Macrophages: supportive cells for tissue repair and regeneration.

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Short title: Macrophages in tissue repair and regeneration

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

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.

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, III 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 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 lipolysaccharide (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, 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 of tissues having permanent renewal; an example is given as the erythroblastic island.

Liver regeneration

Kupffer cells are the resident macrophages of the liver, which activate upon liver injury. Both Kupffer cells and monocyte-derived macrophages are involved in liver regeneration: macrophage depletion leads to a delayed regeneration associated with a decrease in the rate of mature hepatocyte proliferation and a down regulation of various pro- and anti-inflammatory cytokines (Meijer et al., 2000). In 2005, Duffield et al. showed for the first time the existence of a biphasic curve of macrophage activation/function during liver regeneration by ablation of monocytes/macrophages at different time points after liver injury (Duffield et al., 2005). To this purpose, they used the transgenic mouse CD11b-DTR (receptor for diphteria toxin expressed under the control of CD11b promoter) in which monocytes/macrophages can be selectively depleted upon diphteria toxin injection (granulocytes and lymphocytes are not targeted by the toxin). During the

fibrogenic phase, macrophage depletion results in reduced scarring and fewer myofibroblasts. Conversely, during the phase of recovery, macrophage depletion leads to a failure of matrix degradation and fibrosis. These data were pioneers in evidencing functionally distinct subpopulations of macrophages in the same tissue depending on the phase of tissue remodelling (Duffield et al., 2005). Under conditions where proliferation of mature hepatocytes is inhibited, liver progenitor cells (LPCs), also known as oval cells, expand and differentiate into hepatocytes and biliary epithelial cells in order to regenerate liver after damage. Models of LPC expansion (*e.g.* choline-deficient ethionine-supplemented diet, 2-acetylaminofluorene (2-AAF) administration combined to partial hepatectomy) are characterized by the presence of huge number of macrophages. Several studies have explored the trophic functions of macrophages on LPCs, which are always located in close vicinity (Lorenzini et al., 2010). Macrophage depletion is associated with an altered LPC fate, although absence of the evidence of direct interactions does not allow to conclude whether macrophage effects on LPCs are direct or indirect (Thomas et al., 2011). Delivery of bone marrow-derived macrophages (BMDM) in injured liver triggers a decrease of myofibroblast number and an expansion of LPCs, which are associated with an increase of metalloproteinases (MMPs), of IL-10, and of the LPC mitogen TNF-like weak inducer of apoptosis (TWEAK), which is known to be secreted by macrophages (Thomas et al., 2011). However, macrophage depletion has not been found to directly alter LPC proliferation rate, but to reduce their invasiveness into the parenchyma, which is necessary for their differentiation into hepatocytes. This effect may be due to either a direct chemotactic effect of macrophages on LPCs or an alteration of extracellular matrix (ECM) and of myofibroblasts by macrophages (Van et al., 2011). Anyway, macrophage depletion induces a decrease in LPC number and impairment in their differentiation and maturation, as almost no formation of small hepatocyte-like cells being observed (Xiang et al., 2012). Since LPC proliferation and apoptosis rates are not altered by macrophage depletion, this suggests that macrophages are required for the very early phase of LPC priming after acute injury to enter the cell cycle (Xiang et al., 2012). This hypothesis was further confirmed by a very smart study which deciphers some early signalling pathways the environment, including

macrophages, delivers to LPCs to induce them in the hepatocyte route. After a hepatic damage, macrophages are induced to secrete Wtn3a upon phagocytosis of liver debris. Macrophage-derived Wnt3a triggers the expression of the Notch signalling inhibitor Numb in LPCs to prevent their differentiation into biliary cells, while inducing their differentiation in hepatocytes by the activation of the Wnt-βcatenin pathway (Boulter et al., 2012). Altogether, these studies show the importance of macrophages during liver repair and regeneration. Although the M1/M2 paradigm has not been investigated in this context, there is evidence for several functional populations of macrophages in the regenerating liver, specifically acting on both hepatocytes and progenitor cells to recover liver homeostasis.

Skeletal muscle regeneration

Contrary to many tissues which repair after an injury, skeletal muscle is capable of fully regeneration, with recovery of its function. This is due to the properties of satellite cells, the main adult muscle stem cells, which activate after damage and expand before differentiating and fusing to form new myofibres (Wang and Rudnicki, 2012). Macrophages have always been observed during muscle regeneration. Specific depletion of circulating monocytes with diphteria toxin in the CD11b-DTR mouse (which spares all types of granulocytes (Duffield et al., 2005)) or with clodronate-encapsulated liposomes shows a very severe impairment of muscle regeneration (Bryer et al., 2008; Summan et al., 2006; Arnold et al., 2007), indicating the crucial involvement of these cells in muscle regeneration. The first studies have suggested that macrophages that are present during the first phases of muscle regeneration (time of necrosis and phagocytosis of muscle debris) differ from those that accompany the late phases of the process (formation and growth of the new myofibres) (McLennan, 1993; McLennan, 1996). This sequence was recently confirmed, thanks to new immunological tools, together with cell sorting techniques and the use of lineage tracing mouse strains. Soon after injury, $LyC6^{pos}CCR2^{pos}CX3CR1^{lo}$ blood-derived monocytes/macrophages infiltrate the injured muscle area. These macrophages switch their phenotype towards a M2

resolving/wounding phenotype (Ly6C^{neg}CCR2^{neg}CX3CR1^{hi}), likely upon phagocytosis of muscle debris (Arnold et al., 2007). Thus skeletal muscle regeneration is characterized by a sequence of M1 then M2 macrophages, which has been confirmed during human muscle regeneration (Saclier et al., 2013b). The phenotype of these populations evolves with time. Soon after injury, a majority of M1 cells are present, which express higher amounts of TNFα and IL-1β. Then M2 cells appear rapidly, expressing higher amounts of IL-10 and TGFβ and these cells predominate during several days. At the end of muscle regeneration, not only the number of macrophages decreases but the phenotype of both Ly6C^{pos}F4/80^{pos} (F4/80 is a macrophagic marker) and LY6C^{neg}F4/80^{pos} cells changes towards a dampening of all cytokine markers, suggesting a skewing into M2 resolving/silencing macrophages (Perdiguero et al., 2011). Very importantly, perturbing this kinetics strongly alters muscle regeneration. Indeed, a too early anti-inflammatory signal (*e.g.* injection of IL-10 or blocking IFNγ few days after injury) as well as blocking later anti-inflammatory signals (injection of anti-IL-10 antibodies during the last phase of muscle regeneration) impedes muscle regeneration (Cheng et al., 2008; Perdiguero et al., 2011).

The pioneer studies on macrophages during muscle regeneration suggested various effects of macrophage subsets on myogenic cell populations. These have been confirmed through a series of *in vitro* and *in vivo* analyses. Macrophages stimulate myogenic precursor cell (MPC) chemotaxis, growth, and survival through the delivery of anti-apoptotic cues (Saclier et al., 2013b; Sonnet et al., 2006). *In vitro* cocultures experiments using M1 and M2 polarised macrophages helped to finely analyse their role on MPCs. Pro-inflammatory M1 macrophages stimulate MPC proliferation and inhibit their fusion. Conversely, M2 macrophages (both M2a and M2c) stimulate myogenesis by both promoting MPC commitment into terminal differentiation and the formation of large myotubes (Saclier et al., 2013b). Accordingly, *in vivo* depletion of intramuscular macrophages during the late phase of muscle regeneration leads to a decrease of the size of the newly formed myofibres (Arnold et al., 2007), confirming the role of M2 macrophages in differentiation of myogenic precursors. The effectors that drive these multiple effects of macrophages on MPCs start to become known (review

in (Saclier et al., 2013a)). Anti-apoptotic contacts between macrophages and myogenic cells involves at least four cell to cell molecular couples, VCAM-1(CD106)/VLA-4(CD49d), ICAM-1(CD54)/LFA-1(CD11a), CX3CL1/CX3CR1 and PECAM-1(CD31)/PECAM-1(CD31) (Sonnet et al., 2006). Among molecules that stimulate myogenic cell proliferation, IL-6, TNFα, IL1-β, Granulocyte-colony stimulating factor (G-CSF) have been shown to be highly secreted by M1 macrophages; M2 macrophages secrete TGFβ, Insulin Growth Factor (IGF)-1 and low TNFα which stimulate the formation of myotubes and promote regeneration (Hara et al., 2011; Lu et al., 2011; Saclier et al., 2013b).

Skeletal muscle regeneration is sustained by the sequential presence of first M1, then M2 macrophages that deliver specific cues to promote expansion, than differentiation of muscle progenitors.

Kidney regeneration

After damage (experimentally, most of the studies uses the ischemia/reperfusion model injury), the renal parenchyma can regenerate. It has also been shown that just after injury, resident and recruited macrophages may directly damage the tissue through their M1 phenotype (they produce oxygen radicals, hydrogen peroxide, nitric oxide, IL-1, TNFα). Therefore, depletion of macrophages before or during the first steps after injury improves tissue repair (Nelson et al., 2012; Lee et al., 2011). In contrast, later phases of injury are associated with M2 macrophages, which depletion is associated with persistent kidney injury, increased apoptosis, impaired tubular cell proliferation and sustained inflammation (Lee et al., 2011; Kim et al., 2010). In injured kidney, as in skeletal muscle, $Ly6C^{pos}$ bone marrow monocyte population is selectively recruited and gives rise to several macrophage populations, among them $Ly6C^{neg}$ cells that exhibit an M2 phenotype (Lin et al., 2009). M2 macrophages (expressing arginase 1 and the mannose receptor) actively promote repair of injured renal tubules. They clear damage associated molecular patterns (DAMPs) and other cell and matrix debris, they stimulate proliferation of surviving cells through the elaboration of Wnt ligand,

and they promote angiogenesis (review in (Nelson et al., 2012)). During kidney repair, macrophage populations follow a biphasic response suggestive of the presence of subpopulations fulfilling disparate functions. Functional *in vivo* studies showed that infusion of IFN-stimulated BMDM worsens kidney damage (Lee et al., 2011) while IL-10-transduced macrophages modify the inflammatory milieu, induce tubular cell proliferation and protect them from apoptosis (Jung et al., 2012). IL-4-stimulated (M2) macrophages, but not IFNγ-stimulated ones, promote renal tubular cell proliferation (Lee et al., 2011). Molecular effectors involved in these effects are poorly known. Macrophage-derived CSF-1 (M-CSF) treatment increases tubular epithelial cell proliferation and decreases their apoptosis (Menke et al., 2009). Macrophages also secrete Wnt7b, which has been shown to promote regeneration in kidney by directing epithelial cell-cycle progression and basement membrane repair. Specific depletion of Wnt7b in macrophages (in Csf1R-iCRE;Wnt7$^{fl/fl}$ mouse) induces a delay in regeneration, an increase in the expression of the epithelial cell injury-associated marker (injury molecule-1 Kim1), and a blockade of epithelial cell progression into G2/M phase of the cell cycle (Lin et al., 2010). Anti-inflammatory (IL-10 stimulated) macrophages protects epithelial cells from apoptosis and stimulates their proliferation through the increase of intracellular iron pool and the increased expression of lipocalin-2, which is, in an iron-dependent way, a growth and differentiation factor (Jung et al., 2012). The biphasic response of macrophages, from M1 to M2 during kidney regeneration is due to a switch of their phenotype. Tracking IFN-stimulated macrophages that have been adoptively transferred into injured recipient shows that these cells can switch to an M2 phenotype at the onset of kidney repair (Lee et al., 2011). Taken together, these studies show that macrophages undergo a switch from a proinflammatory to a trophic phenotype that supports the transition from tubule injury to tubule repair during kidney regeneration.

Nerve regeneration

In the central nervous system (CNS), first evidence of a role of macrophages came from *in vitro* culture systems in which dorsal root ganglia neurons were cultured in conditioned media from

peritoneal macrophages. The presence of macrophages more than doubles the proportion of surviving neurons and neurite extensions. When macrophages had phagocytosed a myelin fraction, macrophage efficiency is increased. Of interest, LPS-stimulated macrophages do not present this trophic effect (Hikawa et al., 1993; Hikawa and Takenaka, 1996). A breakthrough in the understanding the supportive role of macrophages in CNS repair has been made by Michal Schwartz's team in 1998, in a study that opened the way of a long series of investigations. This study shows that transplantation of macrophages - that were previously activated with peripheral sciatic nerve debris - in the lesion after complete transection of spinal cord leads to partial functional recovery and regrowth and reconnexion of neural fibres. The phagocytic activity of macrophages during the *in vitro* phase of the experiment is crucial for their activation and their subsequent trophic activity *in vivo* (Rapalino et al., 1998). Monocyte-derived macrophages are essential for recovery, as demonstrated by adoptive transfer experiments (Shechter et al., 2009). Several experiments indicate the sequential presence of M1, then M2 microglial/macrophages at the lesion site, suggesting that, as in other tissues, post-injury CNS repair follows a "wound" process (Shechter and Schwartz, 2013; Kigerl et al., 2009). A recent study explored the recruitment of blood-derived monocytes after spinal cord injury. Only M1 Ly6C^{pos}CXRCR1^{lo} monocytes are recruited *via* the leptomeninges through CCR2 signalling at the site of the injury (Shechter et al., 2013). At the same time, M2 macrophages are recruited from blood at the level of the choroid plexus, which provides a M2 environment. Adoptive transfer experiments suggest that here again, only Ly6C^{pos}CXRCR1^{lo} monocytes are recruited, but through VCAM-1 and CD73, they are rapidly educated into M2 cells. These cells then migrate from the choroid plexus through the cerebrospinal fluid to the injury site where they can promote axonal growth and tissue repair (Shechter et al., 2013). Some molecular mechanisms have been identified in the instruction of macrophagic cells into M2 cells, such as the glial scar matrix chondroitin sulphate proteoglycan (CSPG). It promotes IL-10 production by macrophages, which in turn produce MMP-13 that is essential for functional recovery (Shechter et al., 2011). Similarly, substance P triggers and anti-inflammatory milieu in spinal cord injury, which induces IL-10 production by macrophages, and

the reduction of inducible nitric oxide synthase and TNFα synthesis (Jiang et al., 2012). In the same way, introducing anti-IL-6 antibodies switches macrophages from a haematogenous phenotype to a resident-microglial-like phenotype, which is associated with a decrease of pro-inflammatory markers and an increase in axonal regeneration and sprouting (Mukaino et al., 2010). Similarly, the overexpression or the injection of the anti-inflammatory molecule secretory leukocyte protease inhibitor (SLPI) triggers a protective effect after spinal cord injury with an improvement in locomotor control, associated with downregulation of the NFκB pathway and of TNFα (Ghasemlou et al., 2010).

The interactions between macrophages and neural progenitors have also been explored *in vivo* and *in vitro*. Transplanted neural stem/precursor cells establish contacts with phagocytes and skew the inflammatory cell infiltrate by reducing the proportion of M1 macrophages, thus promoting the healing of the injured cord (Cusimano et al., 2012). Cocultures of macrophages ($OX42^{pos}$) and neural progenitors ($NG2^{pos}$) isolated from injured spinal cord showed that macrophages secrete factors inhibiting neural progenitor growth (Wu et al., 2010). Indeed, M1 macrophages are neurotoxic or block both neurogenesis and oligodendrogenesis of adult neural progenitor cells. Inversely, M2 macrophages promote a regenerative growth response in adult sensory axons (Kigerl et al., 2009). Microglia activated by cytokines stimulates neurogenesis. The IL-4-activated microglia shows a bias towards oligodendrogenesis while the low level IFNγ-activated microglia shows a bias towards neurogenesis (Butovsky et al., 2006b). These properties were confirmed in *in vivo* experiments, in which injection of IL-4-activated microglia into the cerebrospinal fluid in an Experimental Auto Encephalitis (EAE) model results in an increased oligodendrogenesis in the spinal cord, associated with improved clinical symptoms (Butovsky et al., 2006a). Nevertheless, the molecular activity of macrophages on neural stem cells is poorly known. M1 neurotoxicity is likely due to their secretion of several cytokines, among which TNFα (Butovsky et al., 2006b), and thrombospondin-1, tissue inhibitor of MMP (TIMP)1 and MMP9, which are also expressed by microglial cells *in vivo* (Wu et al., 2010). Beneficial effects of IL-4 activated microglia have been shown to involve IGF1 (Butovsky et al., 2006a). Ferritin, which is a macrophage-derived signal that promotes oligodendrogenesis, may be

also involved in the trophic role of macrophages. Indeed, after transplantation of ferritin-loaded macrophages into intact spinal white, proliferating $NG2^{pos}$ neural stem cells migrate into the macrophage transplants and accumulate fluorescently labelled ferritin (Schonberg et al., 2012).

Central nervous system repair is sustained by macrophages that in parallel to their classic inflammatory roles, delivery specific cues to neural progenitor cells to ensure both resolution of inflammation and tissue repair.

Erythropoiesis – the erythropoietic island

50 year ago, macrophages have been found associated with maturating erythroblasts in the bone marrow, thus forming the erythroblastic island (reviews in (Manwani and Bieker, 2008; Chasis and Mohandas, 2008)). They form distinct anatomic structures composed of developing erythroblasts surrounding a central macrophage. Usually, islands include one or more synchronously maturing cohorts of erythroid cells undergoing four or five divisions between proerythroblast and normoblast stages. At the end of terminal differentiation, expelled nuclei of erythrocytes are phagocytosed by the central macrophage. Erythroblastic island provides a unique site for erythropoiesis, in which the central macrophage plays a crucial supportive role. Disrupting island integrity by disrupting links between maturating erythroblasts and macrophage leads to increased erythroblast apoptosis and to decreased proliferation, maturation and enucleation. Macrophage provide various interactions, including cell:cell binding and secreted factors to finely modulate erythropoiesis and erythroblast/cyte number through the control of proliferation, survival, terminal differentiation (promotion of enucleation and phagocytosis of extruded nucleus), iron transfer and nutrient supply (reviews in (Manwani and Bieker, 2008; Chasis and Mohandas, 2008)). Erythroblast macrophage protein (EMP) has been shown, through homophilic interactions, to prevent apoptosis of maturating erythroblasts (Hanspal et al., 1998). Later during erythropoiesis, EMP, which plays a role in actin organization, is involved in nucleus partitioning and its recognition by engulfing macrophage at the time of enucleation, phagocytosis being driven by MFG-E8. α4β1/VCAM1 and ICAM4/αv bindings are

also involved in island integrity. A soluble form of ICAM4 is secreted at terminal differentiation and may participate to mature erythrocyte detachment from the central macrophage (Lee et al., 2006). Iron transfer to maturating erythroblast is believed to occur through the central macrophage, since ferritin is localized between the two cell types and macrophages bear ferroportin, responsible of the export of iron (Manwani and Bieker, 2008). CD69, CD163 are also involved in the function of the erythroblastic island, as well as Ephrin2/EphB4 (in the proliferation of erythroblasts). Moreover, several locally secreted molecules positively and negatively regulate erythroblast maturation, such as IGF-1 (positive regulation), TGFβ, IL-6, TNFα, IFNγ (negative regulation). Indeed, macrophage may exert negative role on erythroblast survival. It has been shown that immature erythroblasts express the RCAS1 receptor (receptor binding cancer antigen expressed in Siso cells). Macrophages secrete soluble RCAS1, thus activating apoptosis in erythroblasts (Matsushima et al., 2001). This complexity of erythropoiesis regulation by macrophages in the island has been very recently highlighted by two *in vivo* studies aiming at specifically depleting macrophages under various conditions. Specific depletion of bone marrow macrophages (in the CD169-DTR mouse) triggers both a decrease in the number of erythrocytes/erythroblasts in the bone marrow and an increase of their lifespan. As a result, mice do not suffer from anaemia (Chow et al., 2013). In pathological conditions, the supportive role of macrophages has been clearly evidenced *in vitro* and *in vivo*. After an acute anaemia, macrophages are essential for recovery and erythrocyte development. Inversely, polycythemia, which is characterized by elevated erythropoiesis, is improved by macrophage depletion (in the CD169-DTR mouse or with clodronate-liposomes) (Ramos et al., 2013; Chow et al., 2013). The immune signature of macrophage in the erythroblastic island is very particular. These cells are very large (more than 15 µm diameter), do not express Mac1, and do express F4/80 and a series of markers, some of them not being usually expressed by macrophages in other tissues: CD16, CD32, CD64, CD4, CD31, CD11a, CD11c, CD18, and HLA-DR (Manwani and Bieker, 2008). Further studies will indicate whether this signature is altered under different conditions of erythropoiesis homeostasis, or upon inflammation.

Concluding remarks

From the recent studies investigating the roles of macrophages after injury in various tissues, some common features arise that suggest that post-injury inflammation follows a "wounding" or "healing" kinetics with the sequential presence of pro-inflammatory M1 then M2 macrophages. After a sterile injury, so in the absence of immune challenge, the M1 pro-inflammatory phase is likely very short and resolution of inflammation quickly takes place. Then the proresolving/healing M2 macrophages sustain tissue repair and/or regeneration (Lucas et al., 2010). The next challenges include the deciphering of both the molecular regulation of these macrophages subsets and their precise signalling on precursor cells in the tissues. This will be of importance for attempting of manipulation of the inflammatory compartment for the improvement of regeneration and of some diseases associated with chronic inflammation. In these contexts, both M1 pro-inflammatory and M2 resolving macrophages coexist and are not more able to promote tissue repair and homeostasis recovery.

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