Macrofage Function and Its Impact on Atherosclerotic Lesion Composition, Progression, and Stability

The Good, the Bad, and the Ugly

Jeffrey G. Dickhout, Sana Basseri, Richard C. Austin

The role of the macrophage is of fundamental interest in understanding atherosclerotic lesion development and thrombogenicity. After differentiation from blood peripheral monocytes, intimal macrophages incorporate modified lipoproteins through the scavenger receptor pathway. This transforms the macrophage into a lipid-rich foam cell which is a hallmark feature of atherosclerosis and leads to lesion expansion. Macrophage activation results in the excretion of proinflammatory and cytotoxic substances, including peroxynitrite, an early inducer of atherosclerosis through the endoplasmic reticulum (ER) stress pathway. Further, the accumulation of free cholesterol or uptake of oxidized LDL induces macrophage apoptosis. Cytokine release from macrophages augments the inflammatory response and increases lesion size. Cytotoxic substances, including peroxynitrite and tumor necrosis factor (TNF)-α, released by the macrophage results in cell death of lesion-resident endothelial and smooth muscle cells, thereby disrupting vessel structure. Macrophages can also alter the extracellular matrix of the vessel by releasing matrix metalloproteinases (MMPs), thereby leading to lesion breakdown and predisposing the lesion to fissure or rupture. These aspects of the macrophage in atherosclerotic lesion biology drive the progression of the disease and lead to the decomposition of the arterial wall into atheroma, the ugly gruel remaining after foam cell decomposition.

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The macrophage may also have good effects within the blood vessel wall. Macrophages remove debris through the process of phagocytosis. One aspect of macrophage phagocytosis, namely efferocytosis, is a process that prevents secondary necrosis and inflammation by removing apoptotic bodies before their decomposition. Efferocytosis has been shown to be mediated by 3 tyrosine kinase receptors, Axl, Tyro3, and Mer receptor tyrosine kinase (Mertk) in cells of hematopoietic lineage. Mertk is the primary mediator of efferocytosis in macrophages. Despite the presence of all 3 receptors on macrophages, Mertk has a unique and nonredundant role in apoptotic clearance. Seitz and colleagues have shown that even in the absence of Axl and Tyro3, Mertk has the full potential for clearing apoptotic cells in the thymus and retina of mice. Interestingly, the ability of Mertk knockout macrophages to clear nonapoptotic cells and particles is not affected, suggesting that Mertk is specifically involved in efferocytosis.

Apoptosis is a well-characterized feature of atherosclerotic lesions. It has been suggested that apoptosis increases the risk of lesion rupture by decreasing the number of viable smooth muscle cells necessary for collagen production and by compromising the structural integrity of the fibrous cap after release of MMPs from apoptotic macrophages. Plaque thrombogenicity is also enhanced because lesion-resident cells undergoing apoptosis express active tissue factor on the cell surface, the major physiological initiator of the coagulation cascade. Thus, clearance of these apoptotic particles through efferocytosis would reduce plaque thrombogenicity.

To determine whether the role of the macrophage in the development of the lesion is good or bad, studies have removed the macrophage from the lesion through monocyte colony stimulating factor (M-CSF) mutation and found reduced lesion size, consistent with the idea that macrophages augment lesion progression. Further, the removal of specific proapoptotic factors such as p53, Bax, or Rb from the macrophages of atherogenic-prone mice has been found to accelerate lesion development, suggesting that macrophage apoptosis suppresses atherosclerotic lesion size. Detection of macrophage apoptosis seems to be a rare event in early atherosclerotic lesions from Apoe−/− mice, but is observed in and around the necrotic core of advanced lesions.

Studies on the good role of macrophages have also been conducted. In 2006, Li and colleagues demonstrated that the presence of the Mer receptor enhances efferocytosis of free cholesterol-laden macrophages in vitro. Phagocytes from Merk-deficient mice were incubated with macrophages that were rendered apoptotic through free-cholesterol loading. Compared to wild-type macrophages, Merk-deficient macrophages were defective in the uptake of apoptotic macrophages. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Thorp and colleagues provide novel in vivo evidence for the role of Merk in necrotic core formation in advanced atherosclerotic lesions. Kinase-defective Merk (MerkKDa) mice were crossed onto an Apoe−/− background to generate MerkKDa/Apoe−/− mice. These mice, as well as control Apoe−/− mice, were fed a Western-type diet, and aortic lesion development, efferocytosis, and plaque necrosis were assessed at 10 and 16 weeks. The authors showed that the MerkKDa/Apoe−/− mice had more terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive apoptotic cells in the plaques and importantly that most
of these cells were not associated with phagocytic macrophages. This in vivo data confirmed their previous in vitro findings regarding the compromised efferocytosis of Mer-defective macrophages.4 However, Thorp and colleagues for the first time demonstrated that the advanced atherosclerotic plaques of MertkkDa/Apoe−/−H11002/H11002 mice were more necrotic, linking the compromised efferocytosis mediated by defective Mertk to the necrotic core expansion in advanced atherosclerotic lesions. This expansion of the necrotic core did not result in a significant increase in MertkkDa/Apoe−/−H11002/H11002 mouse lesion size. However, it is unknown whether a difference may have been observed if the study had been extended beyond 16 weeks. The MertkDa mutation did not affect body weight, plasma cholesterol, lipoprotein profile, or TNFα mRNA levels in the aortae. The authors provide strong support for the hypothesis that the Mer tyrosine kinase receptors are important for effective efferocytosis of apoptotic cells in vivo and that compromised efferocytosis attributable to a Mertk mutation leads to enhanced plaque necrosis in advanced atheromata of Apoe−/− mice.

Also reported in this issue of ATVB, Ait-Oufella and colleagues26 have shown that Mertk-deficient bone marrow transplanted into bone marrow–irradiated ldlr−/− mice leads to the accumulation of apoptotic bodies within atherosclerotic lesions. These findings are in agreement with Thorp and colleagues demonstrating in another murine model of atherosclerosis the negative effect of Mertk deficiency on macrophage efferocytosis. Mertk was found to be expressed in the macrophages of human atherosclerotic lesions and its removal in female ldlr−/−Mertk−/− mice led to an increase in TUNEL staining, the expansion of necrotic core size at 8 weeks on a high-fat diet, and an increase in lesion size at 8 and 15 weeks on a high-fat diet. Ait-Oufella et al suggest this increase in lesion size is related to increased inflammation in ldlr−/−/Mertk−/− mice as indicated by increased macrophage and T cell accumulation in these expanded lesions. Further, lesion progression in ldlr−/− mice from 8 to 15 weeks led to an increase in lesion collagen content, a cellular protein thought to preserve lesion structural integrity. In

Figure. Schematic diagram of the various roles played by the macrophage (MΦ) within the atherosclerotic lesion. Early lesion development is augmented by the macrophage through the unregulated uptake of oxidized LDL (oxLDL) via the scavenger receptor pathway, leading to foam cell formation. Macrophage activation leads to the secretion of proinflammatory cytokines and cytotoxic substances such as TNF-α and peroxynitrite (ONOO−), respectively. The accumulation of free cholesterol or oxidatively modified cholesterol loading of macrophages, including 7-ketocholesterol, also produces cytotoxicity. Apoptosis generated by these processes contributes to necrotic core formation. However, the macrophage also counteracts this process of lesion expansion through Merk-mediated efferocytosis of apoptotic bodies (AB). This can prevent their secondary necrosis and release of lipids and MMPs which may accelerate the breakdown of collagen that maintains the structural integrity of the vessel wall.
contrast, lesion expansion in \( ldlr^{−/−}/Merk^{−/−} \) mice was not accompanied by this increase in collagen-based sclerosis. Ait-Oufella et al found increased proinflammatory cytokines TNF-α, interleukin (IL)-12, and IFN-γ and decreased antiinflammatory cytokine IL-10 in the splenocytes and spleen-derived T cells of \( ldlr^{−/−}/Merk^{−/−} \) mice. Further, it has been shown that mice lacking the related tyrosine receptor kinases, Tyro 3, Axl, and Mer (TAM) develop autoimmunity.27 Axl and Tyro3, the other 2 tyrosine kinase receptors present on the macrophage cell surface, result in rapid phosphorylation of Merkt on binding of apoptotic cells to macrophages.9 Double or triple \( axl/merkt/tyro3 \) knockout mice have shed light on the possibility of interactions between the TAM receptor family.9,27 Because all 3 receptors are present on macrophages and they share similar ligands with various binding affinities,8,23 it is possible that there may be cooperative signaling among this receptor family. This requires further investigation in the context of atherosclerotic lesion development if Merkt is to become a therapeutic target.

In summary, Throp et al and Ait-Oufella et al have demonstrated the good role of macrophages in the atherosclerotic lesion through the process of efferocytosis using Merkt knockout mice. Considering the results of earlier studies demonstrating the bad role of macrophages in early lesion development, we begin to develop a clearer model of the impact of macrophage function on lesion composition and stability. Our model illustrated in the Figure shows how the macrophage augments lesion development at the early stage of disease through the unregulated uptake of oxidized LDL and foam cell formation as well as through the secretion of proinflammatory cytokines such as TNF-α and cytotoxic substances such as peroxynitrite. Apoptosis generated by these processes leads to necrotic core formation. However, the macrophage also counts this process of lesion expansion through Merkt-mediated efferocytosis of apoptotic bodies. This can prevent their secondary necrosis and release of lipids and MMPs which may accelerate the breakdown of collagen that maintains structural integrity of the vessel wall. These studies published in this issue of \( ATVB \) provide compelling evidence that macrophage efferocytosis may prevent the ugly prospect of lesion rupture and thrombosis.

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**Disclosures**

None.

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