Macrophages and Inflammation

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Inflammation is a normal physiological response to an infection or injury, such as aggression by microbes, trauma, or heat and radiation. Inflammation works to maintain homeostasis and is a highly regulated process with both pro- and anti-inflammatory components to ensure the prompt resolution of noxious conditions. In the initial stages of inflammation, macrophages destroy the abnormal stimuli, and remove the apoptotic bodies of the dead neutrophils as well as any remaining hazard factor. The macrophages then present the antigen to T lymphocytes to initiate the mechanisms of acquired immunity, which leads to the production of antibodies, cytokines and memory cells. The macrophage activity then switches from pro-inflammatory to anti-inflammatory to remove any elements of aggression, thereby achieving homeostasis. Macrophages play a key role in the innate immune response and form a bridge between the innate and acquired immune response. In certain circumstances, however, when chronic inflammation is produced, macrophages may have a harmful effect and cause lesions. Therefore, inflammation is the classic “double-edged sword”, in which macrophages cut both ways. Activated macrophages have two different phenotypes related to different stimuli: M1 (classically activated) and M2 (alternatively activated). M1 macrophages are pro-inflammatory and play a key role in the host defense mechanism, while M2 are associated with the responses to anti-inflammatory reactions and tissue remodeling. The transformation of different phenotypes of macrophages regulates the initiation, development, and cessation of inflammatory diseases. An imbalance of macrophage M1 ~ M2 polarization is often associated with a range of diseases or inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus. (J Rheum Dis 2018;25:11-18)

Key Words. Macrophage, Inflammation, Disease, Rheumatoid arthritis

INTRODUCTION

Inflammation is an adaptive response to noxious stimuli and conditions such as infection and tissue injury, and serves the purpose of repairing any damage and returning the damaged tissue to healthy state. In the early stages of inflammation, the size of the vessels and the release of liquids increase around the inflammatory loci. Afterwards, diverse kinds of cells reach these loci: neutrophils (first 24 hours), macrophages (48 hours), and lymphocytes (several days later). In this stage, macrophages express a high density of surface pattern recognition receptors, and like neutrophils, respond rapidly to the presence of microbes and variable stimuli in order to initiate innate immunity by removing apoptotic bodies of dead neutrophils or tissue damages; macrophages then present antigen to T lymphocytes to initiating the mechanisms of acquired immunity by which antibodies, cytokines, and memory cells are produced. In addition, macrophages secrete over 100 kinds of proteins that mediate host defense and inflammation, including potent cytokines. They also participate in the regulation of inflammation by turning to tissue replacement and remodeling. Therefore, a controlled inflammatory response is beneficial for restoration of homeostatic balance. In contrast, disruption of this process can lead to chronic inflammation. This self-perpetuating mechanism is now recognized as the basis of a wide range of diseases, including rheumatoid arthritis, inflammatory
bowel disease, asthma, atherosclerosis, neurodegenerative diseases, and cancer, all of which may disrupt the transition to repair processes and the restoration of normal tissue architecture [1].

In this review article, we therefore focus on the latest advancements in our knowledge of macrophage and its role in inflammation and as a bridge between innate and acquired immunity.

**MAIN SUBJECTS**

**Macrophage in inflammation**

Acute inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of infection or injury [2]. This response is best characterized in cases of microbial infections (particularly bacterial infections), which involves receptors of the innate immune system including toll-like receptors (TLRs) and nucleotide-binding oligomerization-domain protein-like receptors 3 [3]. This initial recognition of infection is mediated by tissue-resident macrophages and mast cells that mediate the production of a variety of inflammatory mediators such as chemokines, cytokines, vasoactive amines, eicosanoids and products of proteolytic cascades.

Chronic inflammatory conditions such as rheumatoid arthritis, type 2 diabetes, and cardiovascular disease are less affected by the classic instigators of inflammation—infection and injury. Instead, they are more associated with malfunction of tissues that result in homeostatic imbalance of one physiological systems that are not directly related to host defense or tissue repair [4]. Regardless of the cause, inflammation is presumed to have evolved as an adaptive response for restoring homeostasis, a process that is primarily carried out by macrophages. Therefore, we will emphasize the diverse sequential roles of macrophages as the conductor of inflammation.

**Macrophage as an inducer of the inflammation**

Macrophages originate from bone marrow and reach body tissues by infiltrating through blood vessels in order to act as guards against various kinds of damage [5]. Under normal conditions, most macrophages are removed by apoptosis and only a small portion are differentiated in response to certain stimuli and become mature cells or tissue-specific cells such as Kupffer cells, microglia, etc. Resting macrophages produce only low levels of pro-inflammatory mediators. When an inflammatory process occurs, tissue-resident and recruited macrophages proliferate, differentiate, or become activated under the effect of interleukins or growth factors. Subsequently, they take on the role of antigen-presenting cells, cease to respond to proliferative stimuli and become efficient phagocytes, a process by which they remove unwanted material including apoptotic cells. Macrophages then secrete various types of cytokines and chemokines that direct inflammatory responses and aid in tissue repairing process [5]. In certain circumstances such as chronic inflammation, macrophages have a destructive effect and cause lesion.

A successful acute inflammatory response results in the eradication of the infectious agents followed by resolution and repair phase, which is mainly mediated by tissue-resident and recruited macrophages [1]. If acute inflammatory response fails to eliminate the pathogen or tissue damage, neutrophil infiltrate is replaced by macrophages, and in the case of infection also with T cells. If the combined effect of these cells is still insufficient, a chronic inflammatory state ensues, which involves the formation of granulomas and tertiary lymphoid tissues. Unsuccessful attempts by macrophages to engulf and destroy pathogens or foreign bodies can lead to the formation of granulomas, in which the invaders are separated by layers of macrophages in a final attempt to protect the host [2]. In addition to persistent presence of pathogens, chronic inflammation can result from other causes of tissue damage such as autoimmune responses (owing to the persistence of self-antigens) or undegradable foreign bodies.

Macrophages have several different phenotypic states and acquire distinct functional phenotypes as directed by tissue types and environmental cues [5]. This enables the macrophage to play multiple roles in inflammatory response and repair of tissue, both encouraging and discouraging these processes. The heterogeneity in function is thought to be pivotal to the successful resolution of inflammation and the restoration of healthy tissue.

**Polarization of macrophages**

Macrophages are the chief cells in most tissues, and their numbers increase immensely in inflammation, autoimmune diseases, and cancers. Their progenitor cell is CD34+ cells in the bone marrow, which differentiates into monoblasts and then into pro-monocytes, and finally into monocytes (M0) that are released into the bloodstream. In the role of macrophage colony-stimulating fac-
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In response to various environmental signals (e.g., microbial products, damaged cells, activated lymphocytes) or under different pathophysiologic conditions, macrophages can acquire distinct functional phenotypes by undergoing different phenotypic polarization. When macrophages are recruited into tissues, they become “activated macrophages” and can have two different phenotypes related to different stimuli: M1 (classically activated) and M2 (alternatively activated) (Figure 1) [7].

Microbial products or pro-inflammatory cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF), and TLR ligands stimulate M1 phenotype which is characteristics of high antigen presentation, high production of interleukin (IL)-12 and IL-23, and high production of nitric oxide (NO) and reactive oxygen intermediates. They are generally involved in removal of intracellular pathogens and resistance to tumors in the context of TH1-driven responses and chronic M1 macrophage activation can cause tissue damage, especially under aseptic condition [8]. In contrast, M2 activated macrophages are effectors of resistance to parasites, harbor immunoregulatory properties, promote tumor growth and invasiveness, and orchestrate tissue repair and remodeling (including fibrosis) [9]. M2 can be classified into M2a, M2b, M2c and M2d subtypes. M2a macrophages are activated by IL-4 and/or IL-13 and secrete a series of chemokines that promote the buildup of Th2 cells, eosinophils, and basophils. M2b macrophages are activated by combination of TLR, IL-1R ligand or immune complexes. They secrete high levels of IL-10, but also proinflammatory cytokines such as TNF and IL-6, and express iNOS. In addition, M2b macrophages elicit a Th2 response by recruitment of eosinophils and Tregs. M2c macrophages are induced by IL-10, transforming growth factor (TGF)-β and glucocorticoid. They secrete high levels of IL-10 and TGF-β, including M2b macrophages and so-called ‘regulatory macrophages’. They also express high levels of arginase and promote tissue regeneration and angiogenesis [10]. M2d macrophages are induced by co-stimulation with TLR and adenosine A2A receptor agonists, which are characterized by pro-angiogenic capacity (Table 1) [11].

Several functional properties discriminate M1 from M2 polarized macrophages, including their repertoires of cytokines (IL 12 hi IL 23 hi IL 10 lo vs. IL 12 lo IL 23 lo IL 10 hi), chemokines (chemokine [C-X-C motif] ligand [CXCL]9 and CXCL10 vs. chemokine [C-C motif] ligand [CCL]17 and CCL22), micro-RNA (miR 155 vs. miR 223), iron, glucose, and folate metabolism, scavenger receptors and mannose receptors.

### Table 1. Subset of macrophage polarization

<table>
<thead>
<tr>
<th>Variable</th>
<th>M1</th>
<th>M2a</th>
<th>M2b</th>
<th>M2c</th>
<th>M2d</th>
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<tbody>
<tr>
<td>Stimulus</td>
<td>Lipopolysaccharide (LPS)</td>
<td>IL-4, IL-13</td>
<td>TLR, IL-1R</td>
<td>IL-10, TGF-β</td>
<td>TLR, A2A</td>
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<td>PAMPs/DAMPs</td>
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<td>IFN-β and IFN-γ</td>
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<td></td>
<td>GM-CSF</td>
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<tr>
<td>Products</td>
<td>TNF-α</td>
<td>IL-10</td>
<td>IL-6, IL-10</td>
<td>IL-10, TGF-β</td>
<td>IL-10, VEGF</td>
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<td></td>
<td>IL-23</td>
<td>Arg-1</td>
<td>IL-1-β</td>
<td>Low TGF-β</td>
<td>Low TNF</td>
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<tr>
<td></td>
<td>IL-12</td>
<td>Chi313</td>
<td>low IL-12</td>
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<tr>
<td>Functions</td>
<td>Release proinflammatory</td>
<td>Debris removal</td>
<td>Regulatory macrophages</td>
<td>Regulatory macrophages</td>
<td>Proangiogenic capacity</td>
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Toll-like receptor signaling, particularly TLR4 stimulated by lipopolysaccharide (LPS) and other microbial ligands, preferentially drives macrophages to the M1 phenotype. Two adaptors—MyD88 and TRIF—mediate the downstream signaling of TLR4. The signaling pathway through the MyD88 adaptor results in the activation of a cascade of kinases including interleukin-1 receptor-associated kinase (IRAK)4, TNF receptor-associated factor (TRAF)6, and IκB kinase (IKK)b, which leads to the activation of nuclear factor kappa B. Signaling pathway via the TRIF adaptor pathway promotes the transcription factor interferon-responsive factor 3 (IRF3), leading to the expression and secretion of type I interferon such as IFN-α and IFN-β. Secreted type 1 interferons bind to type I interferon receptor with consequent activation of the transcription factor signal transducer and activator of transcription (STAT)1. IRF3 and IRF5 are involved in regulation of M1 polarization and M1-associated gene induction [12]. Members of the suppressor of cytokine signaling (SOCS) family regulate STAT-mediated activation of macrophages. In macrophages, SOCS proteins not only modulate signaling through TLRs but also regulate the sensitivity of cells toward cytokines. Because SOCS3 is a downstream molecule of Notch signaling [13], it is likely that Notch signaling determines M1 ~ M2 polarization through SOCS3 [14]. However, the role of SOCS3 in modulating macrophage M1 ~ M2 polarization is under debate: although the unique expression pattern of SOCS3 was reported to be crucial for classic macrophage activation [15], SOCS3 paucity also encourages M1 macrophage polarization and inflammation [16].

IL-4 and IL-13 activate macrophages to have M2 phenotype by triggering STAT6 through the IL-4 receptor alpha (IL-4R α), whereas IL-10 activates M2 phenotype via initiating STAT3 signaling pathway through its receptor (IL-10R). In IL-4 and IL-13 pathway, receptor binding of IL-4 activates janus kinase (JAK)1 and JAK3 [17], leading to STAT6 activation and translocation. Several transcription factors, including peroxisome proliferator activated receptor γ (PPAR γ) and Krueppel-like factor 4 (KLF-4), promote macrophage M2 phenotype as well [18,19].

Hypoxia achieves its effect on macrophages through two isoforms of hypoxia-inducible factor (HIF)—HIF-1α and HIF2. HIF-1α is able to mediate the effects of tumor-derived lactic acid and cytokines ( Oncostatin M and Eotaxin) in promoting M2-like phenotype. In contrast, myeloid-specific HIF-2α deletion demonstrated the role of HIF2 in mediating macrophage inflammatory responses rather than HIF-1α [20,21]. Functional miRNAs associated with polarized macrophages have been identified. The expression of miR-155 is found to be repressed in naïve macrophages or LPS-stimulated Akt2/-/- macrophages. In this process, miR-155 targets transcriptional factor C/EBP-β, a hallmark of M2 macrophages [22,23]. Overexpression and depletion of miR-155 promotes macrophages to M1 or M2 phenotype, respectively confirming that miR-155 plays a central role in regulating Akt-dependent M1/M2 polarization of macrophages. It has also been reported that miR-155 can directly block IL-13-induced macrophage M2 phenotype via suppressing the expression of IL-13R α1 [23]. Taken together, this hypothesis supports the idea that miR-155 is a key molecule in causing macrophage polarization toward M1-type activity. Zhuang and colleagues [24] reported that miR-
223 modulates obesity-associated adipose tissue inflammation by regulating macrophage activation; the authors found that miR-223 was upregulated in LPS-treated macrophages but downregulated in IL-4-treated bone marrow-derived macrophages. In agreement with the observation of differential expression of miR-223 in various macrophages, the miR-223-deficient macrophages were hypersensitive to LPS stimulation, whereas such macrophages showed delayed responses to IL-4 compared with controls. Moreover, miR-223-deficient mice exhibited increased adipose tissue inflammatory response but decreased adipose tissue insulin signaling. Based on these results, miR-233 seems to promote macrophage to M2 phenotype.

Transition between the M1 and M2 phenotypes

During disease, transition of macrophage can decrease bactericidal function and induce allergic Th2 reactions. However, if phenotype does not change from the M1 to the M2 type at the end of an inflammatory reaction after the elimination of pathogenic microbes, excessive production of the proinflammatory M1 mediators, followed by tissue damage, and inflammatory diseases can develop [25]. Upon resolution of inflammation, switching from the M1 to the M2 phenotype occurs under M2 stimulus to keep from excessive inflammation. Also, switching from the M2 to the M1 phenotype prevents from allergic Th2 reactions and increases bactericidal activities of macrophage [26]. So this transition is a gradual and ongoing process. However, in many cases, M1 phenotype can be switched to the M2 phenotype even before inflammation comes to an end, whereas the M2 phenotype can be transformed to the M1 under anti-inflammatory M2 stimuli. This condition could be referred to as paradoxical plasticity [26]. Obviously phenotype transition may play an important role in the inflammatory, autoimmune, oncologic, and other diseases. Thus, our understanding of the mechanism underlying phenotype switching will assist in ameliorating impaired immunity.

Macrophages as a sentinel of inflammation in variable cell states

When tissues are under stress or are malfunctioning, they send a different set of signals to tissue-resident macrophages which in turn produce increased amounts, or different sets, of growth factors and other signals that are relevant for the particular tissue. When the stress or malfunction is extreme, local macrophages may be insufficient to resolve the situation, in which case additional macrophages might be recruited. Thus, malfunctioning adipocytes in obese animals secrete chemokine CCL2, which recruits more macrophages to the adipose tissue [27]. The main purpose of these interactions is to help the tissues adapt to the stressful conditions and restore their functionality. If tissue malfunction or stress is even more excessive and adaptation is no longer possible, the cells die by apoptosis or necrosis. Macrophages monitor and interpret the different kinds of cell death, and in addition to the removal of apoptotic and necrotic cells, macrophages make one of several possible ‘decisions’, ranging from the silent removal of dead cells to the induction of inflammatory response. Because necrotic cell death is generally associated with tissue damage, the outcome of necrotic cell recognition by macrophages is usually an inflammatory response [4,28]. Apoptosis can occur for several reasons, and macrophages therefore need to be able to decipher the cause of death to take the appropriate course of action. Thus, different outcomes are possible for diverse situations through which apoptosis occurs (Figure 2). Apoptosis induced by inflammatory or immune responses should have an opposite effect to infection-induced apoptosis, and in this case, the recognition of apoptotic cells by macrophages results in the induction of anti-inflammatory and immunosuppressive pathways [29].

Macrophages and rheumatoid arthritis

Macrophages also play a key role in the pathogenesis of rheumatoid arthritis (RA). Increased number of macrophages found in the synovial tissue can be activated to produce inflammatory cytokines and then contribute to the cartilage and bone destruction. Therefore, the amount of synovial sublining macrophages can be used as a biomarker for disease severity as well as a predictor of responsiveness to disease-modifying anti-rheumatic drug (DMARD) therapy [30]. A strong correlation between number of macrophages, the mean change in the disease activity score, and the degree of joint erosion has been demonstrated [31,32]. There is a lack of evidence for macrophage polarization in either direction in the inflamed joint; however, TNF and IL-1, which are characteristically released in higher quantities by M1 phenotype, are abundant in RA, whereas IL-10 activity, which is characteristic of M2, is relatively diminished in patients with RA [33]. Macrophages in patients with highly active RA show a prevalence of M1 phenotype; on the other hand, macrophages in RA patients with low disease activity
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Figure 2. Macrophage and apoptosis. Apoptotic cells are recognized by their lipid phosphatidylserine (blue) on the plasma membrane and, subsequently, are removed by phagocytosis. In addition, apoptotic cells produce other signals (colored rectangles) that control the outcome of their recognition by macrophages, but its consequences are likely to rely on the cause of cell death.

score or clinical remission predominantly have M2 phenotype. Furthermore, steroid induce the M2 state, and DMARDs such as methotrexate and leflunomide induce M2 state as well; moreover, they impede cell replication and recruitment of immature and inflammatory monocytes to the sites of inflammation [34]. Recently, Choi and colleagues [35] reported that proinflammatory M1-polarizing stimuli and hypoxic conditions induces nuclear factor of activated T-cells 5 (NFAT 5) expression so that enhances chronic arthritis by conferring apoptotic resistance to activated macrophages. Recent animal study reported the therapeutic efficacy in rheumatoid arthritis by targeting macrophage polarization by systemic delivery of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs). In a collagen-induced arthritis mouse model, hUCB-MSCs polarize naive macrophages toward an M2 phenotype by regulating the actions of cyclooxygenase-2 and TNF-stimulated gene/protein 6 responding to TNF, and further by inhibiting the activity of nucleotide-binding domain and leucine-rich repeat pyrin 3 inflammasome. hUCB-MSCs alleviated the severity of arthritis to a similar degree as etanercept-treated groups [36]. Targeting specific macrophage subsets present a potential novel therapeutic strategy for RA, but this approach should be preceded by incorporating a broad understanding of the nature of RA and factors such as age, gender, genetics, diet, microbiology, and medication.

Macrophages and SLE

Systemic lupus erythematosus (SLE) macrophages cannot remove apoptotic cells appropriately. In SLE, there is an altered pro-/anti-inflammatory macrophage status that induces overproduction of inflammatory cytokines such as TNF-α, IL-6, IL-10, and type I IFNs [37,38]. They present self-antigens to autoreactive T cells rather than the immunosilent presentation normally associated with material from apoptotic cells [38]. Moreover, myeloid cells (including dendritic cells) induce overproduction of type I IFNs that lead to overproduction of antibodies (classes immunoglobulin (Ig)G, IgA, IgM) and class-switching from B cells [38]. Several markers of M1 macrophages are elevated in SLE macrophages, which correlates with the severity of renal pathology: IFN-γ, IL-6, CCL2, and CXCL10 from circulating macrophages; CXCL10 from neurological lupus macrophages; and CCL2 from intrarenal macrophages. M1 macrophages are also favored by the milieu they reside in. SLE serum contains copious amounts of TNF-α, GM-CSF, and IFN-γ,
macrophages respond to the environment by acting as a “danger signals” for macrophage cell signaling pathway [39]. Specific B cell subtypes in the marginal zone of the spleen, so-called marginal zone B cells, are involved in SLE pathogenesis. Marginal zone B cells are in close contact with highly phagocytic macrophages called marginal zone macrophages, which are characterized by high expression of class A scavenger receptor macrophage receptor with collagenous structure (MARCO) and scavenger receptor A (SR-A). MARCO and SR-A bind a variety of self- and foreign-ligands, and marginal zone macrophages activate marginal zone B cells through MARCO and SR-A [40]. Taken together, the role of macrophages in SLE pathogenesis has gained attention and deserves more focused research.

CONCLUSION

Macrophages are an integral part of the innate immune system and provide protection against pathogens and noxious stimuli. Activated macrophages have two different phenotypes related to different stimuli: M1 (classically activated) and M2 (alternatively activated). M1 macrophages release high levels of pro-inflammatory cytokines, reactive nitrogen and oxygen intermediates to eliminate microorganisms and tumor cells; on the other hand, M2 macrophages are involved in resolution of inflammation through phagocytosis of apoptotic neutrophils, reduced production of pro-inflammatory cytokines, and increased synthesis of mediators important for tissue remodeling, angiogenesis, and wound repair. Macrophages remove apoptotic load, handle cellular stress that might be associated with the initiation of an inflammatory response, and eventually achieve homeostatic control. Macrophages are also involved in rheumatic diseases, and their role in different rheumatic diseases is diverse according to M1/M2 phenotype. It is thought that heterogeneity in function of macrophage is important for successful resolution of inflammation and renewal of healthy tissue. Therefore, more research on macrophage and their role in inflammation is needed.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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