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Forum Editorial

Macrophage Migration and Function: From Recruitment in Vascular Disease to Redox Regulation in the Immune and Neuroendocrine Networks

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THE MACROPHAGE IS MORE THAN A "PHAGOCYTOSIS" MACHINERY

THE TERM "MACROPHAGE" is derived from the ancient Greek words "μακροσ" (macros; stands for large/big) and "φαγοσ" (phagos; stands for eater/glutton). Thus, literally, macrophages are cells specialized in "rapidly eating" surrounding material of a relatively "large" size. Yet macrophages are much more than cellular phagocytosis machineries. In fact, it is well known today that phagocytosis is only one of hundreds of distinct functions that a macrophage performs. For example, macrophage migration, although often a prerequisite for a subsequent phagocytotic process, is one other typical distinct macrophage activity that is closely associated with various functions that these cells pursue.

Macrophage biology goes back at least to the beginning of the century, but our specific understanding of "immune activation" of macrophages as part of our innate and cellular immune system dates from studies in the late 1950s and early 1960s from Waksman, Matoltsy, Mackaness, George, Vaughan, and colleagues (19, 29, 30, 48). These early studies were concerned with the antimicrobial activities of macrophages against mycobacteria and listeria and already included detailed observations on macrophage migration and phagocytosis.

Today, macrophages are understood as a heterogeneous population of resident and recruited phagocytic immune cells that are found in all organs and are involved in immune defense and tissue homeostasis. They express a variety of surface receptors, which mediate recognition of endogenous and microbial ligands. Activation of macrophages by microbial components and endogenous surface ligands, as well as numerous cytokines, induces a wide spectrum of pro- and anti-inflammatory settings, which are responsible for immune activation and deactivation (22).

Activation of macrophages occurs by both innate and acquired modes. Recently, five distinct types of macrophage activation have been proposed (21). Innate activation prototypically is induced by microbial toxins, such as endotoxin [lipopolysaccharide (LPS)], that activate the macrophage through the so-called pattern recognition receptors, the Toll-like receptors, in conjunction with CD14, LPS-binding protein, and several opsonic receptors. This kind of activation leads to the production of proinflammatory cytokines, α/β interferons, reactive oxygen species (ROS), and nitric oxide (NO), which serve to contain an infectious and harmful stimulus but, when deregulated, trigger a deleterious cascade that can eventually lead to exuberant chronic local inflammation or acute systemic processes, such as septic shock. Humoral activation of macrophages encompasses both acquired (through Fc receptors) and innate arms (through the antibody-independent complement pathway), with both triggering mechanisms leading to the activation of the cytolytic machinery of the macrophage and the secretion of a variety of pro- and antiinflammatory cytokines. The "classical" acquired activation mode is mediated by a priming stimulus of interferon-γ (IFN-γ), followed by microbial triggers such as from LPS, leading to major histocompatability complex (MHC) class II up-regulation, proinflammatory cytokine release [*e.g.,* interleukin-6 (IL-6), tumor necrosis factor (TNF), IL-1, macrophage migration inhibitory factor (MIF)], NO release, and activation of the respiratory burst. In addition, an "alternative" activation pathway for macrophages was recently defined that is characterized by macrophage activation through IL-4 and IL-13 and that involves antigen endocytosis. This pathway predominantly leads to MHC class II and mannose receptor up-regulation, but also features the activation of several chemokines and arginase by the macrophages (21). A fifth mode can be summarized as the innate/acquired deactivation pathway. Deactivation is initiated by uptake of apoptotic cells and lysosomal storage of host components, as well as by a variety of interactions with T cells, extracellular matrix components, and pathogens. Macrophages

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"stimulated" through this pathway respond by MHC class II down-regulation, antiinflammatory cytokine release [IL-10, transforming growth factor-β (TGF-β)], or prostaglandins.

Thus, together, important macrophage functions encompass cell migration, phagocytosis, production of ROS, NO, arginase, a battery of pro- and antiinflammatory cytokines, fibrogenic growth factors, collagenases, coagulation factors, cytolytic and microbicidal activities, but also participation in cellular immunity, humoral immunity, antiparasitic responses, and immunosuppression. These functions are normally part of the physiological immune and inflammatory response of the host that leads to the control and containment of pathogens and to tissue repair. However, deregulation can result in chronic and acute inflammation leading to septicemia, tissue damage including atherosclerosis and fibrosis, delayed-type hypersensitivity (DTH), or allergic reactions (6, 14, 20, 21).

MONOCYTE DIFFERENTIATION AND THE HETEROGENEITY OF MACROPHAGE-FAMILY CELLS

The marked phenotypic heterogeneity and functional specialization of macrophages are due to their cellular differentiation, tissue distribution, and differential responsiveness to a variety of endogenous and exogenous stimuli. In that respect, it is remarkable that the cells of the macrophage superfamily are probably all derived from circulating monocytes from which, by mere function, they often do not differ a lot and which, in turn, develop from a common myeloid progenitor through stimulation with lineage-determining cytokines, such as macrophage colony-stimulating factor or IL-3. Circulating blood monocytes can undergo three different fates: (a) they can become endothelium-resident macrophages; (b) they can convert into a resident macrophage in several tissues; and (c) they may get attracted for a "short-term" stay in the tissue to become a recruited macrophage. The latter two populations especially are often not easily distinguishable from one another. Similarly, differentiation by function between the circulating monocyte and the macrophage state *per se* is difficult, as both populations share numerous activities. Nevertheless, a range of parameters, in particular those within the local microenvironment of the terminal site, contribute to macrophage recruitment and tissue-specific phenotypes. These include surface and secretory products and the extracellular matrix of the neighboring tissue cells, as well as a variety of cytokines and chemokines from immune cells recruited to the tissue site ahead of the macrophage.

Resident macrophages can be Langerhans cells (in the skin), alveolar macrophages (in the lung), Kupffer cells (in the liver), mesangial cells (in the glomeruli), osteoclasts (in bone), or microglial cells (in the CNS). They cease to proliferate, but they have active gene expression activity. Resident macrophages normally die *in situ*, but they can be induced to migrate to lymph nodes or they may reenter the blood stream and differentiate into dendritic cells. Recruited macrophages can serve a range of functions, and they may be grouped into antigen-nonspecific elicited macrophages, alternatively antigen-activated macrophages, and classically antigen-activated cells. In contrast to the resident macrophages, it is now known that proliferation of recruited macrophages is not an unusual event, but prominently occurs in chronic local inflammatory processes. This is especially true for the recruited macrophages in atheromatous plaques (14). Three articles in this *Forum* are concerned with recruited macrophage activity during inflammatory activity under atherogenic conditions (41, 43, 46).

MACROPHAGE MIGRATION AS A KEY PROCESS IN MACROPHAGE DEVELOPMENT AND FUNCTION

Cell migration is a fundamental initial step during the development of tissue macrophages from monocytes that contributes to determining the final phenotype of a macrophage cell as it is controlled by endothelium- and tissue-specific adhesion molecules. Migration is thus a critical first step and also constitutes a central process through which macrophages can later on actively participate in host defense and tissue repair in a targeted fashion. This is why in this *forum* issue, macrophage migration is one of the central themes.

MACROPHAGE MIGRATION INHIBITORY FACTOR

Macrophage migration is historically linked to the protein by the name of "macrophage migration inhibitory factor" (MIF). In fact, MIF was one the first soluble immune mediators to be discovered in the beginning of the 1960s, and early work on macrophage activation and its role in disease was closely associated with studies on MIF (8, 15, 16, 19). The initial discovery of MIF as a secreted lymphocyte mediator that acted to inhibit the random migration of peritoneal macrophages out of capillary tubes not only set the stage for unraveling various other activities of macrophages, but was also part of the initial efforts at that time to understand cellular immune processes in general. As such, MIF was found to be a determining mediator of DTH reactions and other cell-mediated immune processes (8, 15). Subsequently, several cytokines were found to inhibit the migration of macrophages, and the T-cell supernatants studied in the early investigations on MIF, turned out, of course, to contain numerous protein species (28). The molecular identity of MIF remained obscure for close to a quarter of a century and was only to be affirmed 23 years later (50). Nevertheless, it turned out that the original observations on MIF were true and that a 12.5-kDa protein factor secreted from T cells had macrophage migration inhibitory properties and mediated DTH immunity (3, 4).

Today, MIF is known as a pleiotropic, mainly proinflammatory, cytokine. MIF is structually unique within the cytokine protein family and, lacking an N-terminal signal sequence, is secreted by an unconventional export pathway. It is ubiquitously expressed in a wide range of cell types and tissues, where it is usually present in a preformed manner. Constitutive expression of basal MIF levels and intracellular

localization (mostly cytosolic) have pointed to intracellular functions of MIF (9, 12, 28, 33). In fact, MIF modulates the activities of the coactivator and signalosome component JAB1/CSN5 and exhibits a catalytic thiol-protein oxidoreductase (TPOR) activity. With respect to the latter property, MIF shares both functional and structural homology with thioredoxin (Trx) (23, 25). Both proteins have a CXXC redox motif and, upon secretion, can function as cytokines (23, 35, 38, 39). Novel aspects of these cytokine functions of MIF and Trx are reported on in this *Forum* (7, 43), and one report discusses new findings on the structure-function properties of Trx as a monocytic chemokine (7). The extracellular, cytokine-like activities of MIF are mediated through the plasma membrane protein CD74 and in part via redox signaling (24, 26, 37). CD74 is the invariant chain (Ii) of MHC class II proteins; it has therefore been realized that MIF function is closely linked to macrophage activation in general (34).

Being a widely expressed cytokine that is predominantly secreted by monocytes, macrophages, and T cells, MIF contributes to host defense and tissue homeostasis. Moreover, MIF is a key mediator of the innate immune response of the host (12, 40) and controls the innate activation status of macrophages (see above). MIF's role in homeostasis is not restricted to the local tissue site. In contrast, some of its properties, such as glucocorticoid overriding and its secretion by endocrine tissues, suggest that this mediator is more widely involved in maintaining host homeostasis (2, 13). Due to its inflammatory properties, MIF is a key mediator of a number of immune and inflammatory diseases, such as septic shock, colitis, inflammatory lung disorders, atherosclerosis and vascular disease, tumorigenesis, and rheumatoid arthritis (for reviews, see 12, 28, 33). MIF's role in atherosclerosis is dealt with in two original research communications of this issue (43, 46) and is discussed in a review (41).

CHEMOKINES AND MIF AS REGULATORS OF INFLAMMATORY MACROPHAGE ACTIVITY IN VASCULAR DISEASE

Although a number of mediators, including growth factors, can regulate the migration of monocytes and macrophages, chemokines are the master regulators of this particular macrophage function. Chemokines are a family of small proteins that have chemotactic activity toward a wide range of cell types and that are characterized by a certain structural feature. That is, chemokines feature defined N-terminal cysteine residues. Depending on the number of residues that are situated between these cysteines, the CXC-, CC-, CXXXC, and C- subclasses of chemokines have been defined (31). Owing to their central role in monocyte recruitment and macrophage movement, chemokines have a pivotal role in diseases in which recruited tissue macrophages are involved. For example, chemokines are needed to induce the firm arrest of rolling monocytes by the activation of integrins and are centrally involved in monocyte recruitment in atherogenesis (49). In their *Forum* review, Schober and Weber focus on the mechanisms of monocyte recruitment in vascular repair after injury (41). The inflammatory response to acute vessel wall injury, such as upon angioplastic balloon dilatation, has increasingly been recognized to play a decisive role in neointima formation. In particular, the marked infiltration of monocytes aggravates neointimal growth and can thereby promote a state known as restenosis. The adhesion of circulating monocytes to the site of the mechanical injury represents the key event in monocyte recruitment. Therefore, Schober and Weber, highlight the role of adhesion molecules and chemokines in monocyte adhesion. They also pay attention to the recent observation that MIF is markedly up-regulated at inflamed vascular sites and seems to be a crucial player in monocyte recruitment and macrophage activation in atherogenesis and restenosis (11, 42). Verschuren *et al.,* in their original research communication in this issue, add another facet to this picture in demonstrating that MIF is up-regulated in human abdominal aortic aneurysms (46). These authors also confirm in their study earlier proposals that MIF could regulate matrix stability in atherosclerotic plaques (42).

Vascular smooth muscle cells (VSMCs) closely act in concert with monocytes/macrophages to aggravate the local inflammatory state in the atherogenic vessel wall (27). Schrans-Stassen and co-workers have picked up this feature of the atherogenic inflammatory reaction and have investigated the role of MIF in VSMC migration (43). Interestingly, they demonstrate that under certain stimulatory conditions, MIF can potentiate platelet-derived growth factor-BB-mediated VSMC migration. Thus, it is suggested that MIF not only acts to inhibit the random migration of macrophages, but, probably through an indirect mechanism, can amplify the chemotactic movement of cells toward inflammatory sites. Through the former mechanism, MIF could thus assist in the immobilization of macrophages at inflammatory loci, whereas by enhancing monocyte adhesion and directed cell migration, MIF appears to be part of the molecular recruitment machinery involving macrophages. Obviously, such a bimodal behavior is typical for cytokine functionality that, depending on the local microenvironment and temporal context, may affect cell activities in various ways.

CRUCIAL ROLE OF CHEMOKINES IN MACROPHAGE MIGRATION

Whereas Schober and Weber, Schrans-Stassen *et al.*, and Verschuren *et al.* have looked at and studied the biological and disease aspects of macrophage migration and the roles of chemokines therein (41, 43, 46), Bizzarri and co-workers in their original research communication have investigated the structure-function properties of a particular atypical chemokine (7). Although lacking the N-terminal consensus cysteine site, Trx was recently shown to behave functionally as a monocyte chemokine (5, 38). In this issue, the authors have extended their initial studies and have examined which of the cysteine residues present in Trx are required for the chemotactic activity of Trx toward monocytes. Moreover, they wanted to find out whether the structure-function profile of the five cysteine residues of Trx with respect to the chemotactic effect *per se* is discernible from that associated with another typical chemokine function, namely, desensitization.

Cells exposed to a chemokine or to another G protein-coupled receptor ligand will not respond to subsequent stimulation with the same chemokine or ligand (homologous desensitization) or with a different one (heterologous desensitization) (32). Bizzarri *et al.* find that, whereas the chemotactic effect of Trx is dependent on the presence of the CXXC redox center cysteine residues and on the presence of yet another cysteine, Cys^{62}, Trx-mediated desensitization toward MCP-1 chemotactic activation of monocyte activity is redox-independent. Of note, MIF and Trx thus seem to control cell behavior by distinct redox-signaling mechanisms. The molecular mode of this action is still unknown, as neither a Trx nor MIF receptor for these redox effects has been identified.

REDOX REGULATION BY MACROPHAGES IN TISSUE HOMEOSTASIS AND DISEASE

The redox state of both extra- and intracellular Trx switches between an oxidized disulfide form and a reduced dithiol form, depending on the redox state of the environment (35). The same is probably true for MIF (23). Redox dependency of Trx in controlling monocyte/macrophage migration (7) emphasizes an important mechanism by which the overall redox state of an organism, organ, tissue, or microenvironment can contribute to macrophage behavior. Two *Forum* reviews and two original research communications in this issue have more generally addressed the role of redox regulation on and by macrophages.

Alvarado *et al.* have directly studied the link between the overall redox state of the organism and macrophage function, including macrophage migration (1). By devising and applying a specialized animal model of young prematurely aging mice, they demonstrate that variation of the redox state of the organism (by supplying a diet enriched in several potent antioxidants) results in altered immune cell function (1). For example, macrophage migration and phagocytosis in this model are markedly increased upon dietary supplementation with antioxidant-containing biscuits. Similar effects were observed for lymphocytes and natural killer cells, indicating that a favorable dietary redox state may directly improve the immune status of the host, especially in aging individuals.

That the microglial cell is a specialized resident macrophage that is phenotypically adapted to the extraordinary environment of the CNS is discussed by Dringen (17). Upon activation, microglial cells produce a number of substances, including cytokines and proteases. Importantly, activated microglial cells release radicals, such as superoxide and NO, which are products of the enzymes NADPH oxidase and inducible NO synthase, respectively. Although in infection and upon other stress stimuli, circulating monocytes are also recruited to the CNS, the microglial cell constitutes the main cellular defense unit at this site. Accordingly, innate defense mechanisms, such as the oxidative burst and inducible NO synthase activity, are highly elaborated in these cells. In the *Forum* review by Dringen, particular attention is paid to yet another aspect of macrophage redox function. This is that activation of a defense mechanism by a microglial cell or macrophage "is not only a good thing," but "can do considerable harm" to the macrophage itself and surrounding cells. This two-sided coin principle was already eluded to, when the dual role of inflammatory macrophage cytokines, such as TNF or MIF, was discussed in the context of their roles in host defense and disease (see above). Dringen discusses, for the microglial cell, that raising a respiratory burst and NO response confronts these resident macrophage cells with chemically hazardous radicals, as well as their reactive reaction products hydrogen peroxide and peroxynitrite. Although microglial cells produce only a fraction of the superoxide radicals that are produced by neutrophils, the damage may be substantial. Together, these agents have been implicated in oxidative cell damage and neuronal cell death in neurological diseases, such as Alzheimer's disease and Parkinson's disease. However, for self-protection, microglial cells are equipped with efficient antioxidative mechanisms. These cells contain reduced glutathione in high concentrations and antioxidative enzymes such as superoxide dismutase or glutathione reductase. Their superior antioxidative potential protects microglial cells against oxidative damage. Thus, macrophage functions not only encompass a diverse range of activatory responses, but include efficient built-in safety features. As discussed earlier in this Editorial, this may more generally be the innate/acquired deactivation pathway of macrophages or, at the mere level of the cytokine response, the production and secretion of the antiinflammatory cytokines IL-10 and TGF-β. Again, it appears that the widely expressed macrophage mediator MIF may obey this principle, too. Short-term activation by low concentrations of MIF, as encountered in the beginning of an elicited immune response, are proinflammatory, whereas higher doses later on may be involved in antioxidative redox signaling (10, 37). Our current knowledge on the redox-regulating properties of MIF is summarized in the *Forum* review by Thiele and Bernhagen (45).

Down-regulation of an originally actively raised macrophage response is also a central theme in the original research communication by von Knethen and Brüne in this issue (47). These authors have investigated the so-called desensitized phenotype that monocytes/macrophages exhibit during prolonged or chronic inflammatory conditions. For example, this phenotype is characterized by attenuated ROS production and is observed in monocytes derived from the blood of septic patients. Desensitization is associated with a depletion in monocyte/macrophage signaling of a prominent kinase, protein kinase Cα (PKCα). As the initiating stimulus for these effects has been unknown, the authors have examined the stimulation mechanisms and kinase responses in more detail. Interestingly, IFN-γ, the cytokine that is responsible for kick-starting the "classical" acquired mode leading to macrophage activation, was found by von Knethen and Brüne to mediate macrophage desensitization. IFN-γ, but not the prototypical activators of innate macrophage responses, LPS or gram-positive bacterial toxins, led to a dose-dependent depletion of PKCα (47). IFN-γ-mediated PKCα depletion was dependent on prior phosphatidylcholine-phospholipase C activation. Importantly, in line with the notion that PKCα mediates NADPH-oxidase assembly and ROS production in macrophages, it was observed that depletion of PKCα by IFN-γ concomitantly attenuated superoxide radical formation. Thus, in this article, another mechanism was identified by which

macrophages can protect themselves from their own potentially hazardous prooxidative processes.

FUTURE DIRECTIONS

Naturally, any scientific representation on macrophages can only feature and discuss a minute portion of the complex biological network governing macrophage function and migration. In focusing on macrophage migration, on the role of redox regulation for macrophage activation and deactivation, and by highlighting just one of the numerous inflammatory diseases associated with deregulation of macrophage function, in this issue it is attempted to exemplify the complexity of this cellular system. Clearly, in the future, the great variety of macrophage functions will need to be explored more broadly by systemic methodological approaches, such as macrophage transcriptomics and proteomics. Although these are already being pursued (18, 36, 44), it is obvious that today the processes regulating macrophages are only partially understood. For example, macrophages can enter the uninjured CNS, but why are they refractory to inflammatory stimuli, such as injected chemokines? Infectious stimuli can overcome this deactivated state, probably through the intermediate activation of microglial cells, which, as locally adapted resident macrophages, can respond differently. Such stimuli can then enhance monocyte migration to expand the population of recruited macrophages. What are the desensitizing stimuli? What is the molecular identity of the promigratory stimulants that lead to macrophage recruitment? Is IFN-γ-mediated desensitization involved initially, and then overcome by an exuberant innate stimulus? Again, these questions can only exemplify the numerous questions that need to be addressed in the future to understand better the role and functioning of macrophages. The recent discovery of Trx as a novel regulator of macrophage migration as eluded to in this *Forum* is a surprising example that indicates the necessity for broader approaches to identify yet unrecognized regulatory mechanisms of macrophage functions.

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ABBREVIATIONS

CXXC, Cys-Xaa-Xaa-Cys redox motif of TPOR proteins; DTH, delayed-type hypersensitivity; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharide/endotoxin; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; NO, nitric oxide; PKCα, protein kinase Cα; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; TPOR, thiol-protein oxidoreductase; Trx, thioredoxin; VSMC, vascular smooth muscle cell.

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