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The role of immune-related myeloid cells in angiogenesis

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ABSTRACT

Macrophage function is not restricted to the innate and adaptive immune responses, but also includes host defence, wound healing, angiogenesis and homeostatic processes. Within the spectrum of macrophage activation there are two extremes: M1 classically activated macrophages which have a pro-inflammatory phenotype, and M2 alternatively activated macrophages which are pro-angiogenic and anti-inflammatory. An important property of macrophages is their plasticity to switch from one phenotype to the other and they can be defined in their polarisation state at any point between the two extremes. In order to determine what stage of activation macrophages are in, it is essential to profile various phenotypic markers for their identification. This review describes the angiogenic role for myeloid cells: circulating monocytes, Tie-2 expressing monocytes (TEMs), myeloid-derived suppressor cells (MDSCs), tumour associated macrophages (TAMs), and neutrophils. Each cell type is discussed by phenotype, roles within angiogenesis and possible targets as a cell therapy. In addition, we also refer to our own research on myeloid angiogenic cells (MACs), outlining their ability to induce angiogenesis and their similarities to alternatively activated M2 macrophages. MACs significantly contribute to vascular repair through paracrine mechanisms as they lack the capacity to differentiate into endothelial cells. Since MACs also retain plasticity, phenotypic changes can occur according to disease states and the surrounding microenvironment. This pro-angiogenic potential of MACs could be harnessed as a novel cellular therapy for the treatment of ischaemic diseases, such as diabetic retinopathy, hind limb ischaemia and myocardial infarction; however, caution needs to be taken when MACs are delivered into an inflammatory milieu.

Origin of macrophages

Macrophages are key regulators of the innate and adaptive immune responses, having an essential role in inflammation and acting as the first line of host defence. Besides this well-known function, macrophages also play an important role in homeostatic functions such as wound healing, tissue remodelling, angiogenesis, and apoptosis (Mosser and Edwards 2008).

Macrophages are classically thought to originate from haematopoietic stem cells which commit to a myeloid and then monocytic lineage. Beginning in the bone marrow, myeloid progenitor cells differentiate into monocytes. These myeloid progenitors represent a common precursor to neutrophils, eosinophils, basophils, dendritic cells and mast cells. Monocytes are released from the bone marrow into the blood circulation, upon their production. Some monocytes migrate into tissues to become resident macrophages that acquire specific phenotypes according to their localisation. For example, osteoclasts in bone, Kupffer cells in the liver and alveolar macrophages in the lung consistently adopt distinct morphological and functional properties which are related to their specific tissue and microenvironment (Gordon 2003). In the context of the CNS, the origin of microglia (as CNS resident macrophages) remains controversial and these cells were originally thought to originate from the bone marrow. However, recent studies have shown that microglia arise from progenitors within the embryonic yolk sac during early development and these progenitors may seed the developing brain where they generate microglia which are maintained into adulthood (Ginhoux et al. 2013).

Macrophage polarisation

Resident macrophages become activated in response to various stimuli and polarise towards classical M1 or alternatively activated M2 macrophages. Such cells have been further characterised in relation to their function as either host defence (M1), wound healing (M2a), immunoregulation (M2b) or regulation of tissue remodelling (M2c) (Martinez et al. 2008). Indeed, macrophages

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can share one or more of these characteristics at any one time and there may be overlap among the various phenotypes (Mosser and Edwards 2008). In response to stimuli, whether endogenous or during injury, macrophages undergo physiological reprogramming. Following activation, macrophages are classed as M1 or M2 reflective of the Th1 to Th2 linear scale of activation (Mantovani et al. 2013). The Th1/Th2 balance hypothesis originates from findings that CD4+ T-cells expressed differing cytokine profiles (Kidd 2003). While Th1 cells direct the cellular immunity pathway, Th2 cells drive humoral immunity and both cell types suppress one another and maintain an important balance within the immune system. Likewise, M1 and M2 macrophages remain distinct in their cytokine production profiles, in addition to the surface markers they express. Although M1 and M2 polarisation represents either end of the polarisation scale, it is possible that macrophages can be activated towards a phenotype at any point between these two extremes. Evidence also suggests that macrophages are extremely plastic between activation states, switching rapidly from one to another, and fully reversible upon stimulation with a cytokine that has an opposite effect (Porcheray et al. 2005; Pelegrin and Surprenant 2009).

Classically activated M1 macrophages

Tissue resident macrophages undergo activation by inflammatory stimuli following stress or infection. Treatment with IFNγ (released *in situ* by natural killer cells as part of the innate immune response, and T helper cells in the more stable adaptive immune response) together with LPS activates macrophages towards the classically activated M1 phenotype (Mosser and Edwards 2008). There is also evidence that treatment with granulocyte macrophage colony stimulating factor (GM-CSF) can direct monocytes/macrophages towards the M1 phenotype (Xu et al. 2013; Svensson et al. 2011; Sierra-Filardi et al. 2010). The transcriptome from M1 macrophages shows increased expression levels of CD68 and CD80 with relatively lower levels of CD163 and CD206 and they participate in host defence, by their release of pro-inflammatory cytokines IL12, IL23, TNFα, IL1β with low level release of IL10, in addition to their expression of Th1-attracting chemokines CXCL9 and CXCL10. M1 macrophage activation is usually tightly controlled to avoid collateral damage from the pro-inflammatory cytokines and chemokines they produce. For example, an unrestrained pro-inflammatory phenotype is a key component of autoimmune diseases such as type 1 diabetes (Patel et al. 2013) and arthritis (Hofkens et al. 2013).

Alternatively activated M2 macrophages

To generate alternatively activated macrophages in culture, CD14+ monocytes can be treated with IL4 and IL13 (akin to their activation *in vivo* via IL4 release from granulocytes). Their treatment with macrophage colony stimulating factor (M-CSF) can also direct these cells towards the M2 macrophage path (Xu et al. 2013; Svensson et al. 2011; Sierra-Filardi et al. 2010). In contrast to the M1 macrophage pro-inflammatory phenotype, M2/M2-like subtypes demonstrate pro-angiogenic potential, with high expression of IL10 (anti-inflammatory cytokine), and reduced release of TNF α and IL12 (Gordon 2003). M2 cells generally have high expression of CD163 (scavenger receptor), CD206 (mannose receptor) and CD209 (dendritic cell-SIGN) with an absence of CD80 expression. Chemokine synthesis of M2 macrophages comprises CCL13, CCL18 and CCL23 (Martinez et al. 2006).

Macrophage plasticity and disease

Macrophages maintain their plasticity throughout their lifespan and phenotypic changes occur in response to innate and adaptive signals which allow the cells to contribute to homeostatic repair and host defence, in addition to tissue remodelling and wound healing following injury. These changes need to be carefully regulated because an imbalance in M1/M2 polarisation state can lead to excessive inflammatory responses. For example, the inflammatory environment associated with obesity, polarises macrophages towards a pro-inflammatory M1 phenotype which may enhance progression towards overt type 2 diabetes (Lumeng et al. 2007; Patel et al. 2013). This occurs because of pro-inflammatory cytokine release (including TNF α and other cytokines) from M1 macrophages which serve to activate inflammatory kinase pathways and subsequently induce insulin resistance. Concomitantly, IL10 which usually compensates for TNF α, is greatly reduced in the pro-inflammatory state.

Chronic venous leg ulcers are another inflammatory condition where high levels of iron and erythrocyte engulfment by macrophages induce a population of "unrestrained proinflammatory" M1 macrophages (Sindrilaru et al. 2011). Characterisation of these cells revealed expression of both M1 (TNFα, iNOS, IL12 and CCR2) and M2 (CD163 and CD206) markers, suggesting that at the site of disease, cells with an M1 phenotype sometimes persist and fail to efficiently switch to the healing M2 phenotype and this significantly impairs wound healing.

In response to such an imbalance, it has been suggested that a therapeutic approach which promotes M2 phenotype polarisation would be efficacious. Although it would seem that a drive towards the pro-angiogenic phenotype could rectify the imbalance, it is important to note that wholesale blockade of the M1 phenotype causes immune compromise and individuals would become more susceptible to infection and tumour formation. Indeed, cancer tumour growth can be promoted by a reduction in M1 macrophages that are instead skewed towards a regulatory M2 macrophage (Colombo and Mantovani 2005).

Considering the plasticity of macrophages, it is difficult and controversial to define a specific marker to identify macrophage subtypes, and therefore a phenotypic profile of various markers is required. Indeed, expression of macrophage markers is not as simple as positive and negative, rather it is the difference in their basal expression that determines if the cells are more akin to M1 or M2 phenotypes. For example, high levels of expression of CD68, CD16 and CD32 are generally thought of as typical markers for M1. On the other hand, higher expression of CD163, CD206, CD209 and CXCR4 are representative of M2 macrophages (Martinez et al. 2006).

Myeloid cells and angiogenesis

Recent reviews have discussed in detail the role of macrophages in tissue repair and chronic inflammation (Mantovani et al. 2013; Novak and Koh 2013); therefore, this review will focus in examining the role that myeloid cells, including macrophages, play in vascular repair and cancer (Table 1). The potential use of various myeloid cell populations for therapeutic angiogenesis will also be described.

Circulating monocytes

As the precursor cells to macrophages and dendritic cells, monocytes can adopt three distinct states: classical (CD14++CD16−), intermediate (CD14++CD16+) and non classical (CD14+CD16++), and each of these has a distinct gene profile (Fadini et al. 2013; Zawada et al. 2011). Another degree of complexity also exists since monocytes can have different activation phenotypes like macrophages. In particular, classical monocytes (CD14++CD16−) are more M1, expressing CD68 and CCR2, while non-classical monocytes (CD14+CD16++) are more M2, expressing CXCR1 and scavenger receptors CD163 and CD206. Classical monocytes