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Review

Macrophages: Supportive cells for tissue repair and regeneration

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ABSTRACT

Macrophages, and more broadly inflammation, have been considered for a long time as bad markers of tissue homeostasis. However, if it is indisputable that macrophages are associated with many diseases in a deleterious way, new roles have emerged, showing beneficial properties of macrophages during tissue repair and regeneration. This discrepancy is likely due to the high plasticity of macrophages, which may exhibit a wide range of phenotypes and functions depending on their environment. Therefore, regardless of their role in immunity, macrophages play a myriad of roles in the maintenance and recovery of tissue homeostasis. They take a major part in the resolution of inflammation. They also exert various effects of parenchymal cells, including stem and progenitor cell, of which they regulate the fate. In the present review, few examples from various tissues are presented to illustrate that, beyond their specific properties in a given tissue, common features have been described that sustain a role of macrophages in the recovery and maintenance of tissue homeostasis.

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Introduction

Macrophages, first identified – and named – as large phagocytes, play a myriad of roles during innate and adaptive immunity. In addition, the last decade has seen the emergence of a multiple properties of macrophages, showing that they are more than immune cells (Stefater et al., 2011). As the presence of macrophages is associated with most diseases, these cells were firstly thought to be deleterious, as was thought "inflammation" in the broad sense. However, macrophages are also present during the full process of tissue repair and/or regeneration (Murray and Wynn, 2011; Sica and Mantovani, 2012). This led to the identification of macrophages as key players in the orchestration of the resolution of inflammation and of the restoration of the tissue integrity/function. These beneficial effects of macrophages are mainly due to the trophic factors they release in the environment, and particularly on parenchymal cells. The wide range of active molecules secreted by macrophages likely explains their wide roles in tissue development, repair and homeostasis that have been demonstrated in various tissues (Pollard, 2009). The development of techniques and tools including transgenic mouse strains to specifically deplete or

Abbreviations: 2-AAF, 2-acetylaminofluorene; BMDM, bone marrow-derived macrophages; CNS, central nervous system; CSPG, chondroitin sulphate proteoglycan; DAMPs, damage associated molecular patterns; EMP, erythroblast macrophage protein; EAE, experimental auto encephalitis; ECM, extracellular matrix; G-CSF, granulocyte-colony stimulating factor; IGF, insulin growth factor; IFNγ, interferon; IL, interleukin; LPS, lipolysaccharide; LPCs, liver progenitor cells; MMPs, matrix metalloproteinases; MPC, myogenic precursor cell; SLPI, secretory leukocyte protease inhibitor; TIMP, tissue inhibitor of MMP; TWEAK, TNF-like weak inducer of apoptosis; TGF, transforming growth factor; TNF, tumour necrosis factor.

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trace macrophages or macrophage subpopulations, combined to flow cytometry analysis and cell sorting, allowed to investigate the diversity of functions of macrophages in several tissues and diseases (Chow et al., 2011). Moreover, *in vitro* cocultures performed in parallel to the exploration *in vivo* led to the identification of specific cell interactions macrophages develop with other cells and particularly with stem and/or progenitor cells.

Referring to macrophages, one has to keep in mind that the term "macrophages" encompasses a variety of cells harbouring distinct functional phenotypes. Indeed, depending on the environmental cues they received, macrophages may adopt various phenotypes and functions (Stout et al., 2005; Gratchev et al., 2006). This versatility makes macrophages efficient regulators of tissue homeostasis. In an attempt to understand their roles and functions, macrophages have been classified into several subpopulations according to their activation (polarisation) state. These populations were defined *in vitro*, under well-defined stimuli and mainly used human monocyte-derived macrophages. Therefore, these phenotypes likely not correspond to what occurs *in vivo*, were concomitant cues may interfere, leading to a variety of intermediate phenotypes (review in Mosser and Edwards, 2008; Mantovani et al., 2013). Classically activated human M1 macrophages (induced *in vitro* by Interferon (IFN)γ or IFNγ plus lipopolysacharide (LPS) or tumour necrosis factor (TNF)α) secrete interleukin (IL)-12, IL-23, reactive oxygen and nitrogen intermediates, and inflammatory cytokines (IL-1β, TNFα, IL-6) and chemokines (CXCL9, CXCL10). M1 macrophages are associated with the first phases of acute inflammation. Mirroring Th1/Th2 immune response, M2 alternative activation state of macrophages (triggered by IL-4 and IL-13) was first described. M2 macrophages highly express YM1, arginase 1, CCL24 and CCL17 (Gordon and Martinez, 2010; Stein et al., 1992). Then, a series of *in vitro* stimuli, mimicking *in vivo* cues, has been found to induce an M2-like phenotype. Glucocorticoids, transforming growth factor (TGF)β, IL-10 or immune complexes plus LPS or IL-1 trigger M2 phenotypes. M2 phenotype is characterized by low levels of pro-inflammatory cytokines (IL-1, IL-12), elevated CD206 (mannose receptor), IL-1ra and IL-1 decoy type II receptor, IL-10 expression and secretion of CCL17, CCL22, and CCL24 chemokines. However, depending on the stimulus which is used to polarise the cells, some differences are observed, notably in the capacity to produce inflammatory effectors. Other notable differences between M1 and M2 macrophages are related to metabolic regulation. M1-polarized macrophages present an anaerobic glycolytic pathway while M2 polarisation is characterized by oxidative glucose metabolism (fatty acid oxidation), which is believed to sustain their long-lasting functions such as tissue remodelling, repair and healing. Iron metabolism also differs according to the state of polarisation of macrophages. M1 macrophages store iron through high levels of ferritin while M2 cells express high level of ferroportin, the main iron exporter (review in Mantovani et al., 2013; Biswas and Mantovani, 2012; O'Neill and Hardie, 2013; Cairo et al., 2011).

Some attempts have been made to further classify M2 macrophages into subfamilies such as M2a, M2b, and M2c, depending on the stimulus used for polarisation (Martinez et al., 2008). However these subgroups, defined *in vitro* in human, only partially overlap with those that were described in *in vivo* murine models, and that were named wounding/healing/resolving macrophages, as opposed to classical proinflammatory M1 macrophages. Indeed, M2 macrophages cells take part in polarized Th2 responses, parasite clearance, the dampening of inflammation, the promotion of tissue remodelling, angiogenesis and tumour progression (Mantovani et al., 2013).

To add complexity, it has been recently showed that tissue macrophages may come from different sources. In the mouse, most of the tissue resident macrophages have an embryonic origin while most of the macrophages infiltrating the tissues during inflammation come from blood-derived monocytes (Schulz et al., 2012; Hashimoto et al., 2013; Hoeffel et al., 2012). Two main populations of monocytes have been described in mouse circulation. Ly6C^{pos}CCR2^{pos}CX3CR1^{lo} monocytes have a short half-life, migrate to inflamed tissues where they produce TNFα, IL-1 and nitric oxide. Ly6C^{neg}CCR2^{neg}CX3CR1^{hi} cells are found in inflamed and resting tissues and their recruitment depends on the tissue and type of injury (Geissmann et al., 2003; Shi and Pamer, 2011). There is no strict matching between Ly6C^{pos} monocytes and M1 macrophages and between Ly6C^{neg} monocytes and M2 macrophages. In almost all tissues, damage or infection is followed by the rapid entry of LyC6^{pos} monocytes that become M1 macrophages. In some tissues, Ly6C^{neg} monocytes have been shown to invade the repairing/regenerating tissue after the first Ly6C^{pos}/M1 wave of infiltration (Auffray et al., 2007; Tacke et al., 2007; Nahrendorf et al., 2007; Shechter et al., 2013). In other tissues, at rest or after an injury, Ly6C^{pos} monocytes can give rise to both M1 macrophages, which then switch (or skew) into M2 macrophages (Rivollier et al., 2012; Bain et al., 2012; Lin et al., 2009; Arnold et al., 2007). The relative contributions of blood-derived macrophages *versus* tissue resident macrophages during tissue repair or chronic inflammation have not been established yet.

The molecular regulation of macrophage polarisation is starting to be explored. Different regulation pathways have recently been associated with either the M1 or the M2 activation states. They involve a variety of molecular machineries, at the genomic, transcriptomic and post-transcriptomic levels (reviewed in Lawrence and Natoli, 2011). For instance, NFκB has both pro- and anti-inflammatory functions depending on the pathophysiological context. STAT signalling is involved in the M1 (STAT1) and M2 (STAT6) polarization (Ohmori and Hamilton, 1997; Takeda et al., 1996; Varinou et al., 2003), whereas different interferon regulatory factors (IRFs) are associated with M1 (IRF5) and M2 (IRF4) gene expression (Krausgruber et al., 2011; Satoh et al., 2010). Several molecular systems have been shown to be associated with the expression of the M2 phenotype by macrophages, such as PPARs (particularly PPARγ) and the CREB-C/EBP axis (Odegaard et al., 2007; Bouhlel et al., 2007; Ruffell et al., 2009; Marigo et al., 2010). At the DNA level, promoters of some genes characterising macrophage inflammatory profile are specifically associated with histone demethylases or nucleosome remodelling complexes (Lawrence and Natoli, 2011; Satoh et al., 2010). Finally, by controlling the stability and translation of mRNAs, post-transcriptional regulons allow the coordinated expression of chemokines and cytokines involved in the initiation as well as the resolution phases of inflammation (Anderson, 2010).

In vascularised tissues, damage is followed by an inflammatory response, which is characterised by the presence of M1 macrophages (Chen and Nunez, 2010). This response is necessary for limiting the area of tissue damage, for preventing leakage and for cleansing cell/tissue debris. The second phase is the tissue repair, or regeneration when the parenchyma is able to recover function. This process is possible thanks to the resolution of inflammation, where M2 macrophages play an important role. Beside the regulation of inflammation *per se*, M1 and M2 macrophages have been shown to exert specific effects on stem/precursor cells in various tissues. Their role in the coordination of the repair/regeneration process and the recovery of tissue homeostasis is emerging. In this review, we will present few examples of tissue repair/regeneration after a sterile damage, in which macrophages have been shown to play important trophic roles. Although fine tuning of repair/regeneration in a given tissue likely requires specific and orchestrated signals, common features of the kinetics of macrophage polarisation and properties can be observed in various tissues. Macrophages are also involved in the homeostasis