Chapter X

Hepatic Macrophages and Macrophages with Different Functions in Hepatic Fibrosis

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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 from 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
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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].

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 (FcγRII), human monocytes are subdivided into two subsets: one subtype (CD14<sup>high</sup>CD16<sup>+</sup> cells) is often called classical monocytes, because this phenotype resembles the original description of monocytes, whereas other subtype (CD14<sup>+</sup>CD16<sup>+</sup> cells) is called non-classical monocytes [16]. The CD14<sup>+</sup>CD16<sup>+</sup> 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<sup>+</sup>CD16<sup>+</sup> monocytes express CC chemokine receptor 5 (CCR5),
whereas CD14<sup>high</sup>CD16<sup>−</sup> 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<sup>+</sup> 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<sup>+</sup> blood monocytes [23]. CCR2<sup>−</sup>CD62L<sup>−</sup>CX3CR1<sup>low</sup>Ly6C<sup>+</sup> mouse blood monocytes corresponds to CD14<sup>high</sup>CD16<sup>−</sup> (classic) human blood monocytes (which are also CCR2<sup>+</sup>CX3CR1<sup>low</sup>), whereas CCR2 CD62L CX3CR1<sup>high</sup>Ly6C<sup>−</sup> mouse blood monocytes are corresponding to CD14<sup>+</sup>CD16<sup>−</sup> 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<sup>low</sup> blood monocytes express higher amount of CCR2 and CD62L, showing their similarity to CCR2<sup>+</sup>Ly6C<sup>+</sup> (inflammatory) mouse blood monocytes; CD43<sup>high</sup> blood monocytes express higher amount of CX3CR1, indicating that this population is analogous to Ly6C<sup>−</sup> (resident) macrophage population in mice [6]. The blood monocyte subset expressing CCR2<sup>+</sup>Ly6C<sup>(Gr1<sup>high</sup>)</sup> infiltrate into tissues at early phases of inflammation and become exudate macrophages and dendritic cells, whereas blood monocyte subset expressing CCR2<sup>−</sup>Ly6C<sup>(Gr1<sup>low</sup>)</sup> 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**

<table>
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<tr>
<th>M1 Mediators</th>
<th>Toxicants</th>
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| 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] |

<table>
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<tr>
<th>M2 mediators</th>
<th>Toxicants</th>
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| 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 Th17 cells; the Th17 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 hepatotoxins [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 la) 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).
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 II+ 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


