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Atherosclerosis and immunity: A perspective☆

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ABSTRACT

Atherosclerosis is an inflammatory and multifaceted disorder resulting from the accumulation of lipid droplets and several types of immune cells, including macrophages, T and B lymphocytes in the arterial walls. A wide variety of macrophage subtypes with different functions is implicated in the development and progression of atherosclerotic lesions. The prevalence of specific macrophage subtypes, which is influenced by cytokines, mediators, and substances composing atherosclerotic lesions, has been suggested to be an appropriate indicator of transition from a stable to an unstable plaque phenotype. Thus, a better understanding of the mechanisms underlying the differentiation of macrophage subpopulations in relation to the plaque phenotype would help to develop novel approaches aiming at slowing-down the progression of atherosclerotic disease by modulating the polarization of these cells. In addition, many arms of the adaptative immune system, which are regulated by different subtypes of T and B lymphocytes, are involved in atherosclerosis progression and there is an increasing effort to identify immune-modulating therapies targeting either T or B cells with a potential anti-atherosclerotic impact. This paper summarizes the pathophysiology of atherosclerotic disease as it relates to the contribution from the immune system, reviewing the crucial role of macrophages, T and B lymphocytes.

Atherosclerosis: a consecutive inflammation

Atherosclerotic cardiovascular disease remains among the major causes of morbidity and mortality worldwide [1]. Atherosclerosis is a complicated and progressive disorder leading to an asymmetric thickness of the most inner layer of arteries: the intima. This silent progressive disease, affecting large and medium-sized arteries, does not manifest its symptoms until the narrowing of arterial lumen due to atherosclerotic lesions become severe, blocking blood flow and causing ischemic impairment of downstream tissues, or until a sudden rupture of atherosclerotic plaques triggers thrombosis inside arteries [2,3].

The pathogenesis of atherosclerosis is characterized by abnormal lipid deposition within the intima and aberrant inflammatory responses. Previous studies have shown that vascular wall cells, including smooth muscle cells and endothelial cells, can produce mediators of inflammation and cytokines, setting the stage for the initiation of atherosclerotic process [4,5]. In fact, abnormal lipid deposition stimulates vascular wall cells to produce cytokines [6,7]. The generation of cytokines via local vascular wall cells is a stimulus for mobilizing professional inflammatory cells to the atherosclerotic lesions. The combination of these factors may contribute to endothelial injury and impaired endothelial repair [8], early appearance of reversible arterial dysfunction [9] and to an increased risk of cardiovascular disease events [10]. Conversely, controlling both dyslipidemia and low-grade systemic inflammation may reverse early arterial dysfunction [11] and cardiovascular disease risk [12].

Different molecular steps are crucial to the initiation of atherosclerotic process: activation of endothelial cells leading to the expression of endothelial-selectin (E-selectin) and intercellular adhesion molecule-1 (ICAM-1), adhesion/infiltration of monocytes

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under the stimulus of monocyte chemoattractant protein-1 (MCP-1), monocyte maturation into macrophages expressing scavenger receptors [13], CD36-mediated internalization of oxidized LDL, cholesterol efflux regulated by ATP-binding cassette transporter A1 (ABCA1) controlling the addition of cholesterol esters (CE) in macrophages[14] and the storage of CE in macrophages leading to the formation of foam cells.

These steps result in the initial formation of fatty streaks, which are early flat atherosclerotic lesions characterized by the accumulation of foam cells containing lipid droplets beneath the endothelium, at locations where there exists branching of the arteries or areas with turbulent flow [15,16].

Subsequently, due to progressive accumulation of lipids and inflammatory cells, fatty streaks tend to progress to advanced lesions, namely atherosclerotic plaques, containing lipid droplets, foam cells, macrophages and lymphocytes. These inflammatory cells, expressing and releasing different cytokines and mediators, have a crucial role in atherosclerosis progression [17–19].

Other factors such as growth of smooth muscle cells [20] and synthesis of collagens, matrix metalloproteinases (MMPs), fibronectin, and elastin are also accountable for plaque expansion [21–23].

Specifically, macrophages expressing pro-inflammatory cytokines can promote a high-level expression of MMPs contributing to plaque instability [24,25]. Moreover, within progressing atherosclerotic lesions, smooth muscle cells, macrophages, and foam cells are subjected to apoptosis, contributing to the formation of a lipid core [26].

Immune system and atherosclerosis

Studies have suggested that both innate and adaptive immunity play a critical role in the advancement and expansion of atherosclerosis [27,28]. The innate response begins with the stimulation of monocytes/macrophages in the vessel wall and is followed by many adaptive responses, which are modulated by T and B cells [28,29]. Also, some effector cells such as mast cells and eosinophils may contribute to atherosclerotic disease [30]. It has been determined that mast cell-derived IFNγ and IL-6 have a pro-inflammatory role with regard to lesion development. In addition, also eosinophils and activated IgE can promote atherosclerosis progression [31].

In the 'early' atherosclerotic lesion, a large number of macrophages activated by different cytokines and mediators are detectable. These cells, by manipulating their recognition receptors and through lipoprotein internalization, become foam cells [32].

Within early atherosclerotic lesions, macrophages express and release different inflammatory cytokines, including TNF, which mediate the chemotaxis of B cells, T cells [33], and more macrophages into or near the lesion, fostering an inflammatory environment. As a result of a constant influx of inflammatory cells and impaired clearance of dead cells (efferocytosis), atherosclerotic plaque develops [34–36].

Although macrophages constitute the greatest portion of inflammatory cells in atherosclerotic plaque, also T and B lymphocytes (cells of adaptive immunity) have a crucial role in the developing plaque. Despite their minority. lymphocytes, especially T cells, have a very significant function in the modulation of immune responses during atherosclerosis development and progression [37,38]. All subsets of T cells ($CD4^+$, $CD8^+$, NK and follicular helper,T cells) have been recognized in human atherosclerotic plaque [27,39–41].

Naïve $CD4^+$ T cells can differentiate into various subclasses of T helper (T_h) cells including T_{h1}, T_{h2}, T_{h17}, and T_{reg} cells. The variable expression of cytokines in the biological milieu of atherosclerotic plaques contribute to the polarization of Th cells into the various subtypes [42] (Fig. 1). T_{h1} lymphocytes are more abundant than T_{h2} lymphocytes in atherosclerotic plaques [43,44]. T_{h1} differentiation occurs as a result of the activation of T box transcription factor expressed in T cells (T-bet) under different stimuli, including IL-12 and IL-18 cytokines, which are produced by activated macrophages [45]. It has been shown that IFNγ, a cytokine expressed by T_{h1} cells, may promote the development and progression of atherosclerotic plaques [46]. Moreover, it has been shown that both IL-12 and IL-18, by stimulating IFNγ production, may promote atherosclerosis progression [47].

T_{h2} lymphocytes produce IL-5, IL-13 and IL-4, which neutralizes the effects of IFNγ. It has been demonstrated that T_{h2} cells have an anti-atherosclerotic role [48]. Specifically, studies have shown that IL-4 can down-regulate the expression of CD36 [49], vascular cell adhesion molecule-1 (VCAM-1) [50], monocyte chemoattractant protein-1 [51], matrix metalloproteinase 1 [52], and class A scavenger receptor on macrophages [53], thus inhibiting the development of atherosclerosis. In addition, it has been reported that IL-5 may promote the production of natural IgM antibodies against oxLDL by B lymphocytes [54].

The expression of IL-6, TGF-β, IL-17A, IL-17F, IL-22, IL-23, STAT3, and retinoic acid-related orphan receptor γT (ROR γT) [55] skew T_h lymphocytes toward a T_h17 phenotype. The six different members of the IL-17 family (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F) activate ERK1/2, NF-κB, CCAAT/enhancer-binding protein β (C/EBPβ), and C/EBPδ signaling pathways in various target cells, such as endothelial cells, smooth muscle cells, macrophages and Th1 cells [56], leading to the production of pro-inflammatory cytokines such as IFN-γ [57], IL-1β, and TNF [58], and granulocyte colony-stimulating factor [59].

The differentiation of T_h cells into T_{reg} cells is regulated by IL-10, TGF-β [60], and by TLR-activated $CD_{11c} + CD_{103} +$ dendritic cells (DCs) [61]. T_{reg} cells are considered as negative directors of immune effector cells. Different phenotypes of T_{reg} cells have various critical roles in atherosclerosis.

Natural T_{reg} cells (nT_{reg} cells) express CD25 (IL-2R), CD4, and FOXP3. Natural T_{reg} cell possess TCRs with high affinity for self-antigen; they are able to negatively control immune responses through the expression of IL-10 and TGF-β cytokines [42].

Inducible T_{reg} cells (iT_{reg} cells) comprise type 1 regulatory T cells (Tr1 cells) and T_h3 cells. Inducible T_{reg} cells are $CD4^+CD25^+$ and do not need FOXP3 expression for their functionality. They originate from effector T cells, just after exposure to antigens [62]. Typically, Tr1 cells secrete IL-10, while T_h3 cells produce TGF-β. Both iT_{reg} and nT_{reg} cells can restrict autoimmunity by stimulating the expression of the inhibitory receptor CTLA-4, by competing with other T cell subtypes for the set of antigens and MHC class II (MHC II) on antigen-presenting cells (APCs), by direct cytotoxic and/or inhibitory effects on other effector cells, and by down-regulating the expression of co-stimulatory molecules (CD80/CD86) [42].The expression of IL-10 by T_{reg} cell is atheroprotective and slows down the progression of atherosclerotic lesions [40].

Natural killer cells (NKT) represent a particular subclass of T cells. The invariant NKT (iNKT) cells with a defined TCR collection, once stimulated, can release a significant amount of anti-inflammatory cytokines, such as IL-10, IL-4, and IL-13, along with pro-inflammatory cytokines, including IFN-γ [63]. Invariant NKT cells are considered auto-regulatory cells with the ability to promote tolerance in cooperating with T_{reg} cells [64]. Invariant NKT cells have been detected in human atherosclerotic plaques from abdominal aorta [65] and carotid arteries [66]. Further investigation is necessary to discover the role of iNKT cells in atherosclerosis.

A subclass of $CD4^+$ T cells is represented by follicular helper T cells, which are necessary for the constitution of germinal

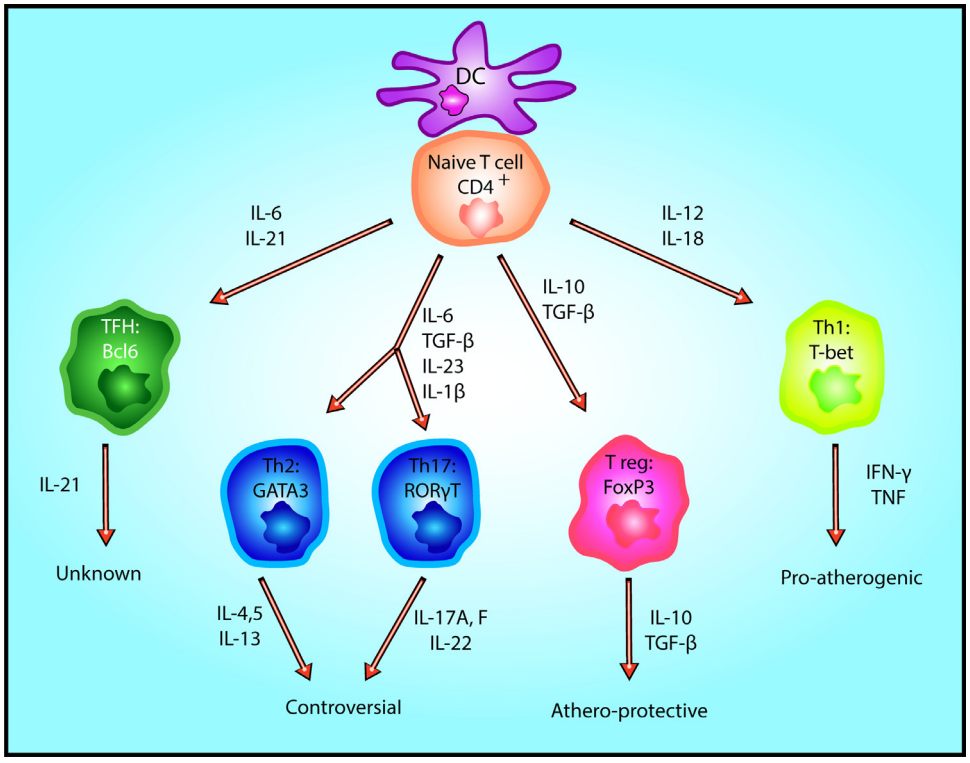

Fig. 1. Different subtypes of T cell in atherosclerosis: Pro-atherogenic: Th1. The expression of IL-12 and IL-18 results in Th1 skewing. IL-12 activates the transcription factor, T-bet, which leads to production of pro-inflammatory cytokines such as IFN-γ and TNF. Atheroprotective: Treg. The presence of IL-10 and TGF-β are necessary for the function of Treg. Treg and some iTreg display the transcription factor FOXP3 and can generate TGF-β and/or IL-10. Controversial: Th17 and Th2. A complex of some cytokines such as IL-23, TGF-β, IL-6, and IL-1β are thought to be Th17 skewing. Th17 expresses the transcription factor RORγT, and generates IL-22 and IL-17A/F. Th2 appears in the process as a result of the impact of IL-4, whereas, in a positive feedback loop, IL-4 influences the production of the transcription factor GATA3. This sequence then results in excess IL-4 and the inhibition of IFN-γ. Unknown: Follicular helper T cell. There are CD4+ T cells in the B cell follicles of secondary lymphoid organs with the potential to release IL-21 and the expression of transcription factor BCL6.

centers and the generation of antibodies by B cells [67]. Their role in atherosclerosis has not been explored. Furthermore, the role of $CD8^+$ T cells in atherosclerosis has not been clarified to date.

B lymphocytes can exert both a protective and a pathogenic role in atherosclerotic disease. Two main subsets of B cells have been identified in human atherosclerotic arteries: B1 and B2 cells [68]. B1 cells derive from fetal hematopoietic stem cells, have a long half-life, are maintained in the periphery by self-renewal within the spleen and produce natural antibodies independent from Th signals. B1 cells are divided into B1a cells, expressing CD5, and B1b cells. B1a cells may produce natural IgM antibodies against oxLDL and antigenic determinants on the surface of apoptotic cells, inhibiting oxLDL uptake and the formation of foam cells and promoting apoptotic cell clearance within atherosclerotic lesions. Overall, available experimental data suggest that B1a cells exert an atheroprotective action [69–71]. By contrast, the role of B1b cells in atherosclerosis needs to be clarified. B2 cells comprise follicular and marginal zone B cells. In contrast to B1 cells, B2 cells have a short half-life and are continuously renewed by bone marrow hematopoietic stem cells. In addition, B2 cells produce immunoglobulins in response to Th signals and their survival is dependent on B-cell activating factor receptor (BAFFR) signaling. Overall, B2 cells seem to exert a pro-atherogenic action [72–74].

From a therapeutic perspective, immune modulation of T ad B cell-mediated responses represents an attractive anti-atherosclerotic therapeutic strategy.

To date, due to their crucial role in controlling immune homeostasis, Treg cells represent the main target of important research efforts focused on the field of immune-modulating therapies against atherosclerosis. Different experimental studies have investigated the potential anti-atherosclerotic impact of different therapies aimed at eliciting CD4+Treg cell responses (e.g., adoptive transfer of T reg cells, induction of polyclonal or antigen-specific Treg cells), showing promising results [75–77]. However, the clinical anti-atherosclerotic benefit of T cell-based therapies in clinical studies needs further investigation.

Furthermore, strategies aimed at expanding B1a cells and increasing natural IgM production might have beneficial anti-atherosclerotic effects and a potential atheroprotective action of putative B2 cell-depleting therapies may be hypothesized.

However, a better understanding of the pathogenic role of B cell responses is warranted before a significant development a B-cell targeting therapies against atherosclerosis will occur.

Macrophage polarization: a general view

Macrophages are classified into different subtypes (M1 and M2 subtypes) on the basis of their biomarker expression and biological function [78,79]. Monocytes can differentiate into M1 and M2 macrophages depending on whether they are exposed to granulocyte-macrophage colony stimulating factor (GM-CSF) or macrophage colony stimulating factor (M-CSF), respectively [80,81]. Macrophage differentiation into totally dynamic macrophages (M1) is usually stimulated through T helper 1 (T_h1) cytokines, such as tumor necrosis factor (TNF), IFNγ, or via lipopolysaccharide recognition. M1 macrophages express large amounts of IL-23 and

IL-12, small amounts of IL-10 [81], and excrete various pro-inflammatory cytokines, including IL-13, IL-6, and TNF [82].

Three subclasses of M2 macrophages have been identified: M2a, M2b and M2c. Macrophage polarization into M2a phenotype is activated by T_h2 cytokines like IL-13 and IL-14. Macrophage switch into M2b phenotype is activated by immune complexes together with lipopolysaccharide or IL-13. Macrophage differentiation into M2c macrophages is activated by transforming growth factor-β (TGF-β), IL10 and glucocorticoids [83]. M2 macrophages produce large amounts of anti-inflammatory cytokines, although an interesting exception exists with M2b macrophages, in that they express high levels of pro-inflammatory cytokines such as TNF, IL-6, and IL-1 [84].

M1 and M2 macrophages' role in homeostatic activities is different: M1 macrophages serve as host defense, M2a macrophages regulate wound healing, and M2b/c macrophages play a crucial role in immune regulation [85]. Functionally, M1 and M2 subtypes of macrophages cooperate in pathogen elimination during infections via the activation of NADPH oxidase and reactive oxygen species (ROS). However, the activation of M1 macrophages may be harmful promoting ROS-mediated tissue injury [86,87]. Instead, the counterbalanced activation of M2 macrophages may protect against such kind of tissue damage [88]. In fact, M2 macrophages can stimulate tissue repair and healing and scavenge dead cells and debris [89]. Moreover, they have profibrotic [90] and proangiogenic features.

Macrophage polarization and functions in atherosclerotic plaques

It is interesting that there is a connection between the cytokines that are expressed in the environment of atherosclerotic lesions and the phenotype of the responding macrophages, in that macrophages can switch from one phenotype to another [91,92]. Furthermore, it should be noted that plaque composition and macrophage polarization are influenced by each other. In fact, based on current research efforts, different subtypes of macrophages have been identified within different atherosclerotic plaques in relation with their heterogeneous composition and complicated milieu [93]. Beyond M1 and M2 subtypes, other macrophages resident in atherosclerotic lesions have been identified including the Mox phenotype, the M(Hb) and Mhem phenotypes and the M4 phenotype. Mox phenotype differentiation is stimulated by the exposure to oxidized phospholipids and by a high-level expression of heme oxygenase-1 (HO-1), through the activation of nuclear factor (erythroid–derived 2)–like 2 (NEF2L2) transcription factor [94]. Mox macrophages express some pro-inflammatory factors such as cyclooxygenase-2 and IL-1β, through a TLR-2-dependent pathway [95]. The M(Hb) and Mhem phenotypes [96] are resistant to lipid loading and stimulated by the exposure to hemoglobin-haptoglobin compounds and heme, respectively. The M4 phenotype is stimulated by the chemokine CXCL4 [97]. In addition, IL-17A-stimulated macrophages have been demonstrated as a new subclass of macrophages within atherosclerotic plaques [98] (Fig. 2).

In atherosclerotic plaques, macrophages adopt their phenotype under the influence of the degree of accumulated lipids and the production of specific mediators and immune factors [99,100]. Accumulation of oxidized lipoproteins induces the M1 pro-inflammatory phenotype by preventing the expression of the transcription factor Kruppel-like factor-2 [101,102]. Cholesterol crystals promote the secretion of pro-inflammatory cytokines by M1 macrophages through the activation of the caspase-1-activating NLRP3 inflammasome [99].

Additionally, cholesteryl esters (such as linoleate and 7-keto cholesteryl-9-carboxynonanoate) may induce the M1 phenotype by the activation of either TLR-4 or nuclear factor (NF-$\kappa\beta$) signaling pathways. In contrast, 9-oxononanoyl-cholesterol, the main product of cholesteryl ester oxidation, promotes the development of an anti-inflammatory macrophage phenotype through an increased secretion of TGF-β [103]. Furthermore, conjugated linoleic acid, by increasing IL-10 secretion, induces the anti-inflammatory M2 phenotype [104]

Within atherosclerotic plaques, the rupture of small vessels can result in the release of erythrocytes and iron-containing pigments, which may be phagocytosed by macrophages [105,106]. Iron loading is known to induce the expression and activation of oxysterol receptor LXR-α (also known as liver x receptor-α), which in turn regulates both iron recycling potency of macrophages, by stimulating iron export, and cholesterol efflux, by inhibiting lipid cellular accumulation within macrophages [107]. Interestingly, in human atherosclerotic plaques in which neovascularization is occurring a population of macrophages with the M2 phenotype co-localizes in close proximity to iron deposits and oxidized lipids. In fact, IL-4-polarized M2 macrophages are capable of processing iron by increasing the expression of ferroportin via an LXR-α-dependent mechanism [107,108].

In addition, iron accumulation in atherosclerotic plaques may lead to the differentiation of the M(Hb) phenotype. M(Hb) macrophages express both scavenger receptor cysteine-rich type-1 protein M130 (CD163) and macrophage mannose receptor 1 (MMR, known as CD206) [109]. One of the key features of the M(Hb) macrophage phenotype is the production of anti-inflammatory factors. In fact, by responding to scavenging of hemoglobin-haptoglobin complexes through CD163, this kind of macrophages produce IL-10 via the phosphoinositide 3-kinase (PI3-K)-AKt pathway [110]. Moreover, M(Hb) macrophages display a decreased intracellular iron accumulation as a result of an increased ferroportin expression, so this subtype of macrophages generates less ROS [111].

Macrophage polarization toward the Mhem subtype is stimulated by the oxysterol receptor LXR-β-mediated activation of the cyclic AMP-dependent transcription factor ATF-1 [112]. LXR-β stimulates the expression of both ATP-binding cassette subfamily A member 1 (ABCA1) and LXR-α [113]. MHem macrophages are identified by increased expression of HO-1.

Altogether, M2, M(Hb) and Mhem may coexist in areas of human atherosclerotic plaques in which there has been some degree of hemorrhage and remain able to accumulate lipids and form foam cells. Mhem and M(Hb) macrophages have the potential to decrease the level of oxidative stress [112,114], while the iron-loaded M2 macrophage phenotype can improve oxidative efficiency.

As mentioned above, there is a relation between cytokines and growth factors, which are expressed in atherosclerotic lesions, and the phenotype of macrophages that respond [115]. IL-4 is an effective inducer of the M2a phenotype in human atherosclerotic lesions and could be considered one of the essential factors that induce $CD68^+MMR^+$ M2 macrophages [116,117]. Overall, there is evidence that macrophages with the M2 phenotype are highly competent in the scavenging process and ultimate phagocytosis of cellular debris in human atherosclerotic injuries [116]. Indeed, they may help to maintain effective efferocytosis and decrease inflammation and autoimmunity [118,119].

Previous studies have shown that both M-CSF and GM-CSF may regulate macrophage polarization toward the M1 and M2 phenotype in atherosclerotic lesions [120]. However, it is also possible that fluctuations in GM-CSF and M-CSF concentrations are related

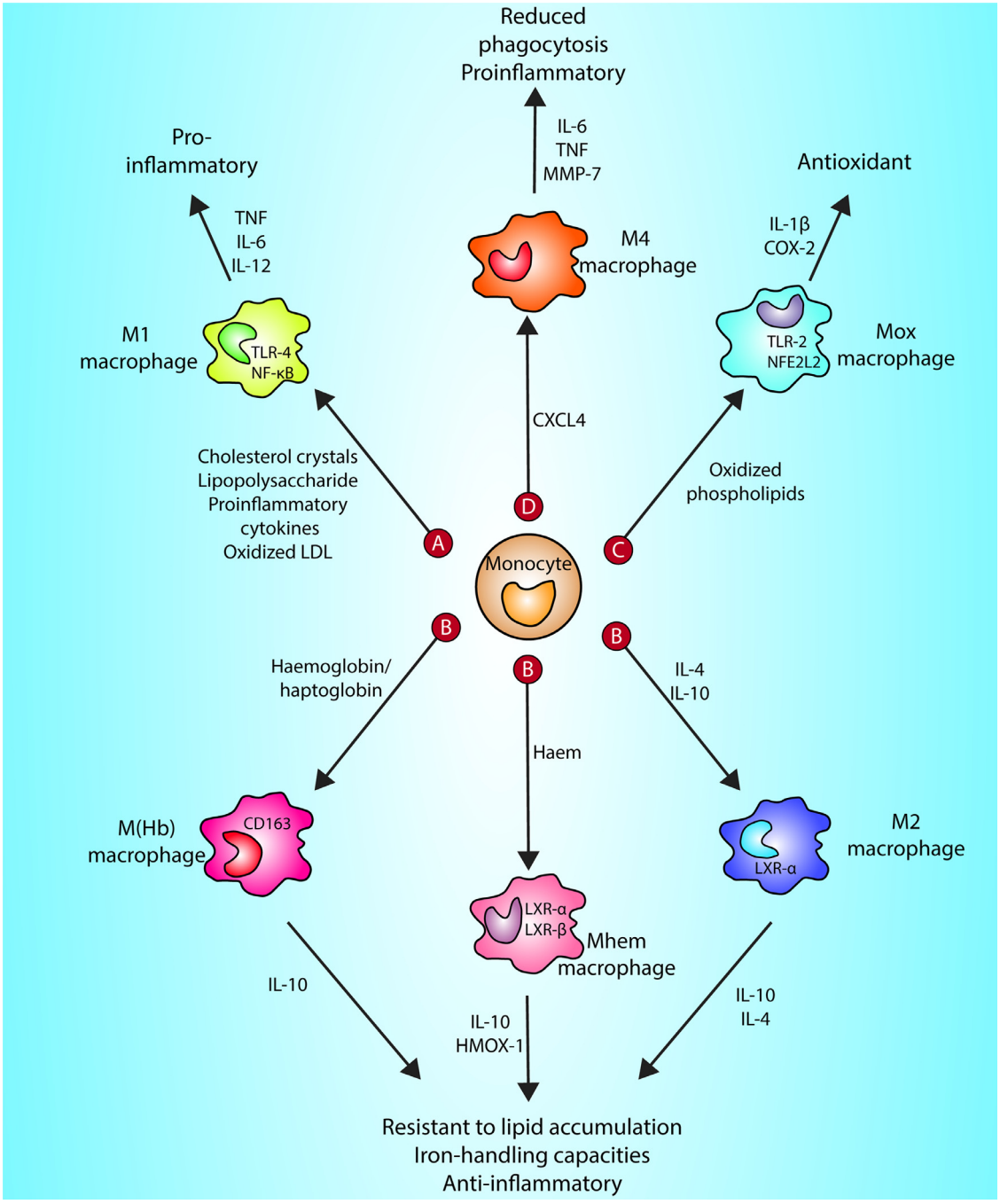

Fig. 2. The main subclasses of macrophages in atherosclerotic lesions. Due to presentation of stimuli in atherosclerotic lesions, the differentiation of monocytes toward various phenotypes of macrophages is strictly controlled, for example, A) M1 phenotype of macrophages discharge pro-inflammatory cytokines, B) Mhem, M (Hb), and M2 phenotypes have anti-inflammatory actions, are stable to lipid accumulation, and contain iron-handling capabilities, C) Mox phenotypes represent an antioxidant gene expression category, and D) M4 macrophages, such as the M1 phenotype, release pro-inflammatory cytokines with a reduced capacity for phagocytosis. Abbreviations: COX-2, cyclooxygenase; CXCL4, C-X-C motif chemokine 4; HMOX-1, haem oxygenase (decycling) 1; LDL, low-density lipoprotein; LXR, liver X receptor; MMP-7, matrix metalloproteinase-7; NFE2L2, nuclear factor (erythroid-derived 2)-like 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TLR, toll-like receptor; TNF, tumor necrosis factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to the prevalence of M1 and M2 macrophages in atherosclerotic plaques at different stages [121].

In addition, two macrophage subpopulations resembling M-CSF-induced and GM-CSF-induced macrophages, $CD68^{+}CD14^{+}$ and $CD68^{+}CD14^{-}$phenotypes, have been observed [80]. It has been shown that the $CD68^{+}CD14^{+}$ phenotype, resembling M-CSF-induced macrophages, exhibits many pro-inflammatory genes and is frequently observed in human atherosclerotic lesions. In contrast, the $CD68^{+}CD14^{-}$phenotype, resembling GM-CSF-induced macrophages, exhibits some genes involved in the regulation of reverse cholesterol transport and migration of macrophages through the vessel wall [80].

Platelet factor 4 (C-X-C motif chemokine 4 or CXCL4), which is extensively expressed in atherosclerotic plaques, may induce the differentiation of M4 macrophages [122,123]. Although the M4 phenotype has some common features with the M1 and M2 macrophage phenotypes, it does not possess the same capacity for phagocytosis [124]. The M4 macrophage restricts the expression of

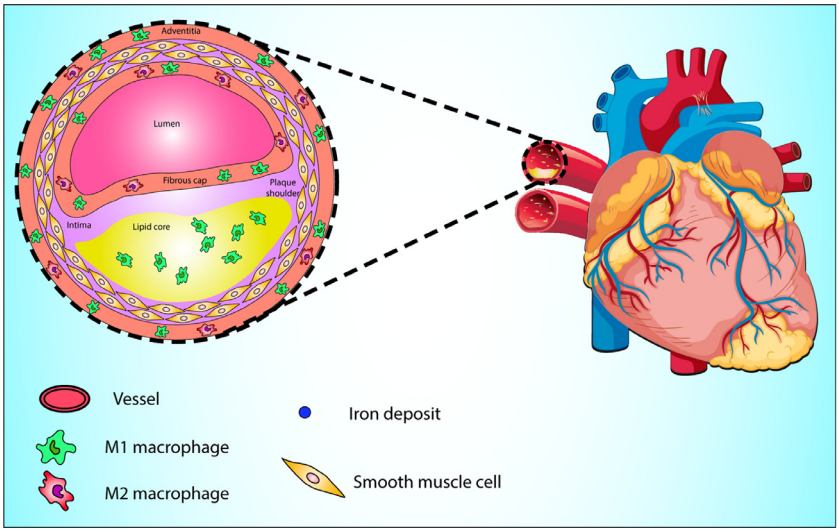

Fig. 3. The distribution of macrophage subclasses in human atherosclerotic lesions. M1 macrophages are significantly present in the plaque shoulder and lipid core, while M2 macrophages are the most common phenotype in the adventitia and areas of hemorrhage (neovascularization), which contain iron deposits. In the fibrous caps, a similar number of both macrophage subclasses exist.

the Mhem phenotype [94], and in spite of the ability of switching between the M1, M2, and Mox phenotype, polarization to M4 macrophages is unidirectional [124].

Simultaneous with plaque development and progression, the number of macrophages within the plaque increases [125]. Much evidence suggests that a number of macrophages with diverse phenotypes coexist within human atherosclerotic lesions (Fig. 3).

At the most unstable sites within the plaque (lesion shoulder), macrophages with M1 polarization are located [126], while in the surrounding necrotic center (fibrous cap) both M1 and M2 phenotypes are expressed [127]. As a result, there may be a balance between the potentially harmful pro-inflammatory impact of M1 phenotype and the potentially advantageous effects of M2 phenotype within the fibrous cap [90]. In the adventitia area, the ratio of M2 macrophages are two- to three-fold more common than M1 macrophages [127].

In different portions of atherosclerotic plaques different specific biomarkers of macrophages are expressed. Such a variability of biomarker expression is due to: 1. Various subtypes of macrophages originating from monocytes, 2. Response to the local microenvironment, resulting into the switch to M1 or M2 phenotype, 3. Continuous recruitment of M1 and M2 macrophages to the plaque [128].

Of note, there is evidence suggesting that also macrophage polarization in extra-vascular tissues (e.g., epicardial adipose tissue), could impact on the progression of atherosclerotic plaques. For instance, macrophage polarization in epicardial adipose tissue could impact on atherosclerosis burden of coronary arteries. Within the epicardial adipose tissue of patients with coronary artery disease, both pro-inflammatory cytokine expression and macrophage infiltration are increased compared to controls [129,130]. As it pertains to the ratio of M1:M2 macrophages in epicardial adipose tissue, the shift toward a pro-inflammatory macrophage phenotype is positively correlated with the severity of coronary artery disease [129].

Conclusion

The studies cited in this review provide evidence that atherosclerosis is an inflammatory disease with a strong immune system component.

Macrophages, the main cellular component of atherosclerotic lesions, participate in all stages of plaque formation and progression [131]. Their functional phenotypes are influenced by many cytokines and signals from atherosclerotic microenvironment *in vivo*. During plaque progression, these stimuli are divergent and occur in different regions of atherosclerotic lesions. Thus, macrophages continuously modify their phenotype and in turn modulate plaque composition and progression.

Substantial progress in the area of immunity and its involvement in atherosclerosis provides insight into the influence of numerous, yet different, subclasses of T and B lymphocytes on atherosclerotic plaque formation and progression. Pro-atherogenic (e.g. Th17 and $CD20^{+}$ B cells) and anti-atherogenic (e.g. Treg and Breg cells) lymphocytes affect atherosclerosis via influencing vascular inflammation and plaque stability.

In coming years, further studies are necessary to understand certain challenges that still remain: 1. How different macrophage phenotypes originate from monocytes, 2. Specific functions of various macrophage subtypes within the plaque, 3. The stimuli for T and B lymphocyte activation and their role in the development and expansion of atherosclerotic plaque. Hopefully, full consideration of these challenges will provide opportunities for the prevention and treatment of atherosclerotic disease.

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