Review

Contribution of Macrophage Polarization to Metabolic Diseases

Yoshihiro Komohara, Yukio Fujiwara, Koji Ohnishi, Daisuke Shiraishi and Motohiro Takeya

Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

Macrophage activation is one of the major immunological events in the pathogenesis of various diseases. Recent studies have disclosed that complicated mechanisms are involved in macrophage activation and polarization, and many published research articles have been based on the M1/M2 polarization concept. It is considered that M1- and M2-like macrophages are associated with T helper (Th)1-type and Th2-type immune responses, respectively, via several immune mediators. In this article, we summarize the correlations between macrophage polarization and metabolic disorders in both humans and mice and discuss the contribution of macrophage polarization to the pathogenic process of metabolic diseases.


**Key words**: Macrophage, Heterogeneity, Non-alcoholic fatty liver disease, Obesity, Atherosclerosis

Introduction

Macrophages are professional phagocytes that act as key regulators of inflammatory responses in several diseases. They are detected in almost all organs and are known to be multifunctional and have heterogeneous phenotypes. They were first described as professional phagocytes by Metchnikoff, who received the Nobel Prize in 1908. Subsequently, macrophages were found to play important roles in inflammation and innate immunity. It is well known that there are two subpopulations of macrophages in humans and animals: resident and exudate macrophages. Resident macrophages are observed in normal, non-inflamed tissues, and their distinct functions are dependent on their anatomical location. Recent evidence demonstrated that some populations of resident macrophages are derived from yolk sac macrophages or their progenitors. In contrast, exudate macrophages are derived from circulating monocytes that infiltrate inflamed tissues where they differentiate into macrophages. In addition to such heterogeneity, recent studies have revealed a new concept for macrophage phenotype based on their activation status. Gordon S and colleagues found that interleukin (IL)-4 induced alternative macrophage activation that differed from classical macrophage activation, which is induced by bacterial products or pathogen-associated molecular patterns by means of the distinct expression of surface receptors and cytokines. Mills CD and colleagues demonstrated that classically and alternatively activated macrophages are distinguishable based on their catabolism of L-arginine, and they suggested naming the classically and alternatively activated macrophages as M1 and M2 macrophages, respectively. M1 cells produce nitric oxide via inducible nitric oxide synthase to prolong tissue damage and suppress tumor cell growth, whereas M2 cells produce ornithine (a precursor of proline and polyamines) via arginase I to promote cell replication (including that in tumor cells) and tissue repair. Following the introduction of these concepts, many molecules have been reported as the markers of M1 or M2 macrophages (Table 1). In addition, many studies using murine models have demonstrated the significance of macrophage polarization in several diseases. Phenotypical changes of macrophages have also been investigated in several human diseases because some specific markers, such as CD163, CD204, and CD206, have been found to be
common liver disorders associated with metabolic syndrome. Non-alcoholic steatohepatitis (NASH) is one of the life-threatening fatty liver diseases that can potentially progress to liver cirrhosis and liver cancer. Lipid accumulation and the inflammatory responses elicited by endotoxins from gut microbiota are considered to be the causes of NASH pathogenesis\(^1\)). Furthermore, dental bacterial infection also promotes the pathogenesis of NASH by activating Kupffer cells\(^2\)). Proinflammatory molecules, including IL-1\( \alpha \), tumor necrosis factor (TNF)-\( \alpha \), IL-6, and ROS, are considered to accelerate hepatic steatosis and insulin resistance, and macrophages preferentially produce these molecules after free fatty acid and endotoxin stimulation via toll-like receptors (TLRs)\(^3, 4\)). Macrophage depletion by clodronate-containing liposomes ameliorates liver damage in a murine NASH model\(^15\)). Macrophage aggregation around degenerated lipids comprise crown-like structures (CLS), and many CLS are observed in the liver of human NASH patients and in a murine NASH model\(^16\)). In a murine NASH model, macrophages in the CLS engulf dead hepatocytes and lipids and they express both the M1 markers\(^16\)); in contrast, macrophages in human CLS express CD163, an M2 marker (Fig. 2). The plasma concentration of soluble CD163 is positively associated with NAFLD activity and fibrosis, and bariatric surgery reduces the serum levels of soluble CD163\(^17\)). M2 Kupffer cells secrete IL-6, which induces hepatocyte senescence and resistance to apoptosis and steatosis in mice\(^18\)). Macrophages of C57BL/6 and BALB/c mice have M1- and M2-like responses, respectively, and NASH is induced more easily in C57BL/6 mice than in BALB/c mice\(^19\)). Therefore, it remains controversial whether macrophage phenotype is associated with the pathogenesis of NAFLD and whether CD163 is useful as a marker for evaluating macrophage or Kupffer cell activation.

### Macrophage Phenotype and Obesity

It is well known that macrophages are present in the adipose tissue (AT) and that an increased number of macrophages is observed in AT of obese patients and a murine obesity model; thus, macrophages are considered to be involved in AT homeostasis\(^20\) (Fig. 3).
Macrophage infiltration and proliferation in AT are mediated by IL-4, osteopontin, and monocyte chemoattractant protein-1 (MCP-1) and are related to AT development and angiogenesis\(^\text{21-23}\). The phenotype of adipose tissue macrophages (ATMs) is considered to be an M2-like phenotype; however, M1-like activation of ATMs is associated with the insulin resistance of adipocytes in obese patients and a murine obesity model\(^\text{20}\). Macrophage-derived TNF-\(\alpha\), IL-6, MCP-1, IL-1\(\beta\), and nitric oxide are suggested to be involved in the insulin resistance of adipocytes\(^\text{24}\). Saturated fatty acids (SFA) derived from adipocytes stimulate ATMs via TLR2/4 and Fetuin-A to express the M1 phenotype\(^\text{24}\), and cytokine secretion by activated macrophages induce SFA release and insulin resistance in adipocytes in a paracrine manner\(^\text{25, 26}\). The activation of nucleotide-binding domain-like receptor protein 3 inflammasomes is well known in M1 activation and is involved in insulin resistance in both humans and mice\(^\text{27}\). In a human study, a decreased number of macrophages and M2-like activation of ATMs was observed after drastic weight loss in obese patients\(^\text{28}\). CLS are also observed in AT of obese patients and a murine obesity model; the macrophages in these CLS express both M1 and M2 markers\(^\text{20}\). CD44 and its ligand, SPP1, are known to be up-regulated in ATMs of obese mice and humans, and blocking CD44 signals ameliorates insulin resistance in obese mice\(^\text{30}\). On

**Fig. 1.** M2-related molecules in humans and mice

(A) Human monocytes were differentiated into mature macrophages using a culture with 2% human serum, M-CSF (50 ng/ml), and GM-CSF (1 ng/ml) for 1 week. Further, the macrophages were stimulated for 1 day with IL-4, IL-10, and the supernatant of the T98G glioma cell line (tumor cell supernatant; TCS) to induce polarization to the M2 phenotype. The expression levels of CD163, CD204, CD206, and \(\beta\)-actin were evaluated using immunoblotting. For immunoblotting of CD204, cell lysates were pre-treated with N-glycosidase. The specific monoclonal antibodies used were anti-CD163 (clone RM3/1), anti-CD204 (clone SRA-E5), and anti-CD206 (clone 5C11) antibodies.

(B) The expression levels of CD163, CD204, and \(\beta\)-actin on murine resident peritoneal macrophages and bone marrow-derived macrophages were evaluated using immunoblotting. For preparing bone marrow-derived macrophages, bone marrow cells were stimulated with M-CSF (50 ng/mL) for 1 week. The antibodies used to stain CD163 and CD204 were rabbit polyclonal antibody (Santa Cruz Biotech) and mouse monoclonal antibody (clone SRA-E5), respectively. For immunoblotting of CD204, cell lysates were pre-treated with N-glycosidase. Tumor cell supernatant (TCS) of the MCA205 sarcoma cell line was used.
and inflammatory mediators secreted by macrophages are associated with additional immune cell infiltration and smooth muscle cell activation. Foamy macrophages in plaque are known to secrete IL-1β and TNF-α, which are markers of the M1 phenotype, indicating that foamy macrophages in plaque are polarized to the M1 phenotype. Interestingly, macrophages located around the lipid core express CD163 and CD206, whereas foamy macrophages hardly express these antigens. In a study using apolipoprotein E knockout mice, macrophages infiltrated in plaque were differentiated to the M2 phenotype and seemed to be involved in vascular cell proliferation, whereas macrophages in the late stage were preferentially shifted to the M1 phenotype. Immune reactions are now considered to be a key mechanism in atherosclerosis, and IL-4 derived from leukocytes, including neutrophils and natural killer T cells, are

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considered to be associated with M2 polarization in the early stage\(^\text{47, 39}\). Pentraxin 3 (PTX3) is suggested to be a novel biomarker for atherosclerosis that is as useful as C-reactive protein\(^\text{40}\) because it is predominantly expressed on activated macrophages and neutrophils in plaque lesions and is closely involved in M1 differentiation\(^\text{41-43}\). PTX3 expression is preferentially detected in iron-rich areas and severe plaque lesions in human atherosclerosis, and interestingly, CD163-positive macrophages in plaque also express PTX3\(^\text{44}\). This discrepancy indicates that CD163 may not be suitable as an M2 marker in atherosclerotic lesions. Moreover, miR-155 is also upregulated in M1 macrophages and is involved in MCP-1 and TNF-\(\alpha\) production\(^\text{45}\); it is involved in the progression of atherosclerotic plaque formation\(^\text{46}\). Taken together, these findings suggest that M2 macrophages play a protective role in the development of atherosclerosis.

**Glucagon-Like Peptide-1 Agonist and Oncostatin M may be Useful for the Treatment of Metabolic Syndrome by Regulating Macrophage Polarization**

Glucagon-like peptide-1 (GLP-1) is a hormone secreted from the L-cells of the small intestine, and it regulates systemic glucose metabolism. As such, GLP-1 agonists are now used for the treatment of diabetes\(^\text{47}\). The GLP-1 receptor (GLP-1R) is expressed on many cell types, including macrophages, and we
previously found that GLP-1 or a GLP-1R agonist induces macrophage polarization to the M2 phenotype via signal transducer and activator of transcription 3 (STAT3) activation\(^{48}\). In a previous study, macrophages inhibited the secretion of adiponectin by adipocytes, and the inhibition was significantly recovered by treatment with a GLP-1R agonist\(^{49}\). It has also been reported that a GLP-1R agonist improved steatohepatitis in a murine NASH model by regulating hepatic fatty acid metabolism\(^{50}\). In addition, treatment with a GLP-1R agonist decreased weight gain in obese mice by activating fat metabolism\(^{51}\), and in obese women, it reduced the body mass index (BMI)\(^{51}\). Furthermore, a GLP-1R agonist reduced serum concentrations of TNF-\(\alpha\), IL-1\(\beta\), IL-6, and soluble CD163, all of which are known macrophage-derived inflammatory molecules in patients with type 2 diabetes mellitus\(^{52}\). GLP-1 and GLP-1R agonists have been demonstrated to suppress atherosclerosis in rodents, and the clinical study of preventive effects of GLP-1R agonist is now undergoing\(^{53}\).

Oncostatin M (OSM) is a member of the gp130 family that is involved in M2 polarization via STAT3 signaling, and macrophages stimulated by OSM over-express IL-10 and arginase I\(^{54, 55}\). A lack of OSM receptor signaling induces M1 activation of macrophages and leads to AT inflammation, insulin resistance, and hepatic steatosis in obese mice\(^{55}\). Treatment with OSM improves obesity, insulin resistance, and lipid metabolism in hepatocytes by regulating the expression levels of the genes related to lipolysis and lipogenesis in obese mice\(^{56, 57}\). OSM is produced by ATMs in obese mice, and a higher level of OSM expression in human subcutaneous AT significantly correlates with a higher BMI\(^{59}\). OSM increases plasminogen activator inhibitor-1 and IL-6 expression in adipocytes stimulated by TNF-\(\alpha\)\(^{58}\). These findings indicate that OSM secreted by ATMs plays important roles in AT inflammation via autocrine and paracrine mechanisms. It remains unclear whether these beneficial effects against metabolic syndrome occur because of macrophage polarization to the M2 phenotype. Nonetheless, agents such as GLP-1 agonist or OSM that are involved in the regulation of macrophage activation or differentiation may be applied as a new tool for the treatment of obesity-induced metabolic disorders.

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All authors declare no conflict of interest regarding this manuscript.

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