Macrophage Diversity and Polarization in Atherosclerosis
A Question of Balance

Alberto Mantovani, Cecilia Garlanda, Massimo Locati

Abstract—Diversity and plasticity are hallmarks of mononuclear phagocytes, which are reflected in plaque formation and evolution. Different monocyte subsets, which differentially contribute to plaque infiltration and to atherosclerosis complications, have been identified. Similarly, depending on different environmental signals plaque-associated macrophages can express polarized pro- and antiatherogenic programs by influencing lipid metabolism, inflammatory responses, and plaque stability. Thus, a “macrophage balance” plays a major role in the pathogenesis of atherosclerotic plaques and affects evolution and complications of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2009;29:1419-1423.)

Key Words: macrophage ■ inflammation ■ atherosclerosis

Diversity and plasticity are hallmarks of the mononuclear phagocyte system. 1–6 Mononuclear phagocytes develop into morphological and functional distinct cell types in response to the tissue microenvironment (eg, lung alveolar macrophages, Kupffer cells, decidual macrophages). Moreover, inflammatory signals of microbial or leukocyte origin drive different forms of macrophage activation and polarization. 2,7 A key component of the chronic inflammation characteristic of the atherosclerotic plaque is a persistent influx of mononuclear phagocytes, which are the major leukocyte population present in atherosclerotic lesions. 8,9 Here, they ingest lipoprotein particles and give rise to foam cells and contribute to the atherogenic process by regulating lipid metabolism and orchestrating inflammatory responses. 10,11 In particular, they contribute to evolution of the plaque by secreting cytokines, and reactive oxygen species, and play a major role in weakening and destabilization of the fibrous cap by releasing proteases. 8 Here we will concisely review selected aspects of monocyte heterogeneity and macrophage activation and discuss mononuclear phagocyte diversity and plasticity in the context of atherosclerosis.

Monocyte Diversity
Monocytes are not born equal. 6 Differential expression of selected surface molecules have been used to identify monocyte subsets in mouse and man. In humans, expression of the FcγRIII receptor (CD16) distinguishes 2 monocyte subsets. 12 CD14+/CD16- cells represent 80% to 90% of circulating monocytes and express high levels of the chemokine receptor CCR2 and low levels of CX3CR1. In response to lipopolysaccharide (LPS), they are poor producers of inflammatory cytokines but release IL-10. Conversely, CD16+ monocytes have a CX3CR1high/CCR2low phenotype and account for inflammatory cytokine production (in particular the CD14- ones).

In the mouse, monocyte subsets can be distinguished on the basis the Ly6C antigen expression (identified by the anti-Gr1 RB6–8C5 antibody), which also recognizes Ly6G expressed on neutrophils, and of the chemokine receptors CCR2 and CX3CR1. 13,14 Gr1+/CCR2high monocytes, originally called “inflammatory,” are recruited at sites of inflammation and in lymph nodes and produce high levels of inflammatory cytokines. Gr1– monocytes patrol blood vessels in a seemingly random fashion under steady state conditions. Here they may well scavenge oxidized lipids. In response to infection, Gr1– extravasate rapidly and sustain the initial inflammatory response. 6,15 This observation is consistent with the fact that the CX3CR1 ligand CX3CL1 (also known as fractalkine) as well as the CCR2 ligand CCL2 (also known as monocyte chemotactic protein 1 [MCP-1]) are induced by inflammatory signals. In particular, CX3CL1 is induced in endothelial cells by IFNγ and microbial ligands and suppressed by IL-4 and IL-13. 16 Thus, both in mouse and man different monocyte...
subsets can be identified by surface markers, but there is no clear-cut unambiguous relationship between murine and human subpopulations.

Based on expression of the angiotropin receptor Tie2, a small monocyte subset (~1%) oriented to promotion of angiogenesis has also been identified. Although there is evidence for differential involvement of the “classical” monocyte subpopulations in atherosclerosis, the role of Tie2 monocyte remains to be established.

Macrophage Activation and Polarization

Diversity is a hallmark of mononuclear phagocytes, and diverse are the forms of macrophage activation induced by different environmental signals, including microbial products and cytokines (Figure 1). In response to some bacterial moieties (eg, LPS) and IFNγ, macrophages undergo classic activation (or M1). Selected properties of these cells include production of copious amounts of reactive nitrogen and oxygen intermediate and IL-12. M1-activated macrophages are part of polarized Th1 responses and are oriented to mediate resistance against intracellular parasites and tumors and to elicit tissue disruptive reactions. Alternative macrophage activation (or M2) was originally discovered as a response to IL-4. M2 activated macrophages come in different flavors depending on the eliciting signals: IL-4/IL-13; immune complexes and ligands of MyD88-using receptors (IL-1 or LPS); glucocorticoid hormones; TGF-β; IL-10. M-CSF–cultured monocytes have a transcriptional profile close to IL-4 activated cells, suggesting that this is a default pathway of differentiation. In general, M2-activated cells share high expression of scavenger, mannose, and galactose receptors, and an IL-12low, IL-10high, IL-1 decoy receptor (IL1R2)high, IL-1 receptor antagonist (IL1RN)high phenotype. Different forms of macrophage activation also have a distinct chemokine repertoire (eg, CCR4-agonists CCL17 and CCL22 for M2 cells; CXCR3-agonists CXCL9 and CXCL10 for M1 cells).

The various versions of M2 activation are oriented to the promotion of tissue remodeling and angiogenesis, parasite capsulation, regulation of immune responses, and tumor promotion. Recent results have highlighted the integration of M2-polarized macrophages with immunoregulatory pathways. M2 cells were shown to induce differentiation of regulatory T cells; conversely, regulatory T cells have been reported to induce alternative activation of human mononuclear phagocytes. Although general properties are retained from mouse to man, there are significant differences, such as the association of the chitinase 3-like 3 lectin (also known as Ym1), the transcription factor found in inflammatory zone 1 (FIZZ1), and arginase 1 (ARG1) with M2 polarization in the mouse but not in man. In vivo counterparts of M2 macrophage polarization have been observed in tissue remodeling during ontogeny, in placenta, and in various pathological conditions. The M1/M2 macrophage polarization paradigm should be viewed in the light of the plasticity and flexibility of mononuclear phagocytes mirroring the Th1/Th2 paradigm and their activating products (IFNγ and IL-4). Indeed, different signals give rise to a complex multidimensional variety of macrophage activation profiles.

The relation between monocyte subsets and macrophage polarization remains unclear. In a model of infection and acute myocardial infarction, Gr1+ and Gr1− cells have been shown to differentiate into M1 and M2 cells, respectively. However it remains unclear whether this reflects precommitment or temporal recruitment and exposure to different environmental signals.

Monocyte Recruitment in Atherosclerosis

Monocyte recruitment in atheromas fulfills the conventional paradigm of rolling, arrest, and transmigration. Briefly, P- and to a lesser extent E-selectin have an important role in monocyte rolling, and VCAM-1 and ICAM-1 are involved in firm adhesion. Chemokines have complex functions in the regulation of leukocyte traffic in atherosclerosis, CXCL1 and CCL5, interacting with CXCR2 and CCR5, respectively, promote monocyte arrest. In complex with CD74, CXCR2 also recognizes the thrombomodulin (TM), which is expressed by the endothelium during atherogenesis. The CCL2/CXCR2 axis, which is essential for atheroma development, does not promote monocyte arrest but likely governs monocyte transmigration.

Atherosclerosis is associated with profound changes in monocyte numbers and subsets. In hypercholesterolemic ApoE−/− mice, monocytosis is mainly attributable to an increase in the Gr1+/CCR2− subset. The Gr1+ mouse monocytes differentiate into aortic macrophages. Recruitment of Gr1+/CCR2+ monocytes requires also CX3CR1 and CCR5. Gr1− monocytes depend on CCR5 for entry into atheromatous lesions. An increased proportion of CD14+/CD16+ monocytes has been associated with hypercholesterolemia and increased incidence of coronary artery disease. Thus, although human monocyte subsets do not mirror the mouse ones, changes in
Macrophage Heterogeneity in Atheroma

Macrophages in atherosclerotic lesions have long been known to be heterogeneous in terms of tissue factor, myeloperoxidase, and CCL18 production. Compared to M-CSF-driven macrophages, GM-CSF-driven macrophages have high levels of genes that promote macrophage emigration (CCR7) and reverse cholesterol transport, such as peroxisome proliferator activated receptor gamma (PPARγ), liver X receptor alpha (LXRα), and ATP-binding cassette, subfamily G, member 1 (ABCG1). Both macrophage-differentiated cells accumulate lipids, but they are differentially distributed in vascular lesions.

IFNγ-producing Th1 cells dominate during atherogenesis, and atherosclerotic lesions are characterized by macrophages with a classic M1 phenotype producing inflammatory cytokines (Figure 2). T cells in atherosclerotic lesions respond to oxidized LDL (oxLDL) and heat shock proteins, and activation of the T-cell-specific T-box transcription factor T-bet, a key transcription factor in Th1 development, results in loss of class II MHC expression in macrophages in atherosclerotic lesions and protects from atherosclerosis. Other than T cell products, forces driving macrophage activation and polarization in atheromas include oxLDL, microbial products, and necrotic tissue-derived components. Genetic evidence also implies TLR in the pathogenesis of atherosclerosis, possibly because of recognition of microbial ligands, products released from necrotic cells (eg, the high mobility group box 1 protein), and extracellular matrix components. Conversely, Th2 responses have been suggested to be protective, in a delicate balance affecting macrophage activation. Other factors acting on the protective side of this balance also affect macrophage polarization. PPARγ agonists have been shown to directly induce M2-like differentiation in vitro and in vivo. OxLDL uptake by macrophages results in increased concentrations of oxysterols, which are ligands of LXR, and LXR activation has emerged as a key component of the macrophage balance in that in macrophages promotes cholesterol efflux, inhibits NF-kB signal-

Concluding Remarks

Mononuclear phagocytes are diverse and can express a continuum of differentiation/activation programs in response to environmental signals. As summarized in Figure 2, in response to tissue signals, macrophages in plaques can express pro- and antiatherogenic programs. Available information is compatible with a "macrophage balance" view of mononuclear phagocytes in atherosclerosis (Figure 2). Tipping the balance toward pro- or antiatherogenic functions affects pathogenesis, evolution, and complications.

The recognition of diversity and polarization of mononuclear phagocytes in atherosclerosis raises a number of questions. For instance, the relationship between monocyte subsets and plaque-associated macrophages heterogeneity remains to be defined at different stages in the natural history of atheroma. The actual characterization of plaque-associated macrophages remains fragmentary, and "omics" approaches will be required to define heterogeneity and polarization as done for mononuclear phagocytes in other contexts. Answering these open questions may pave the way to better exploitation of macrophages and their products as therapeutic and diagnostic targets.
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