476.

[59] Kreider, T.; Anthony, R. M.; Urban, J. F. Jr. and Gause, W. C. (2007). Alternatively activated macrophages in helminth infections. *Current Opinion in Immunology*, 19, 448–453.

[60] Mosser, D. M. and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology*, 8, 958□969.

[61] Mori, Y.; Izawa, T.; Takenaka, S.; Kuwamura, M. and Yamate, J. (2009). Participation of functionally different macrophage populations and monocyte chemoattractant protein-1 in early stages of thioacetamide-induced rat hepatic injury. *Toxicologic Pathology*, 37, 463-473.

[62] Golbar, H. M.; Izawa, T.; Yano, R.; Ichikawa, C.; Sawamoto, O.; Kuwamura, M.; LaMarre, J. and Yamate, J. (2011). Immunohistochemical characterization of macrophages and myofibroblasts in α-naphthylisothiocyanate (ANIT)-induced bile duct injury and subsequent fibrogenesis in rats. *Toxicologic Pathology*, 39, 795-808.

[63] Dijkstra, C. D.; Dopp, E. A.; Joling, P. and Kraal, G. (1985). The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology*, 54, 589-599.

[64] Yamashiro, S.; Takeya, M.; Nishi, T.; Kuratsu, J.; Yoshimura, T.; Ushio, Y. and Takahashi, K. (1994). Tumor-derived monocyte chemoattractant protein-1 induces intratumoral infiltration of monocyte-derived macrophage subpopulation in transplanted rat tumors. *The American Journal of Pathology*, 145, 856-867.

[65] Tomokiyo, R.; Jinnouchi, K.; Honda, M.; Wada, Y.; Hanada, N.; Hiraoka, T.; Suzuki, H.; Kodama, T.; Takahashi, K. and Takeya, M. (2002). Production, characterization, and interspecies reactivities of monoclonal antibodies against human class A macrophage scavenger receptors. *Atherosclerosis*, 161, 123-132.

[66] Damoiseaux, J. G.; Dopp, E. A.; Calame, W.; Chao, D.; MacPherson, G. G. and Dijkstra, C. D. (1994). Rat macrophage lysosomal membrane antigen recognized by monoclonal antibody ED1. *Immunology*, 83, 140-147.

[67] Polfliet, M. M.; Fabriek, B. O.; Daniels, W. P.; Dijkstra, C. D. and van den Berg, T. K. (2006). The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production. *Immunobiology*, 211, 419-425.

[68] Golbar, H. M.; Izawa, T.; Murai, F.; Kuwamura, M. and Yamate, J. (2012) Immunohistochemical analyses of the kinetics and distribution of macrophages, hepatic stellate cells and bile duct epithelia in the developing rat liver. *Experimental and Toxicologic Pathology*, 64, 1-8.

[69] Zhao, L.; Kaneko, T.; Okiji, T.; Takagi, M. and Suda, H. (2006). Immunoelectron microscopic analysis of CD11c-positive dendritic cells in the periapical region of the periodontal ligament of rat molars. *Journal of Endodontics*, 32, 1164-1167.

[70] Kinoshita, M.; Uchida, T.; Sato, A.; Nakashima, M.; Nakashima, H.; Shono, S.; Habu, Y.; Miyazaki, H.; Hiroi, S. and Seki, S. (2010). Characterization of two F4/80-positive Kupffer cell subsets by their function and phenotype in mice. *Journal of Hepatology*, 53, 903-910.

[71] Kono, H. and Rock, K. L. (2008). How dying cells alert the immune system to danger. *Nature Reviews Immunology*, 8, 279–289.

[72] Erwig, L. P. and Henson, P. M. (2007). Immunological consequences of apoptotic cell phagocytosis. *The American Journal of Pathology*, 171, 2–8.

[73] Su, G. L. (2002). Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. *American Journal of Physiology Gastrointestinal and Liver Physiology*, 283, G256-265.

[74] Gehrie, E.; Van der Touw, W.; Bromberg, J. S. and Ochando, J. C. (2011). Plasmacytoid dendritic cells in tolerance. *Methods in Molecular Biology*, 677, 127□147.

[75] Bataller, R. and Brenner, D. A. (2005). Liver fibrosis. *The Journal of Clinical Investigation*, 115, 209–218.

[76] Svirtlih, N.; Jevtovic, D.; Simonovic, J.; Delic, D.; Dokic, L.; Gvozdenovic, E.; Boricic, I.; Nesic, Z.; Neskovic, G. and Urban, V. (2007). Older age at the time of liver biopsy is the important risk factor for advanced fibrosis in patients with chronic hepatitis C. *Hepatogastroenterology*, 54, 2324–2327.

[77] Yeh, M. M. and Brunt, E. M. (2007). Pathology of nonalcoholic fatty liver disease. *American Journal of Clinical Pathology*, 128, 837–847.

[78] Grossi, S.; Sumberaz, A.; Gosmar, M.; Mattioli, F.; Testino, G. and Martelli, A. (2008). DNA damage in peripheral blood lymphocytes of patients with cirrhosis related to

alcohol abuse or to hepatitis B and C viruses. *European Journal of Gastroenterology and Hepatology*, 20, 22–25.

[79] Lazaridis, K. N.; Strazzabosco, M. and LaRusso, N. F. (2004). The cholangiopathies: disorders of biliary epithelia. *Gastroenterology*, 127, 1565–1577.

[80] Strazzabosco, M.; Fabris, L. and Spirli, C. (2005). Pathophysiology of cholangiopathies. *Journal of Clinical Gastroenterology*, 39, S90–91.

[81] Harada, K.; Chiba, M.; Okamura, A.; Hsu, M.; Sato, Y.; Igarashi, S.; Ren, X. S.; Ikeda, H.; Ohta, H.; Kasashima, S.; Kawashima, A. and Nakanuma, Y. (2011). Monocyte chemoattractant protein-1 derived from biliary innate immunity contributes to hepatic fibrogenesis. *Journal of Clinical Pathology*, 64, 660–665.

[82] Zhang, X. and Mosser, D. M. (2008). Macrophage activation by endogenous danger signals. *Journal of Pathology*, 214, 161–178.

[83] Chen, C. J.; Kono, H.; Golenbock, D.; Reed, G.; Akira, S. and Rock, K. L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nature Medicine*, 13, 851–856.

[84] Marra, F.; DeFranco, R.; Grappone, C.; Milani, S.; Pastacaldi, S.; Pinzani, M.; Romanelli, R. G.; Laffi, G. and Gentilini, P. (1998). Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *The American Journal of Pathology*, 152, 423–430.

[85] Zamara, E.; Galastri, S.; Aleffi, S.; Petrai, I.; Aragno, M.; Mastrocola, R.; Novo, E.; Bertolani, C.; Milani, S.; Vizzutti, F.; Vercelli, A.; Pinzani, M.; Laffi, G.; LaVilla, G.; Parola, M. and Marra, F. (2007). Prevention of severe toxic liver injury and oxidative stress in MCP-1-deficient mice. *Journal of Hepatology*, 46, 230–238.

[86] Martinez, F. O.; Gordon, S.; Locati, M. and Mantovani, A. (2006). Transcriptional profiling of human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *Journal of Immunology*, 177, 7303-7311.

[87] Niino, D.; Komohara, Y.; Murayama, T.; Aoki, R.; Kimura, Y.; Hashikawa, K.; Kiyasu, J.; Takeuchi, M.; Suefuji, N.; Sugita, Y.; Takeya, M. and Ohshima, K. (2010). Ratio of M2 macrophage expression is closely associated with poor prognosis for Angioimmunoblastic T-cell lymphoma (AITL). *Pathology International*, 60, 278-283.

[88] Tidball, J. G. and Villalta, S. A. (2010). Regulatory interactions between muscle and the immune system during muscle regeneration. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*, 298, R1173-1187.

[89] Yamate, J. (2007). Heterogeneity of macrophage populations and myofibroblasts appearing in rat renal interstitial fibrosis. *Journal of Toxicologic Pathology*, 20, 185-195.