Control of iron homeostasis as a key component of macrophage polarization

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In response to microenvironmental signals, innate recognition of tissue damage or pathogen exposure, and signals from activated lymphocyte subsets, macrophages undergo adaptive responses essential for a coordinated immune response, resistance to pathogens, and tissue repair. During the last few years increasing evidence has accumulated indicating that macrophage plasticity can be viewed as a spectrum of activation status between the classic pro-inflammatory (M1) program, induced by bacterial moieties such as lipopolysaccharides and the Th1 cytokine interferon-γ, and the alternative tissue repair-prone (M2) program, originally discovered as a response to the Th2 cytokine interleukin-4, mirroring Th1/Th2 polarization.[1] It is now appreciated that M2-like functional phenotypes can also be induced by other signals, including antibody immune complexes together with lipopolysaccharides/interleukin-1, glucocorticoids, transforming growth factor beta-β, and interleukin-10.[2]

Polarized macrophages differ greatly in expression of immunoregulatory genes and profoundly influence immune responses and tissue homeostasis.[3,4] M1 macrophages are characterized by high levels of pro-inflammatory cytokines (interleukin-12, interleukin-23, tumor necrosis factor-α) and an interleukin-12^{high}/interleukin-10^{low} phenotype, produce reactive nitrogen and oxygen intermediates, express high levels of major histocompatibility class II and co-stimulatory molecules, and display microbicidal activity. In this context, it is relevant to recall that M1 macrophages are also characterized by marked iron sequestration properties, which contribute to the cells' bacteriostatic effects.[5] M1 macrophages are part of polarized Th1 responses and mediate resistance to intracellular pathogens and tumors. Of note, under these activated conditions, M1 cells can also elicit tissue disruptive reactions. Conversely, M2 macrophages show increased phagocytic activity, high expression of scavenging, mannose and galactose receptors, production of ornithine and polyamines through the arginase pathway, and an interleukin-12^{low}/interleukin-10^{high} phenotype. In general, these cells participate in polarized Th2 responses, help in parasite clearance, dampen inflammation, promote tissue remodeling, and possess immunoregulatory functions.[6] Macrophages are also key elements linking inflammation and cancer, and tumor-associated macrophages are also characterized by an alternative-like activation phenotype.[7,8]

In addition to their role in immunity, macrophages are of central importance to body iron homeostasis, as the main iron supply for erythropoiesis derives from the iron recycled by macrophages after phagocytosis of senescent red blood cells.[9] Iron retention in the reticuloendothelial system is a well characterized response of body iron homeostasis to inflammation, as a host's attempt to withhold iron from the invading pathogens. This may eventually restrict iron availability for erythroid precursors and may contribute toward causing the common condition of inflammation-related anemia. However, recent studies have revealed that the role of macrophages in iron homeostasis is multifaceted and more complex than previously suspected.

In this issue of *Haematologica*, Corna and colleagues show that mouse macrophage polarization also affects iron homeostasis.[10] Similar results were reported earlier this year for human polarized macrophages,[11] indicating that differential iron management is a conserved functional property of human and murine polarized macrophages, differently from other functional aspects not conserved across species.[3] Moreover, a recent study showed that glucocorticoids polarize monocytes toward a M2 phenotype characterized by hemoglobin clearance and export of heme-derived iron.[12]

In their study, Corna and colleagues found that M2 cells have lower levels of H ferritin (Ft), the iron storage protein, and higher expression of membrane proteins involved in iron uptake, such as the transferrin receptor (TfR1) and the CD163 hemoglobin/haptoglobin receptor. Moreover, the high expression of ferroportin (Fpn), the only known iron exporter from cells, resulted in elevated iron release activity by M2 cells. In line with the expression of Ft and TfR1, the binding activity of the iron regulatory proteins (IRP), which post-transcriptionally regulate the expression of a number of iron genes,[13] was lower in M1 than in M2 cells. Since IRP-binding activity and the labile iron pool are usually inversely related,[14] this result apparently contrasts with the larger labile iron pool (measured by the calcein method) found in M2 cells. Interestingly, high Fpn mRNA levels counteracted the impaired translation of Fpn mRNA due to increased IRP-binding activity. This finding is in line with the results of a recent study which showed that the selective inactivation of IRP2 in mouse macrophages had no consequences on Fpn-mediated iron handling by macrophages, and thus put into question the role of IRP-mediated control of Fpn expression.[15] Overall, the analysis of M1 macrophages confirmed that pro-inflammatory stimuli trigger changes in gene expression (such as Fpn repression and Ft induction) favoring iron sequestration.[5,16] Conversely, it appears that M2 macrophages are characterized by an iron release-prone phenotype, and thus there are large differences in both intra- and extracellular iron availability between the two populations.

Iron sequestration in M1 macrophages operates as a bacteriostatic mechanism. The functional implications of the iron export activity of M2 macrophages are, in contrast, still undefined. Considering the evidence that M2 macrophages participate in the regeneration of acutely injured mouse skeletal muscle,[17] Corna and colleagues suggested that iron release from M2 macrophages could play a relevant role in muscle repair. Recently, Recalcati and colleagues[11] reported that conditioned medium of M2 macrophages sustained faster growth of malignant and non-malignant cell lines, and because iron is

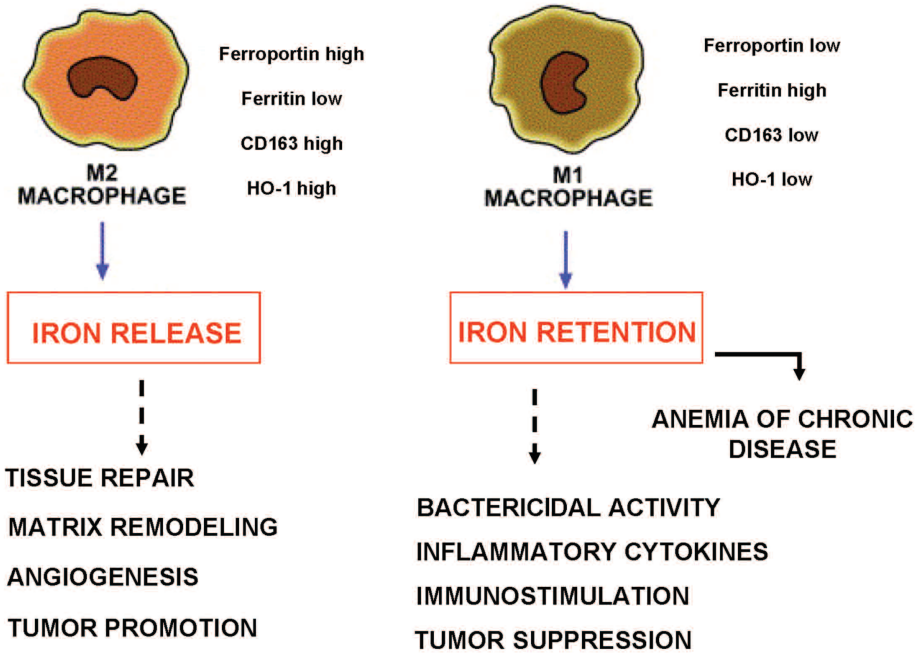

Figure 1. Schematic representation of iron uptake and export in polarized macrophages. M1 macrophages are characterized by the coordinated regulation of genes related to iron metabolism (ferritin^{high}/ferroportin^{low}) which results in iron retention. This is of relevance for their bacteriostatic properties but also represents the cellular basis for the anemia of chronic disease. Conversely, M2 macrophages are characterized by high levels of scavenger receptors (CD163) which enable efficient iron uptake, and a ferritin^{low}/ferroportin^{high} phenotype that supports iron donation to the microenvironment. This may contribute to tissue repair by providing iron to proliferating parenchymal cells and to fibroblasts for collagen synthesis, but also sustain tumor growth in the case of tumor-associated macrophages.

an essential cofactor for DNA synthesis they suggested that tumor-associated macrophages could provide iron to the microenvironment to sustain the high requirements of tumor cells. Fpn appears to play a key role in this process because the conditioned medium of M2 macrophages derived from a patient with loss of function Fpn mutation did not show the cell growth accelerating effect. The presence of functional Fpn on the plasma membrane appears to be a key determinant of iron release not only by macrophages but also by tumor cells. Interestingly, reduced Fpn expression (and hence higher iron content) has recently been found in breast cancer cells compared to in non-malignant breast epithelial cells.[18] Notably, in this extensive study it was also shown that Fpn levels in human tumors were inversely correlated with malignant potential and clinical outcome in large cohorts of breast cancer patients. Altogether, these studies highlight the importance of the Fpn-mediated control of iron availability in the tumor microenvironment.

It is now increasingly appreciated that beyond their long recognized role in promoting inflammation, macrophages undergo alternative activation producing phenotypes with completely different and in some cases opposite biological properties. Their role in tissue homeostasis and in a variety of pathological conditions, ranging from infectious diseases to tumors, has been recognized, and activating signals, surface markers, and molecular pathways associated with different forms of macrophage activation have been progressively characterized. Inspired by the intellectual framework of lymphocyte polarized activation during adaptive immune responses, our understanding of macrophage polarization has been dominated by immunological phenotypes and biological implications. Beyond the immunological phenotypes, the data from Corna and colleagues[10] and others[11,12] now focus our attention on the relevance of the macrophage metabolic profile during activation of these cells. In particular, iron management emerges as a metabolic signature of macrophage activation, with M1 cells committed to reduce iron availability to the microenvironment via a Ft^{high}/Fpn^{low} phenotype and M2 to increase iron availability to tissues via their Ft^{low}/Fpn^{high} phenotype.

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Non-erythroid effects of erythropoietin

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Erythropoietin (EPO) regulates red blood cell production by binding to its cell surface receptor, EPO-R, expressed on erythroid progenitor cells. Although EPO was originally believed to be an erythroid-specific hematopoietic cytokine, for over a decade, a substantial body of scientific evidence has accumulated to demonstrate that the biological effects of EPO are not limited to the erythron (Figure 1). In this issue of the journal, Lifshitz and colleagues[1] report on their most recent contribution to this field of research by demonstrating that, within the hematopoietic system, EPO may exhibit modulatory effects on macrophage number and function. The authors examined *in vivo* effects of EPO on splenic macrophages and inflammatory peritoneal macrophages, as well as *in vitro* effects of EPO on bone marrow-derived macrophages in culture. The experimental data show that splenic macrophage numbers were increased in mice in response to systemic EPO treatment. In transgenic mice engineered to constitutively over-express endogenous EPO, an even more significant increase in the number of splenic macrophages was observed, possibly as part of an adaptive mechanism leading to increased erythro-phagocytosis in severely polycythemic mice.[2] Inflammatory macrophages isolated from murine peritoneum displayed enhanced activation and phagocytic function, both following exogenous EPO treatment and in association with the over-expression of endogenous EPO, but without an increase in the number of macrophages migrating into the peritoneal cavity. The *in vivo* activity of EPO observed in these studies may be associated with direct effects on macrophages, indirect effects of EPO on other cell types that modulate macrophage number and function, or a combination of direct and indirect effects. Additional experiments by the investigators using cultured murine primary bone marrow-derived macrophages revealed enhanced activation and phagocytic function of the cells following EPO treatment. These direct EPO effects were associated with increased macrophage nitric oxide and interleukin (IL)-12 secretion, whereas IL-10 production was decreased, consistent with the generation of a pro-inflammatory phenotype and classical Th1 immune response.

The investigation of non-erythroid biological effects of EPO raises the question of the role of the erythroid receptor EPO-R, which is ubiquitously expressed at relatively low levels in many non-hematopoietic tissues. Lifshitz and colleagues addressed this issue in part by demonstrating that the newly discovered effects of EPO on macrophages were associated with the expression of EPO-R mRNA in cultured murine bone marrow-derived macrophages. The investigators further demonstrated the ability of EPO to mediate the increased phosphorylation of STAT proteins, as well as the induction of AKT and ERK2 phosphorylation and the nuclear translocation of p65 NFκB in macrophages. Although the direct effects of EPO on intracellular signal transduction and the induced changes in macrophage phenotype and function are presumably mediated in part by EPO-R, further studies will be necessary to delineate the structure of the cell surface receptor that mediates the effects of EPO in macrophages. Previous studies investigating non-erythropoietic EPO activities suggested that, in some experimental models, the tissue protective activity of EPO and of some EPO derivatives without erythropoietic activity may be mediated by a heteroreceptor complex between EPO-R and the common β receptor (βC-R) – a signal-transducing component of the cellular receptors for granulocyte-macrophage colony-stimulating factor, IL-3 and IL-5.[3,4] Other studies reported, however, that the βC-R may not be required for EPO-induced signal transduction and its cellular effects in some non-hematopoietic cells.[5,6] The detection of low levels of cell surface EPO-R on non-hematopoietic cells has been made possible by using a novel radiolabeled-EPO binding assay to demonstrate as few as 50 EPO binding