Macrophages play important roles in innate immunity, the first line of host defense against invading pathogens, as well as in inflammation, adaptive immune responses and scavenging responses to maintain tissue homeostasis. Macrophage subsets also participate in inflammatory processes. Th1 cytokines such as interferon-γ and interleukin (IL)-1β, as well as lipopolysaccharide, induce a "classical" activation profile (M1), whereas Th2 cytokines, such as IL-4 and IL-13, induce an "alternative" activation profile (M2). In atherosclerosis, a systemic inflammatory response is combined with an accumulation of immune cells, including macrophages and their subsets. Atherosclerotic lesions contain large numbers of lipid-laden macrophages, known as foam cells. Recently, considerable attention has been focused on the heterogeneity of these macrophages. In this chapter, we describe recent advances in research on macrophages,
especially on their life cycle, functions, and participation in the pathogenesis of atherosclerosis.

Introduction

Inflammation plays an important role in the initiation and development of atherosclerosis. The first step in the formation of atherosclerotic lesions is endothelial injury, which is mediated by various inflammatory stimuli [1]. Epidemiological investigations, including the Framingham study, have demonstrated a close association between serum cholesterol concentrations and atherosclerotic diseases [2]. In individuals with metabolic disorders, including dyslipidemia and glucose intolerance, leukocytes adhere to the injured endothelium and migrate into the arterial wall, where they undergo transformation to macrophages [3]. Macrophages play important roles in innate immunity, the first line of host defense against invading pathogens, and in adaptive immune responses [4, 5]. Recently, considerable attention has focused on macrophage heterogeneity. In this review, we describe recent advances in research on macrophages, especially on their life cycle, functions, and participation in the pathogenesis of atherosclerosis.

Macrophage Contribution to Atherogenesis

During the first step of atherosclerotic plaque formation, circulating monocytes in blood extravasate to arterial intima, where they differentiate into macrophages. (Figure 1) Macrophages express various scavenger receptors, including scavenger receptor A (SRA) and CD36, which bind lipoprotein particles modified by reactive oxygen species (ROS). These scavenger receptors are generally regarded as essential components for foam cell formation. Moreover, scavenger receptors, including SRA, have been associated with the formation of atherosclerotic plaques, a process closely related to critical cardiovascular events such as acute coronary syndrome [6]. We recently reported that PI3kinase-associated cholesterol-enriched microdomains are involved in SRA-mediated uptake of modified low-density lipoprotein (LDL) by human macrophages, suggesting that cholesterol-enriched microdomains may contribute to inflammatory processes [7]. Cellular uptake of LDL-cholesterol via native LDL receptors is subject to negative feedback regulation, whereas the uptake of oxidized LDL (oxLDL)-cholesterol via scavenger receptors is not [8]. This can result in a massive cholesterol uptake by macrophages, which become foam cells due to their foamy appearance under the microscope. These foam cells accumulate in the intima of blood vessel walls, forming fatty deposits. Atherosclerotic plaques consist of these fatty deposits surrounded by a cap of smooth muscle cells and a layer of collagen-rich matrix. Macrophage secretion of matrix-degrading proteases can degrade the fibrous cap, resulting in a thin fibrous cap and necrosis at the center of the plaque. These plaques, called vulnerable plaques, may rupture, triggering the coagulation cascade and causing acute thrombosis of the artery, resulting in acute coronary syndromes and stroke [4]. (Figure 2)
Heterogeneity of Macrophages in Atherosclerotic Plaques

Accumulated evidence has shown that macrophages consist of heterogeneous cell populations. For example, an alternative subset of macrophages, activated by interleukin (IL)-4 and expressing large numbers of mannose receptors (MR), was first identified in 1992 [9]. Macrophages were later categorized as classically (M1) and alternatively (M2) activated macrophages [10]. In response to lipopolysaccharide (LPS) or Th1 cytokines such as interferon (IFN)-γ, macrophages undergo classical activation, with the resulting M1 macrophages producing many types of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, IL-1 and IL-6. In contrast, M2 macrophages, alternatively activated by Th2 cytokines such as IL-4 and/or IL-13, express scavenger receptors and MR on their surfaces and produce anti-inflammatory cytokines, such as IL-10. The characteristics of macrophages fall into a continuum between M1 and M2 macrophages [11]. (Figure 3)

Subsets of macrophages are present in human atherosclerotic plaques. For example, 2 macrophage subsets were observed in human atherosclerotic plaques obtained from carotid endarterectomies, with one subset producing MCP-1 and the other expressing MR [12]. Histological analysis revealed that the macrophages expressing MR were located far from the lipid core of the plaques and contained fewer lipid droplets than the macrophages not.
expressing MR [13]. Using cDNA microarray analysis, we recently assessed the types of macrophages contributing to atherogenesis [14].

Treatment with oxLDL-cholesterol resulted in the polarization of human monocyte-derived macrophages toward the M1 or M2 subset. OxLDL uptake affected TGF-β1- and NF-κB-mediated functions of M1 macrophages, but not those of M2 macrophages, suggesting that M1, but not M2, macrophages are those that likely respond to oxLDL [14].

Macrophage subsets can be generated from peripheral blood mononuclear cells (PBMCs) in vitro. Incubation of PBMCs with IFN-γ and LPS or granulocyte macrophage colony-stimulating factor (GM-CSF) results in M1 macrophages, whereas incubation with IL-4 or macrophage colony-stimulating factor (M-CSF) results in M2 macrophages [15, 16]. Although one study found that M-CSF-induced M2 macrophages may be more susceptible to becoming foam cells than M1 macrophages [17], this result is inconsistent with the in vivo results described above [13, 14]. Determination of the macrophage subset more likely to become foam cells requires further investigation. (Figure 4)

Figure 2. ‘Vulnerable’ plaque and ‘stabilized’ plaque.
Figure 3. Classically activated (M1) and alternative activated (M2) macrophages. IFN = interferon; LPS = lipopolysaccharide; TNF = tumor necrosis factor; iNOS = inducible nitric oxide synthase; RNI = reactive nitrogen intermediates; ROI = reactive oxygen intermediates; MHC = major histocompatibility complex; TLR = Toll-like receptor; MR = mannose receptor; Arg = arginase; SRs = scavenger receptors.
Figure 4. Peripheral blood mononuclear cells recruitment and differentiation. Circulating low-density lipoprotein (LDL) particles enter the arterial wall and accumulate in the intima, where they undergo chemical modifications, such as oxidation. Modified LDL can induce endothelial cell activation and expression of adhesion molecules. Furthermore, intimal macrophages can internalize modified LDL particles through scavenger receptors and become foam cells—a key process in the development of atherosclerotic plaque. T cells enter atheroma by interacting with adhesion molecules on the surface of endothelial cells in response to chemoattractant proteins. Once in the vessel wall, lymphocytes can use the T-cell receptor to recognize different antigens, possibly including modified low-density lipoprotein, presented by the major histocompatibility complex class II on macrophages or other antigen presenting cells. T cells then assume different programs of activation, typically becoming Th1 and Th2 cells. Cytokines from both groups differently influence plaque progression. The Th1 cytokine, interferon-γ, classically activates macrophages, the Th2 cytokines, IL-4 and IL-13, promote alternative macrophage activation. IFN = interferon; IL = interleukin; MHC II = major histocompatibility complex class II; TCR = T cell receptor; Th1 = type 1 T-helper cell; Th2 = type 2 T-helper cell; TNF = tumor necrosis factor.
**Monocytes Are Also Heterogeneous**

Similar to macrophages, monocytes have also been shown to be heterogeneous. A series of *in vivo* experiments revealed the presence of two distinct monocyte subsets in the blood circulation of mice, Gr1⁺/Ly6C<sup>high</sup>CCR2⁺CX3CR1<sup>low</sup> and Gr1⁺/Ly6C<sup>low</sup>CCR2⁺CX3CR1<sup>high</sup> monocytes, which are thought to correspond to CD14<sup>high</sup>CD16<sup>−</sup> and CD14<sup>low</sup>CD16<sup>+</sup> monocytes in humans, respectively [18]. Gr1⁺/Ly6C<sup>high</sup> monocytes extravasate into tissue, differentiating into M1 macrophages and producing many types of inflammatory cytokines. In contrast, Gr1⁺/Ly6C<sup>low</sup> monocytes crawl on the vasculature, patrolling the luminal side of small vessels under normal conditions. In response to inflammation, these Gr1⁺/Ly6C<sup>low</sup> monocytes extravasate into tissue, differentiate into M2 macrophages, and become involved in wound repair and tissue remodeling. (Figure 5)

Although mouse monocyte subsets have been well characterized, those in humans are less well understood. Human monocytes initially identified by their expression of CD14 and CD16, are also known as the LPS receptor and FcγRIII, respectively. Subsequently, two major subsets were identified, CD14<sup>high</sup>CD16<sup>−</sup> and CD14<sup>low</sup>CD16<sup>+</sup> monocytes [19], with the former accounting for 80–95% of human PBMCs. CD14<sup>high</sup>CD16<sup>−</sup> monocytes express CCR2 and have inflammatory characteristics, whereas CD14<sup>low</sup>CD16<sup>+</sup> monocytes lack CCR2, suggesting that CD14<sup>high</sup>CD16<sup>−</sup> and CD14<sup>low</sup>CD16<sup>+</sup> monocytes correspond to murine Gr1⁺/Ly6C<sup>high</sup>CCR2⁺CX3CR1<sup>low</sup> and Gr1⁺/Ly6C<sup>low</sup>CCR2⁺CX3CR1<sup>high</sup> monocytes, respectively [18].

**Figure 5. Heterogeneity of monocytes and macrophages. Tip-DC = TNF and iNOS producing dendritic cells.**
Monocytes Contribution to Atherogenesis

Monocytes were the first inflammatory cells associated with atherosclerosis and were found to be the main component of porcine atherosclerotic plaques [20]. The monocyte subsets responsible for atherogenesis, however, remained unclear.

During inflammation, circulating monocytes are recruited into the intima, where they differentiate into macrophages that accumulate lipids. Gr1−/Ly6C<sup>high</sup> monocytes are able to roll on the endothelium due to their expression of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and lymphocyte function-associated antigen (LFA)-1; and of chemokine receptors, including CX3CR1, CCR2 and CCR5 [21]. Following activation, these monocytes rapidly (µm/min) extravasate into the tissue. In contrast, little is known about the mechanisms of recruitment of Gr1<sup>−</sup>/Ly6C<sup>low</sup> monocytes, although CCR5 is thought to be critical for their migration [22]. In response to inflammation, these cells slowly (mm/h) extravasate through as yet undefined mechanisms. Although Gr1<sup>−</sup>/Ly6C<sup>low</sup> monocytes are thought to patrol the endothelium, it is not clear whether these cells scavenge lipids or dying cells on the endothelium. (Figure 6)

Although the exact role of each monocyte subset in human atherosclerosis remains unclear, clinical evidence provides some clues. For example, patients with coronary artery disease have higher numbers of peripheral blood CD14<sup>+</sup>CD16<sup>+</sup> monocytes than do healthy volunteers [23]. Peak levels of CD14<sup>high</sup>CD16<sup>low</sup> monocytes after acute myocardial infarction correlated negatively with the recovery of left ventricular function [24]. Moreover, an ex vivo study using diagnostic magnetic resonance chips showed that the phagocytic activities of these subsets differed [25]. Immuno-stained human coronary arteries showed that CD68<sup>+</sup>CD14<sup>+</sup> cells were predominant within atherosclerotic lesions, whereas CD68<sup>+</sup>CD14<sup>−</sup> cells were predominant in areas devoid of disease [26].

Where Do Macrophages Come from? Or Do They Proliferate by Themselves?

As shown above, atherogenesis is thought to result from the accumulation of lipid-laden macrophages, and macrophages are thought to be derived from monocytes. Recently, however, murine M2 macrophages were reported to proliferate by themselves in situ, not to be derived from circulating monocytes [27]. Injection of thioglycollate and L. sigmodontis into murine pleural cavities induced M1 and M2 macrophages, respectively. Although clodronate-loaded liposomes (CL-liposomes) have been found to block tissue infiltration by macrophages, these liposomes did not affect the accumulation of M2 macrophages after L. sigmodontis injection, although they blocked accumulation of M1 macrophages after thioglycollate injection. These results indicated that M1 macrophages are recruited from circulating monocytes and lose the ability to proliferate, whereas M2 macrophages can proliferate by themselves in situ.
Figure 6. Gr1+Ly6C<sup>high</sup> and Gr1−Ly6C<sup>low</sup> monocytes in pathogenesis of atherosclerotic formation. Monocytes are recruited (influx) into the intima and may differentiate into macrophages that accumulate lipids and cholesterol derivatives. Gr1−Ly6C<sup>low</sup> monocytes are recruited into the intima, and are believed to differentiate into macrophages that accumulate lipids. Little is known about Gr1−Ly6C<sup>low</sup> monocytes. Crawling occurs in a random behavior and involves leukocyte function-associated molecule-1 (LFA-1, β2-integrin), CX3CR1 and the chemokine fractalkine.

**Conclusions**

Both monocytes and macrophages contain heterogeneous cell populations, and contribute to atherogenesis by interacting with one another. However, the distinct contribution of each subset to atherogenesis requires further investigations. The life cycle and balance of various macrophage subsets are broadly involved in the pathogenesis of atherosclerotic processes. Macrophage biology may be a promising target in future investigations aimed at reducing the morbidity and mortality of atherosclerotic disease.

**References**


