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Heterogeneity of atherosclerotic plaque macrophage origin, phenotype and functions: Implications for treatment

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ABSTRACT

Macrophages are key players in atherosclerotic lesions, regulating the local inflammatory milieu and plaque stability by the secretion of many inflammatory molecules, growth factors and cytokines. Monocytes have long been considered to be the main source of plaque macrophages. However, recent findings provide evidence for proliferation of local macrophages or transdifferentiation from other vascular cells as alternative sources. Recent years of research focused on the further identification and characterisation of macrophage phenotypes and functions. In this review we describe the advances in our understanding of monocyte and macrophage heterogeneity and its implications for specific therapeutic interventions, aiming to reduce the ever growing significant risk of cardiovascular events without any detrimental side effects on the patient's immune response.

1. Introduction

Atherosclerosis is the major underlying pathology of most cardiovascular diseases, though it usually remains asymptomatic for many years. Clinical complications such as stroke or myocardial infarction represent a large social and economic burden and are the leading cause of death worldwide (WHO | The top 10 causes of death). Present treatment strategies are focusing on the reduction of hyperlipidaemia using statins (Endo, 1992; Ray and Cannon, 2005) and hypertension using β-blocker and ACE inhibitors (Sipahi et al., 2007; Yusuf et al., 2008). However, even though the combinatorial use of these treatments and surgical intervention reduces the cardiovascular risk to 30%, this disease and its outcome still stress the need for additional therapeutic options.

Atherosclerosis is characterised as a disease caused by dysfunctions in lipid metabolism and inflammation (reviewed by Hansson et al., 2006; Ross, 1999). The discovery of inflammation as an important driver of plaque growth and rupture suggested that the immune system might be an interesting therapeutic target. Immune cells, and especially macrophages, are highly abundant in the atherosclerotic plaque and have been implicated in all stages of disease (reviewed by Hansson and Hermansson, 2011). Upon injury of the endothelial layer (e.g. by hyperlipidaemia, hypertension, blood flow disturbances or oxidative stress), monocytes enter the vessel wall and differentiate into macrophages. Lipids accumulated and modified in the intima are engulfed by macrophages which subsequently become lipid-laden foam cells that cannot emigrate from the vessel wall anymore and thus form an early subendothelial lesion. Extensive uptake of lipids by macrophages induces their apoptosis, and while in early stages of the disease macrophages are still able to clean dead cells and cell debris in a process called efferocytosis, more advanced plaques have defective efferocytosis, leading to an accumulation of dead cells, secondary necrosis and the formation of a necrotic core. Smooth muscle cells that migrate from the media into the intima are overlaying the necrotic core and form a fibrous cap which stabilises the plaque. In an advanced plaque, the fibrous cap is destabilised by matrix metalloproteinases (MMPs) and matrix degrading enzymes secreted by macrophages increasing the risk for plaque rupture. The opposing roles of macrophages during atherogenesis, on the one hand promoting plaque growth and rupture by the creation and maintenance of an inflammatory environment and on the other hand containing the inflammation through efferocytosis and secretion of anti-inflammatory mediators, show that this cell type controls the delicate balance that determines clinical outcome. Previous studies have unveiled the broad heterogeneity of monocytes and macrophages and the differential contribution of distinct subsets to atherosclerosis.

This review will highlight and update the role of these different subsets in the initiation and progression of atherosclerosis. Moreover, we will discuss promising therapeutic approaches targeting macrophage subsets or -functions to reduce the risk for cardiovascular events.

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2. Monocytes

2.1. Characterisation of monocytes

Monocytes were first described as mononuclear phagocytes originating from the bone marrow (Furth and Cohn, 1968). They have been seen as a homogeneous cell population that differentiates into macrophages or dendritic cells after migration into various tissues (León et al., 2005; Virolainen, 1968). The differential expression of the lipopolysaccharide (LPS) receptor cluster of differentiation (CD) 14 and the FcγIII receptor CD16 have later shown first evidences of a heterogeneous population in humans. The major group of monocytes expresses high levels of CD14 ($CD14^{++}/CD16^{-}$) whereas the additionally described subpopulation of monocytes shows a low density of CD14 in co-expression with CD16 ($CD14^{+}/CD16^{+}$) (Passlick et al., 1989). Regained interest in monocyte subsets revealed an additional discrimination of the subset of CD16 expressing monocytes. Studies have shown that $CD16^{+}$ monocytes do not represent a homogeneous population either and could further be divided into $CD14^{++}/CD16^{+}$ and $CD14^{+}/CD16^{++}$ cells (Ancuta et al., 2003). In 2010, the Nomenclature Committee for the International Union of Immunological Societies proposed the current nomenclature that classifies human monocytes into three subpopulations: classical $CD14^{++}/CD16^{-}$ monocytes, intermediate $CD14^{+}/CD16^{+}$ monocytes and non-classical $CD14^{+}/CD16^{++}$ monocytes (Ziegler-Heitbrock et al., 2014). In mice, the differentiation of monocyte subsets is based on the glycoprotein Ly6C. $Ly6C^{high}$ monocytes are similar to the human classical $CD14^{++}/CD16^{-}$ subset while $Ly6C^{low}$ monocytes are identical to the human non-classical $CD14^{+}/CD16^{++}$ monocytes. All monocyte subsets descend from a common precursor in the bone marrow. Fate mapping studies in mice have unveiled the conversion of $Ly6C^{high}$ to $Ly6C^{low}$ monocytes (Hilgendorf et al., 2014; Sunderkotter et al., 2004; Yona et al., 2013). Observations in patients after transplantation of hematopoietic stem cells exhibit a similar conversion of monocytes starting with $CD14^{++}$ classical monocytes followed by a gradual shift to $CD14^{+}/CD16^{+}$ monocytes and subsequently $CD14^{+}/CD16^{++}$ non-classical monocytes (Rogacev et al., 2015). Besides their described differences in the expression of CD14 and CD16 or Ly6C, monocyte subsets also exhibit disparities in their phenotypic and functional properties. Classical monocytes represent the major subset of circulating monocytes. They exhibit a high expression of the chemokine receptors CCR2, CXCR1, CXCR2 and CXCR4 and high levels of CD62L (Ancuta et al., 2003; Wong et al., 2011), while both CX3CR1 and CCR5 are expressed at lower levels (Stec et al., 2012; Wong et al., 2011). Stimulating them with LPS activates the production of several cytokines and chemokines, including Interleukins (IL) − 10, IL-6, IL-8, IL-1β, Tumor Necrosis Factor (TNF) and CCL2 (Cros et al., 2010; Wong et al., 2011), and transcriptome analysis further revealed the importance of classical monocytes in the antimicrobial immune response. This is underlined by the high expression of myeloperoxidase and several receptors such as CD36, CD32, CD64 and signal-regulatory protein alpha (SIRPα) implicated in phagocytosis. Furthermore, genes associated with angiogenesis and coagulation are enriched in this subset, linking classical monocytes to tissue repair and wound healing (Cros et al., 2010; Wong et al., 2011; Zawada et al., 2011). Similarly, intermediate monocytes are characterised by the expression of CCR1, CCR2 and CXCR2. In addition, this subset expresses CX3CR1 and CCR5 (Ancuta et al., 2003; Zawada et al., 2011) which is expressed by non-classical monocytes. $CD14^{+}/CD16^{+}$ monocytes have been described as main producers of reactive oxygen species (Cros et al., 2010; Zawada et al., 2011) and produce high amounts of the pro-inflammatory cytokines TNF and IL-1β upon LPS stimulation. In contrast, they were also discovered as the main source for the anti-inflammatory IL-10 (Skrzeczynska-Moncznik et al., 2008). Furthermore, it was observed that intermediate monocytes revealed the highest expression of MHC class II compared to the classical and non-classical subset together with a strong capability to stimulate proliferation of $CD4^{+}$ T cells (Wong et al., 2011; Zawada et al., 2011). The characterisation of intermediate monocytes with a mixed expression of markers of classical and non-classical subset especially underlines the transitional state of these cells in the development of the different monocyte subsets. Early studies on non-classical monocyte subsets unveiled phagocytic capacities and the production of reactive oxygen species. In contrast, their cytokine production capacity was strongly reduced (Ziegler-Heitbrock et al., 1991). Furthermore, the expression of Intercellular Cell Adhesion Molecule (ICAM) − 1, opsonin receptors FcγRI and FcγRII, high levels of MHC class II and decreased expression of CD11b and CD33 suggested a more mature phenotype of non-classical monocytes, similar to differentiated macrophages that already reside in the tissue (Ziegler-Heitbrock et al., 1993). They do not express CCR2 and CD62L but instead high levels of CX3CR1 (Ancuta et al., 2003; Wong et al., 2011). The high expression of genes associated with cytoskeleton mobility implicated non-classical monocytes in patrolling the blood circulation to control and remove virally infected or damaged cells (Auffray et al., 2007; Wong et al., 2011; Zawada et al., 2011). In response to Toll Like Receptors TLR7 and TLR8 activation by viruses and nucleic acids, non-classical monocytes produce TNF, IL-1β and CCL3 (Cros et al., 2010) stressing their role as surveillants in the circulation.

2.2. Monocytes in atherosclerosis

2.2.1. Monocytes as risk factor for atherosclerosis

Previous studies already demonstrated an association of monocytes with atherosclerosis in humans. First evidence of a link with atherosclerosis revealed the positive correlation between the number of non-classical monocytes and elevated levels of cholesterol, Low Density Lipoproteins (LDL) and triglycerides (Rothe et al., 1999, 1996). Later studies unveiled a significant role for $CD16^{+}$ monocytes in atherosclerosis, illustrated by the correlation of levels of $CD16^{+}$ monocytes with obesity and diabetes, two classical risk factors for atherosclerosis (Poitou et al., 2011; Timmerman et al., 2008) and the link between $CD16^{+}$ monocyte levels and the diagnosis of coronary atherosclerosis (Schlitt et al., 2004). Likewise, patients with stable or unstable angina pectoris revealed a relationship between enhanced levels of $CD16^{+}$ monocytes and the presence of vulnerable atherosclerotic plaques (Imanishi et al., 2010; Kashiwagi et al., 2010). More recent studies have not just focused on CD16 expressing monocytes but took a deeper look by distinguishing between the intermediate $CD14^{+}/CD16^{+}$ and non-classical $CD14^{+}/CD16^{++}$ subsets of monocytes. A study performed on 951 patients exhibited the intermediate but not the non-classical monocytes as an independent predictor of cardiovascular events (Rogacev et al., 2012). Contrary, a study analysing frozen monocytes of 700 randomly selected subjects concluded that the classical subset is an independent predictor of future cardiovascular risk. In contrast to intermediate $CD14^{+}/CD16^{+}$ monocytes that affect the plaque size, $CD14^{++}/CD16^{-}$ monocytes were associated with inflammation and a thinner fibrous cap (Berg et al., 2012). Nevertheless, the fact that intermediate monocytes produce high amounts of reactive oxygen species, TNF and IL-1β and stimulate $CD4^{+}$ T cells, in combination with the observed association with atherosclerosis indicate monocytes as risk factor for CVD.

2.2.2. Monocyte recruitment

Considering the availability of (receptor) knockouts and their suitability to perform bone marrow transplantations, cell depletion or adoptive transfer, studies on monocyte recruitment are mainly performed in mice. In the steady state, monocytes mainly originate from hematopoietic stem and progenitor cells (HSPCs) in the bone marrow. However, during atherosclerosis HSPCs migrate from the bone marrow niche to seed the spleen where extramedullary haematopoiesis contributes to the generation of $Ly6C^{high}$ monocytes as well (Robbins et al., 2012). Interestingly, myocardial infarction is boosting the process of