Macrophage heterogeneity and tissue lipids

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Macrophages are present as resident cells in adipose tissue, and blood monocytes are recruited in increased numbers to sites of lipid accumulation in atherosclerosis, a modified form of inflammation in the arterial wall. Recent findings reported by 3 separate groups in this issue of the JCI provide evidence for distinct monocyte subsets, differential chemokine receptor usage, and phenotypic modulation of macrophages in murine models of genetic and high-fat diet–induced disease (see the related articles beginning on pages 175, 185, and 195). These studies raise prospects for selective therapeutic targets to ameliorate macrophage hyperinflammatory responses, while sparing host defense and repair mechanisms.

Monocytes and mature macrophages are prominent in the host response to lipid accumulation in major arteries, the development of atherosclerotic plaques, and their complications (1). Less established are the details of their colocalization and possible metabolic interactions with adipocytes in body fat stores (2). New studies reported in this issue of the JCI by Swirski et al. (3) demonstrate that a monocyte subset that expresses high levels of a marker antigen, Ly-6C, dominates hypercholesterolemia-associated monocyteosis and gives rise to macrophages in atherosoma. Also in this issue, Tacke et al. (4) report that monocyte subsets differentially employ the chemokine receptors C-C motif chemokine receptor 2 (CCR2), CCR5, and C-X-C motif chemokine receptor 1 (CX3CR1, also known as the fractalkine receptor) to enter atherosclerotic plaques. They also exploited an mAb, Gr-1, directed against Ly-6 family antigens to distinguish monocyte subsets and used CD11c, a β2 integrin expressed by myeloid DCs and selected tis-

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Nonstandard abbreviations used: CCR2, C-C motif chemokine receptor 2; CX3CR1, C-X-C motif chemokine receptor 1.

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Lipid-laden macrophages, known as foam cells, play a central role in the pathogenesis and repair of atherosclerotic plaques, the product of local cellular inflammatory reactions in arterial walls (6). Macrophages have also been implicated in plaque vulnerability to rupture and thrombus formation. Their recruitment depends on circulating monocyte levels and is greatly reduced in M-CSF–deficient osteopetrotic mice, which display a variable deficiency of tissue macrophage populations in the absence of this important growth and survival factor (7). Recently, interest has grown in the observations by several groups that circulatory monocytes express distinctive chemokine receptors, particularly with respect to CX3CR1 and CCR2 (also known as receptor for monocyte chemoattractant protein 1 [MCP-1]), as well as antigen markers such as Gr-1; CD14, a receptor for LPS-binding protein; and CD16, an Fc receptor (8, 9). Figure 1 summarizes the present consensus that different monocyte subsets give rise to constitutively present, resident macrophages and to tissue macrophages that have been recruited in increased numbers to local sites of infection and immunologic injury. There is evidence that this distinctive recruitment property of CD14⁺CD16⁻, which is not present in CD14⁺CD16⁺ macrophages, mediated by CX3CR1 lo CCR2–CD62L– (CX3CR1 lo CCR2–CD62L–), whereas the CX3CR1 hi subset may give rise to resident tissue cells.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Modulation of macrophage phenotype</th>
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<tbody>
<tr>
<td><strong>Category</strong></td>
<td><strong>Stimulus</strong></td>
</tr>
<tr>
<td>Innate activation</td>
<td>Microbial products, e.g., LPS, other TLR ligands</td>
</tr>
<tr>
<td>Classical activation</td>
<td>IFN-γ</td>
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<tr>
<td>Alternative activation</td>
<td>IL-4, IL-13</td>
</tr>
<tr>
<td>Innate and acquired deactivation</td>
<td>Apoptotic cells, IL-10, glucocorticoids, TGF-β, PGE₂</td>
</tr>
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For further information, see refs. 9, 10. MARCO, macrophage receptor collagenous; MHCII, MHC class II. Ym1, a chitinase-like protein, and FIZZ1, a resistin-like protein are markedly and selectively induced by IL-4 and IL-13, acting through a common receptor chain.
sources of resident tissue macrophages, which turn over more slowly, are less clear. The origins of nonclassical inflammatory macrophages elicited by metabolic stimuli, such as modified (lipid)proteins in atherosclerosis, diabetes mellitus, and Alzheimer disease, have not been hitherto characterized.

Once present in tissues, mature macrophages exhibit another level of heterogeneity. Microbial stimuli acting through a variety of TLRs and non-TLRs, such as dectin-1, induce a range of transcriptional and nontranscriptional effector responses (known as innate activation) (9, 10). The cytokine IFN-γ induces a signature of proinflammatory antimicrobial activities (known as classical activation) that are important in cell-mediated immunity to intracellular pathogens and are associated with Th1 lymphocyte responses. A distinctive, “alternative activation” profile is associated with Th2-type polarized responses. Characteristic features of IL-4- and IL-13–induced (M2) phenotypes include enhanced MHC class II expression, as in IFN-γ-induced M1 macrophages, but distinctive upregulation of endocytic lectin-like receptors (e.g., the mannose receptor) and of arginase, rather than inducible NOS2 (Table 1). Alternative activation is strongly associated with extracellular parasitic infections, allergy, humoral immunity, and fibrosis. Macrophage deactivation is equally complex and characterized by distinctive, partially overlapping signatures of gene expression induced by IL-10, glucocorticoids, TGF-β, and other regulatory mediators.

A further level of heterogeneity to consider relates to the origin, differentiation, and activation of myeloid DCs (11). These cells arise from common progenitors and share many phenotypic characteristics with macrophages, namely phagocytosis, receptor expression, and cytokine secretion, but acquire a unique antigen-presenting capacity for naive T lymphocytes as they undergo a complex process of maturation. They become actively migratory cells, responding to selected chemokines as they deliver antigen to draining lymph nodes. However, DCs display considerable heterogeneity in lymphoid and nonlymphoid tissues, which has been difficult to establish in situ, rather than in the simpler in vitro model systems that are widely employed, and they express many apparently selective markers, such as CD11c, also expressed by tissue macrophages.

Macrophage-lipid interactions

The distinction drawn between innate immunity and lipid homeostasis is artificial and is breaking down. For example, TNF-α, the prototypical proinflammatory cytokine that is critical for host defense, has direct effects on metabolic pathways including lipid metabolism, as originally described by Cerami and colleagues and termed cachectin (12). The scavenger receptors expressed mainly by macrophages and endothelia are implicated in innate recognition of microorganisms, as well as in foam cell formation and uptake of modified lipoproteins and of apoptotic cells (13). Circulating lipoproteins, their cellular receptors and lipid transfer proteins, play an important role in LPS-induced responses, TLR stimulation, activation of intracellular signaling pathways, especially of NF-κB, and activation of gene expression. The roles of nuclear receptors such as PPARγ in inflammation and of lipid metabolites in the initiation and resolution of inflammation fall outside the scope of this commentary. Recently, interest has grown in the possible interactions between macrophages and adipocytes, involving cytokines such as leptin, adiponectin, and effects of these and other paracrine mediators on insulin responses and signaling.

New experimental findings

The study in this issue by Swirski et al. (3) utilized apoE-KO (apoE−/−) mice on a C57BL/6 background, fed a high-fat “Western” diet over a period of many weeks to induce an increase in atherosclerotic lesions throughout the aorta. The authors characterized monocyte numbers mainly in blood and spleen, as well as in bone marrow, and distinguished their phenotype from that of more mature...
splenic macrophages. (In rodents, the spleen is also a major hemopoietic organ). Hypercholesterolemia was associated with gradual monocytosis, with a doubling time of approximately 1 month, due to a selective increase in the Ly-6C–positive subset. Monocytosis was ascribed to increased survival and continued proliferation, in part due to M-CSF, as well as impaired conversion of Ly-6C<sup>hi</sup> to Ly-6C<sup>lo</sup> cells; monocytosis decreased upon statin-induced reduction of cholesterol. Clodronate-laden liposomes were used to deplete monocytes and macrophages in vivo. The recovery of Ly-6C<sup>hi</sup> monocyte numbers, presumably from Ly-6C<sup>hi</sup> precursors, occurred over 5 days in animals on a control chow diet but was impaired in the hypercholesterolemic mice. Monocytes recovered from enzyme-digested aortas and analyzed by FACS showed preferential accumulation of Ly-6C<sup>hi</sup> cells, especially in more severe lesions, localized by immunocytochemistry. Experiments confirmed that Ly-6C<sup>hi</sup> monocytes adhered preferentially to TNF-α-activated endothelium under laminar flow conditions in vitro, irrespective of diet or apoE deficiency. Adoptive transfer of CD45.2 monocytes to congenic CD45.1 mice demonstrated recruitment of Ly-6C<sup>hi</sup> monocytes to atherosclerotic aortas followed by local, rapid differentiation into macrophages; this was confirmed by labeling of splenic monocytes with <sup>111</sup>Inoxine and localized by autoradiography. Transfer studies with CCR2-KO mice confirmed that CCR2 was required.

It is not clear whether the glycosylphosphatidylinositol-linked (GPI-linked) protein Ly-6C is a marker or functional receptor/ligand for an adhesion on endothelial cells and whether it or its human equivalent provides a potential target for inhibition of monocytosis and associated atherosclerosis. Future studies should establish whether CD14<sup>+</sup>/CD16<sup>+</sup> monocytosis is an etiological factor in human disease.

Tacke et al. (4) used a similar apoE<sup>−/−</sup>–plus-diet model and also observed monocytosis with increased frequency of Gr-1<sup>hi</sup> monocytes (their Gr-1 mAb reacts with an epitope on both Ly-6C and Ly-6G, expressed on granulocytes as well). They employed an i.v. labeling method to mark blood monocyte subsets by phagocytosis of 0.5–µm latex particles. A portion of Gr-1<sup>lo</sup> monocytes in apoE<sup>−/−</sup> mice can be specifically and efficiently labeled for more than 5 days; Gr-1<sup>hi</sup> monocytes were labeled by a modification of this procedure, wherein monocytes are transiently depleted by clodronate-loaded liposomes prior to latex injection. Latex is then transferred to bone marrow, and labeled Gr-1<sup>hi</sup> monocytes appear in the circulation after 2 days, remaining Gr-1<sup>hi</sup> for 4 days before converting into Gr-1<sup>lo</sup> cells by 7 days. Normalization of numbers and cell clearance made it possible to compare the tracking of cells of each subset into lesions; the labeling procedure itself did not affect cellular recruitment. Though both subsets entered plaques, initial entry of Gr-1<sup>hi</sup> monocytes was approximately 20-fold greater than that of Gr-1<sup>lo</sup> cells, after normalization for frequency of labeled cells. A fat-rich diet increased rates of recruitment of both subsets. Another observation was that a subpopulation of cells expressing CD68, a pan-macrophage/DC marker, was CD11c<sup>+</sup> and that Gr-1<sup>hi</sup> monocytes were particularly predisposed to become CD11c<sup>+</sup> cells.

Further studies examined the role of chemokine receptors in recruitment. Surgical transfer of donor atherosclerotic aortic arches was used to take advantage of different knockout strains lacking CCR2 and CX3CR1. CCR2 was shown to be required (it also plays a role in monocyte exit from the bone marrow). Unexpectedly, Gr-1<sup>hi</sup> monocyte entry into plaques also depended on CX3CR1, and a potential ligand for this receptor, CX3CL1, was detected on endothelium overlying plaques. Xenogeneic in vitro experiments with HUVECs confirmed that Gr-1<sup>hi</sup> mouse monocytes required both CCR2 and CX3CR1 for in vitro migration. Finally, mAb depletion studies were used to implicate CCR5, in part, in monocyte migration to plaques.

The overall conclusion was that multiple chemokine receptors operate to control the migration of monocyte populations. This study identified a role for CX3CR1, not previously found in acute inflammatory models, giving rise to speculation that CX3CR1 may be selectively involved in atherosclerotic plaque recruitment, whereas CCR2 is more widely required in response to different types of inflammatory stimuli (4). This may account for the observation that polymorphism in human CX3CR1 leads to relative protection from cardiovascular disease (14).

In the third article, Lumeng et al. (5) restricted their studies to analysis of the macrophage phenotype in lean and adipose tissue of wild-type apoE<sup>−/−</sup> C57BL/6 mice. Obesity and insulin resistance were induced in male mice, which were fed a high-fat diet consisting of 45% of calories derived from fat, starting at 8 weeks of age, for up to 20 weeks. Control mice were fed a standard diet consisting of 4.5% of calories derived from fat. Epididymal fat pads were extracted and the stromal vascular fraction isolated after removal of adipocytes by flotation. Digested preparations were analyzed by FACS, using F4/80 and CD11b to identify macrophages, which could be isolated by magnetic immunofluorescence-positive selection.

Adipose tissue from lean mice contained macrophages that expressed several markers of alternative activation, including IL-10 as well as enhanced levels of Ym1, arginase, and mannose receptor (5). By contrast, macrophages isolated from adipose tissue of obese mice exhibited a proinflammatory phenotype, overexpressing IL-6, NO52, and CCR2; this chemokine receptor was required for diet-induced but not constitutive cell recruitment, as shown with CCR2-KO mice. In addition, some of the recruited macrophages expressed the CD11c antigen, although no further attempt was made in the present study to examine other possible DC markers. It was shown that IL-10, which was overexpressed in lean mice, protected adipocytes from TNF-α-induced insulin resistance in vitro. Leptin-deficient ob/ob mice showed a similar increase in F4/80<sup>+</sup>CD11c<sup>+</sup>CD11b<sup>+</sup> cells.

These studies are consistent with a role for CCR2 in the diet-induced recruitment of monocytes that acquired a proinflammatory phenotype, gradually replacing a population with an alternative activation phenotype consisting of CCR2-independent macrophages that are slow to turn over. It is not clear which local stimuli might be responsible for either type of polarization, although lipid-derived metabolites are likely mediators of the proinflammatory phenotype. The nature and role of the CD11c<sup>+</sup> cells also need to be investigated further. One limitation of these results is that ex vivo studies are not a precise reflection of cellular heterogeneity in vivo, although a limited immunocytochemical analysis was performed. This must be done with great care, since, in my own experience, adipose tissue is prone to stain nonspecifically with a range of mAbs. The potentially important surface and metabolic interac-
tions among adipocytes, monocytes, macrophages, endothelial, and other stromal cells also deserve further investigation.

**Conclusion**

These intriguing studies have yielded further insights into the complexity of monocytes and tissue macrophages and the role of different chemokine receptors in constitutive and lipid-induced recruitment (summarized in Figure 2). It is clear that enhanced recruitment induced by metabolic and infectious inflammatory stimuli differs in important aspects that need better definition, in view of their importance in modified, often chronic nature of monocyte/macrophage heterogeneity. Further, the role of different chemokine receptors in constitutive and lipid-induced recruitments among adipocytes, monocytes, and tissue macrophages and the importance in modified, often chronic nature of monocyte/macrophage heterogeneity is also difficult to assess due to the considerable plasticity of macrophages, their repertoire of receptors and extensive biosynthetic capacity. Furthermore, it is important not to overinterpret the expression of a single marker, such as CD11c, as indicative of DC differentiation. A panel of markers should be investigated, in situ if possible, and even then in relation to the complexity of macrophage differentiation and modulation in different tissue microenvironments. As to possible therapeutic exploitation, further investigation is required before different subsets of cells can be selectively, efficiently, and safely targeted. The availability of well-characterized experimental mouse models, as described in the articles under discussion, makes this an attractive goal. Their possible relevance to human disease poses further challenges for pathophysiological, translational research.

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