Minireview

Control of Macrophage Dynamics as a Potential Therapeutic Approach for Clinical Disorders Involving Chronic Inflammation

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ABSTRACT

Macrophages are a well recognized player of both innate and adaptive immunity and have emerged as a key regulator of systemic metabolism, hematopoiesis, vasculogenesis, apoptosis, malignancy, and reproduction. Such pleiotropic roles of macrophages are mirrored by their protean features. Upon environmental challenges, macrophages redistribute and differentiate in situ and contribute to the multiple disease states by exerting protective and pathogenic effects. The environmental challenges include cytokines, chemokines, lipid mediators, and extrinsic insults, such as food and pathogenic bacteria. In addition, homeostasis and the activation state of macrophages are influenced by various metabolites from a commensal microbe that colonizes epithelial and mucosal surfaces, such as the lungs, intestines, and skin. In this review, we describe macrophage differentiation, polarization, and various functions in chronic disease states, including chronic inflammatory bowel disease, tumorigenesis, metabolism and obesity, and central nervous system demyelinating disorders. Controlling the macrophage dynamics to affect the pathologic states is considered to be an important therapeutic approach for many clinical disorders involving chronic inflammation.

Introduction

Macrophages are widely distributed in many organ systems and function as key regulators in both innate and adaptive immunity. They are extremely versatile cells and are exquisitely attuned to their microenvironment. Inflammation resulting from pathogenesis or tissue damage activates resident macrophages to serve as a primary source of proinflammatory cytokines and other inflammatory mediator production. On the other hand, macrophages are equally critical in the resolution of inflammation by producing anti-inflammatory cytokines and ingesting pathogenic microorganisms due to their phagocytic activity. Thus, macrophages play a pivotal role in not only tuning inflammation, but also functioning in constructive processes, such as wound healing and tissue repair.

Macrophages are recruited from precursor monocytes in the circulation, which, in turn, are derived from stem cells in the embryo and bone marrow (Gordon and Taylor, 2005; Mosser and Edwards, 2008). Although distinct subpopulations of monocytes may exist, once recruited to a site of injury or infection, these monocytes differentiate into macrophages, with functionally and phenotypically discrete populations, depending on the cytokine environment (Gordon and Taylor, 2005; Mosser and Edwards, 2008; Lawrence and Natoli, 2011). On the basis of the type 1/type 2 helper T-cell polarization concept (Romagnani, 2000), phenotypically polarized macrophages are now generally classified into two major subtypes termed proinflammatory M1 and anti-inflammatory M2 (Gordon, 2003; Martinez et al., 2008). Macrophages exposed to microbial products and interferon (IFN)-γ become classically activated macrophages (M1), which produce copious amounts of proinflammatory cytokines and chemokines and function predominantly in inflammation, tissue damage, killing of intracellular microbes, and increased tumoricidal activity (Gordon, 2003). Conversely, alternative activation

ABBREVIATIONS: AD, Alzheimer’s disease; CCR2, CC chemokine receptor 2; CNS, central nervous system; CSF, colony stimulating factor; CSF1R, colony stimulating factor 1 receptor; FFA, free fatty acid; HFD, high-fat diet; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MS, multiple sclerosis; PD, Parkinson’s disease; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; Th, T helper; Tie2, tyrosine kinase with Ig-like loops and epidermal growth factor homology domain-2; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α; TZD, thiazolidinedione; VEGF, vascular endothelial growth factor.
of macrophages is canonically defined as that induced by certain stimuli, such as interleukin (IL)-4, IL-10, IL-13, and glucocorticoid (Gordon and Martinez, 2010). Alternatively activated macrophages (M2) are characterized by the production of anti-inflammatory cytokines and expression of cell-surface markers, such as CD206, Ym1, and arginase-1 (Mantovani et al., 2004; Mosser and Edwards, 2008; Ho and Sly, 2009), and are principally associated with allergic and parasitic immune responses, tissue remodeling, angiogenesis, tumor promotion, and humoral immunity (Gordon, 2003). The anti-inflammatory function of M2 macrophages has been confirmed by a recent report showing that endotoxemic lung inflammation can be exaggerated in M2 macrophage-depleted transgenic mice (Kambara et al., 2015). M2-polarized macrophages are categorized into three subtypes, M2a, M2b, and M2c, based on gene expression profiles (Mantovani et al., 2004). The M2a subtype is elicited by IL-4 or IL-13, the M2b subtype is elicited by IL-1 receptor ligands or exposure to immune complexes plus lipopolysaccharide (LPS), and the M2c subtype is elicited by anti-inflammatory stimuli, such as glucocorticoid hormones, IL-10, and transforming growth factor-β (TGF-β) (Hao et al., 2012). However, this subclassification may not fully represent the complexity of the transitional states of macrophage activation, which is finely tuned in response to different microenvironments (Cassetta et al., 2011; Murray et al., 2014; Xue et al., 2014).

Since macrophages are predominant players present in the inflammatory milieu and proinflammatory cytokines and chemokines produced by macrophages are major mediators of tissue injury or damage, macrophages have emerged as key regulators leading to the initiation, promotion, and progression of many diseases. Thus, it has been suggested that macrophages may represent a suitable target for anti-inflammatory therapy. Indeed, macrophage ablation therapy and inhibition of macrophage infiltration have shown efficacy in patients with rheumatoid arthritis and osteoarthritis, respectively (Barrera et al., 2000; Young et al., 2001). Furthermore, several animal studies have revealed that macrophage depletion with clodronate liposomes results in attenuation of lung injury after endotoxin, ischemia reperfusion, or mechanical ventilation (Kooy et al., 2002; Naidu et al., 2003; Frank et al., 2006; Zhao et al., 2006). However, it has been reported that significant depletion of macrophages is associated with immunosuppression, infection, and poor wound healing (Burnett et al., 2004; Caillier et al., 2005; Mirza et al., 2009). In this regard, we could consider the importance of macrophage subsets and their different activated states in disease environments, which may affect the outcome of macrophage-targeted therapy. This review underlines recent advances in the understanding of the role of macrophages in the pathogenesis of chronic inflammatory diseases, with special focus on inflammatory bowel disease (IBD), cancer, diabetes, and degenerative central nervous system (CNS) diseases. We also discuss therapeutic strategies for targeting macrophages to treat chronic disorders, with the potential opportunity and limitations of this clinical translation.

Role of Macrophages in Intestinal Homeostasis and Pathogenesis of IBD

In the intestine, tissue resident macrophages, using scavenger receptors on their surface, monitor and respond to changes in their environment. Some pattern recognition receptors recognize exogenous microbial structures, such as LPS and lipoteichoic acid. Following recognition, they initiate engulfment, which leads to killing and degradation of the microbe. Thus, they play a role in pathogen clearance in the intestine. The scavenger receptors on resident macrophages also recognize endogenous self molecules, such as apoptotic dead cells and their debris. In addition, resident macrophages promote epithelial repair by producing growth factors and maintain immune homeostasis by secreting cytokines, such as IL-10. Therefore, resident tissue macrophages maintain intestinal tissue homeostasis.

The intestinal mucosal barrier provides the first line in host defense against the external environment. The intestinal epithelium forms physical, biochemical, and biologic barriers to pathogenic and commensal bacteria and can reinforce their barriers in response to microbial stimuli. Thus, intestinal epithelium participates in a coordination of immune responses and maintains fundamental immune regulation. In response to bacteria, the intestinal epithelium secretes various factors that switch macrophages to become tolerogenic phenotypes (Rimoldi et al., 2005).

In the intestine, two populations of macrophages are characterized. One population is CD11cCD103+ cells, and the other is monocyte-derived CD11cmonocytes F4/80CX3CR1low macrophages (Bogunovic et al., 2009; Varol et al., 2009). At the steady state, monocyte-derived CD11clow F4/80CX3CR1low macrophages are the most abundant mononuclear phagocytes in the lamina propria of the intestine (Dalerba et al., 2011). Unlike major tissue-resident macrophages that are derived from the yolk sac or fetal liver, the CD11clow F4/80CX3CR1low resident macrophages originate from Ly6C+ monocytes that circulate in the blood (Jung et al., 2002; Varol et al., 2007, 2009; Bogunovic et al., 2009). Hematopoietic stem cells give rise to Ly6C+ monocytes. The recruitment of the monocytes to the intestine depends on the expression of CC chemokine receptor 2 (CCR2), which is the receptor for monocyte chemoattractant protein-1 (MCP-1; also known as CCL2), which is known as an inflammatory chemokine (Zigmond et al., 2012). Moreover, Ly6C+ cells are recruited to the injured site in response to inflammation. Furthermore, germ-free mice harbor fewer CX3CR1high macrophages (Niess and Adler, 2010). Based on these reports, it has been proposed that the sustained and low-grade inflammatory stimuli, including commensal or pathogenic bacteria, induce the recruitment of Ly6C+ monocytes into the intestine and development into resident macrophages. Given that CX3CR1high macrophages have a limited half-life (Jaensson et al., 2008), intestinal resident macrophages seem to be continuously replenished from circulating monocytes. These subepithelial CX3CR1high resident macrophages are associated with the intestinal epithelium and sense luminal bacteria by sampling the lumen through transepithelial dendrites (Niess et al., 2005; Zeuthen et al., 2008). In addition, they produce IL-10, which promotes survival and expansion of regulatory T cells (Hadis et al., 2011). The CX3CR1-deficient mice abrogated the establishment of oral tolerance because of impairment of IL-10 production and regulatory T-cell expansion (Hadis et al., 2011). Furthermore, despite being actively phagocytic and bactericidal, resident macrophages in the intestine fail to produce proinflammatory mediators in response to stimuli, such as the Toll-like receptor (TLR) ligand (Geissmann et al., 2010). Thus, at the steady state, the resident macrophages...
exhibit tolerogenic properties, thereby maintaining intestinal homeostasis.

The dysfunction of the mucosal barrier induces tissue injury and/or bacterial translocation. The risk of developing IBD is associated with increased bacterial translocation. In accordance with this, the intestinal barrier is dysregulated in IBD (Mankertz and Schulzke, 2007). For example, reduced goblet cell mucin synthesis and a damaged epithelial tight junction are observed in IBD (Zeissig et al., 2007; Merga et al., 2014). The dysfunction of the mucosal barrier leads to the infiltration of circulatory macrophages. The recruited macrophages produce proinflammatory cytokines, such as IL-6, tumor necrosis factor-α (TNF-α), IFN-γ, and reactive oxygen species (ROS). These mediators are involved in the activation of multiple antimicrobial mechanisms and contribute to the clearance of invading organisms. On the other hand, TNF-α and IFN-γ modulate a tight junction barrier function (Madara and Stafford, 1989; Turner, 2009). TNF-α and IFN-γ can induce changes in the tight junction barrier function, including the degradation of tight junction proteins, such as ZO-1, and the modulation of myosin light chain kinase activation or cytoskeletons (Turner, 2009). Thus, macrophages appear not only to protect the host from invading microbes, but also to aggravated mucosal damage through secreted cytokines.

Macrophages are essential for the pathology of IBD. The population of macrophages in the intestine of patients with IBD is different from that of normal subjects (Rugtevit et al., 1994, 1997; Rogler et al., 1999; Carlsen et al., 2006; Hetzenecker et al., 2012). The resident macrophages in normal mucosa exhibit low expression levels of CD14; however, macrophages accumulating in inflamed mucosa of patients with IBD have high expression levels of CD14. These CD14+ macrophages in the inflamed mucosa are derived from circulating monocytes. The endothelial cells in the mucosal vessels of patients with IBD exhibit high levels of adhesion molecules. These molecules promote circulating blood CD14+ monocyte rolling and transendothelial migration into inflamed mucosa (Burgio et al., 1995; Inoue et al., 2005). In contrast to resident macrophages that lack the ability to produce proinflammatory mediators, CD14+ macrophages produce larger amounts of mediators, such as IL-1, IL-6, IL-23, TNF-α, ROS, and nitric oxide (Rugtevit et al., 1997). These proinflammatory macrophages have been identified as critical mediators in IBD. For example, IL-23 is responsible for the differentiation of naïve CD4 T cells into Th17 cells. Th17 cells infiltrate the inflamed intestine of patients with IBD, where they produce IL-17, IL-6, and TNF-α, leading to amplification of the inflammatory process (Harrington et al., 2005; Park et al., 2005; Bettelli et al., 2007; Kastelien et al., 2007). IL-23 receptor polymorphisms are associated with susceptibility of IBD (Tremelling et al., 2007). Furthermore, CD14+ macrophages also promote IFN-γ production from lamina propria cells and the produced IFN-γ induces macrophage differentiation with the IL-23–hyperproducing phenotype (Kamada et al., 2008). Thus, macrophages seem to participate in the establishment of persistent inflammation by inducing a positive feedback loop mechanism. Because these proinflammatory cytokines are secreted by classically activated M1 macrophages, CD14+ macrophages might be simplistically regarded as M1 macrophages. However, macrophages in the gut appear to have some of the hallmarks of both M1 and M2 macrophages. Thus, gut-resident macrophages may not fit readily into the M1-M2 paradigm. CD14+ macrophages in humans are the equivalent of Ly6C+ monocytes in mice. Ly6C+ monocytes are highly plastic and give rise to CX3CR1hi macrophages at the steady state, whereas, upon the inflammatory stimuli, these cells differentiate into a spectrum of macrophages that are distinct from resident CX3CR1hi macrophages. Resident CX3CR1hi macrophages lack a migratory ability (Bogunovic et al., 2009; Schulz et al., 2009; Hadis et al., 2011). However, when Ly6C+ monocytes are recruited to inflamed tissue, these cells give rise to CCR7+ macrophages, which have a migratory capacity, thereby inducing adaptive immune responses in the lymph node (Gordon and Taylor, 2005).

Macrophages appear to have both proinflammatory and protective roles in IBD pathogenesis. Impaired proinflammatory cytokine production by macrophages can contribute to IBD by diminishing the capacity to clear potentially pathogenic commensal bacteria from the lining of the bowel (Smith et al., 2009). TLR4-mediated MyD88 signaling in subepithelial macrophages induces cyclooxygenase-2, leading to amplification of mucosal prostaglandin E2 synthesis that supports epithelial survival (Pull et al., 2005; Fukata et al., 2006). Intestinal resident macrophages continuously maintain tissue homeostasis by various mechanisms, including clearance of apoptotic cells and promotion of epithelial growth. Moreover, in addition to secretion of immunoregulatory proteins, such as IL-10, and arginase-1, resident macrophages have an important role in the wound healing process by producing TGF-β and platelet-derived growth factor, which contributes to tissue regeneration by promoting the differentiation of the proliferation of myofibroblasts.

Accordingly, manipulation of the process of generation, differentiation, and activation of macrophages could be a potential therapeutic target for IBD therapy. Macrophages recruited to the site of inflammation secrete an array of proinflammatory chemokines and cytokines, such as TNF-α, thereby enhancing the inflammatory circuit. Since TNF-α plays a pivotal role in the pathogenesis of IBD, TNF-α may be one of the therapeutic targets for IBD. The anti–TNF-α antibodies adalimumab, certolizumab, infliximab, and golimumab could lead to achieving or maintaining remission of IBD. Thus, TNF-α antibodies have emerged as a treatment approach for refractory IBD patients. Treatment with these antibodies may induce systemic inhibition of the host defense function of TNF-α, resulting in systemic immunosuppression. To overcome such an adverse effect, an oral polyclonal anti–TNF-α antibody, AVX-470, has been developed. AVX-470 is delivered orally to neutralizing TNF-α locally in the gastrointestinal tract (Bhol et al., 2013).

Activation of CD4+ T helper (Th) cells contributes to the uncontrolled immune response in IBD. Induction of T-cell apoptosis in the inflamed intestine correlates with the clinical efficacy of IBD therapy (Neurath, 2014). In CD4+ Th cell subsets, Th17 cells have been implicated in the pathogenesis of IBD. Th17 cells secrete IL-17 and other cytokines, which can trigger and amplify the inflammatory process. Commitment and differentiation of Th17 cells are induced by activation of naïve Th cells in the presence of proinflammatory cytokines. IL-6 and IL-23 are required for Th17 cell lineage commitment and Th17 cell differentiation, respectively (Zhou et al., 2007). The deficient conditions of IL-6 or IL-23 lead to the impairment of the generation of Th17 cells. Given that macrophages are a major source of IL-6 and IL-23
in the inflamed intestinal mucosa, pharmacologic manipulation of the secretion of these cytokines from macrophages may be effective in suppressing the uncontrolled T-cell response in IBD.

**Involvement of Macrophages in Tumor Development**

The tumor microenvironment contains various inflammatory cells and mediators (Mantovani et al., 2008; Hanahan and Weinberg, 2011; Coussens et al., 2013). These inflammatory cells play an essential role in tumor pathogenesis. Macrophages are in the tumor microenvironment and can show both protumor and antitumor phenotypes in the different settings. An array of evidence supports the concept of functional plasticity and heterogeneity of macrophages (Mosser and Edwards, 2008; Murray et al., 2014). Most mouse tissue-resident macrophages originate from the yolk sac progenitors, with some exceptions, such as the intestine (Wynn et al., 2013). These tissue-resident macrophages self-maintain independent of adult bone marrow (Wynn et al., 2013) and recruit additional macrophages from circulating bone marrow monocytes in response to pathogens or following injury. Tumor cells also recruit bone marrow monocytes by secreting chemokines, such as colony stimulating factor (CSF) 1 and MCP-1 (Noy and Pollard, 2014). Thus, both yolk sac–derived macrophages and bone marrow–derived macrophages reside in the tumor microenvironment. Both tissue resident macrophages and circulating bone marrow monocytes express high levels of the CSF1 receptor (CSF1R) and circulating bone marrow monocytes express high levels of CCR2. Thus, macrophages in the tumor microenvironment are highly heterogeneous. Intriguingly, these macrophages appear to behave differently. In a mouse glioma model, resident yolk sac–derived microglia and recruited bone marrow–derived macrophages are present in the tumor microenvironment and these cells show distinct responses to antitumor therapies (Pyonteck et al., 2013).

Inflammatory conditions link tumor initiation and progression. Chronic infection, such as *Helicobacter pylori*, in the stomach generates a pool of monocytes that could cause persistent inflammation, resulting in the initiation of tumorigenesis (Cordon-Cardo and Prives, 1999; Peek and Blaser, 2002; Karin et al., 2006; Ferreira et al., 2008; Krueger et al., 2013; Hatakeyama, 2014). Chronic IBD, such as Crohn’s disease and ulcerative colitis, increases the risk of colorectal cancer (Balkwill, 2004; Balkwill et al., 2005; Grivennikov et al., 2009; Grivennikov and Karin, 2010; Terzic et al., 2010; Foersch and Neurath, 2014). Macrophages are involved in the inflammatory responses that promote tumorigenesis by producing a proinflammatory cytokine, such as IL-6, TNF-α, and IFN-γ, and by secreting growth factors that induce epithelial cell growth and promote cancer-associated mutations (Mantovani et al., 2008; Grivennikov et al., 2012; Noy and Pollard, 2014). In general, M1-type macrophages activated by IFN-γ or microbial products show tumoricidal potential (Fig. 1). IFN-γ primed macrophages enhance the capacity of the production of proinflammatory cytokines, such as IL-12 and IFN-γ, and ROS. These mediators have the potential to kill tumor cells. These macrophages also promote Th1 responses by producing various mediators, such as IL-12, C-X-C motif ligand (CXCL) 9, and CXCL10 (Gordon, 2003; Martinez et al., 2006; Biswas and Mantovani, 2010). Given that Th1 responses are involved in antitumor responses (Tsung et al., 1997; Biswas and Mantovani, 2010), promotion of Th1 responses by M1 macrophages may provide a positive feedback loop in antitumor mechanisms.

On the other hand, macrophages observed in the established metastatic tumors lose tumoricidal activity but promote tumor progression (Biswas and Mantovani, 2010; Qian and Pollard, 2010). M2 macrophages produce protumor factors, such as IL-8, vascular endothelial growth factor (VEGF), and matrix metalloproteinases. In addition, tumor-associated macrophages, regulatory macrophages and myeloid cells, and myeloid-derived suppressor cells suppress antitumor immunity by secreting IL-10, TGF-β, programmed death-ligand 1, and arginase 1. These populations also promote tumor growth (Fig. 1) (de Visser et al., 2005; Nardin and Abastado, 2008; Sierra et al., 2008; Andreu et al., 2010; Yang et al., 2011). Besides tumor growth, these protumor macrophages facilitate tumor survival, tumor cell invasion, metastasis, angiogenesis and skew effective T-cell responses by secreting multiple factors, such as VEGF, Wnt, matrix metalloproteinases, cathepsins, and IL-10 (de Visser et al., 2005; Sinha et al., 2005; Nardin and Abastado, 2008; Sierra et al., 2008; DeNardo et al., 2009; Andreu et al., 2010; Gocheva et al., 2010; Qian and Pollard, 2010; Wong et al., 2010; Coussens et al., 2013; Yang et al., 2014). Tyrosine kinase with Ig-like loops and epidermal growth factor homology domain-2 (Tie2)–expressing monocytes/macrophages increase the invasive properties of glioma cells by secreting high levels of geratinase enzymatic proteins (Gabrusiewicz et al., 2014). During the
acquisition of malignancy, the tumor microenvironment appears to become a Th2-type immune suppressive environment. Malignant epithelial cells polarize macrophages to an M2-like phenotype (Hagemann et al., 2005, 2006). The cytokines IL-4, IL-10, and IL-13 produced by immune cells, such as Th2 cells and B cells, and/or tumor cells induce M2 polarization of macrophages. Furthermore, growth factors, such as CSF1 and granulocyte macrophage-CSF, which are produced by tumor cells or helper T cells, promote polarization of macrophages in protumoral phenotypes (Lin et al., 2002; Su et al., 2014). Thus, macrophages are educated in the tumor microenvironment to have a protumor phenotype.

These protumor macrophages are re-educated to be antitumor macrophages (Stout et al., 2009). IFN-γ, CpG-DNA, and IL-10 antibody switch macrophages from an M2-like protumor phenotype to an M1 phenotype (Guiducci et al., 2005; Duluc et al., 2009). By inhibition of nuclear factor-κB and signal transducer and activator of transcription 3, protumor macrophages become cytotoxic to tumor cells and promote regression of tumors in vivo (Kortylewski et al., 2005; Hagemann et al., 2008). Along similar lines, Notch signaling induces antitumor activity in macrophages by promoting M1 polarization (Wang et al., 2010). Thus, the phenotypes of macrophages in the tumor environment are highly plastic (Fig. 1).

Macrophages have a key role in both promoting and preventing tumor progression. Because tumor-associated macrophages promote resistance to various cancer therapies, macrophages constitute an attractive cellular target for cancer therapy in combination with chemotherapy or other strategies. Since CSF1 is the major lineage regulator of macrophages, CSF1 signaling through its receptor CSF1R is a potential target for therapy. Tumor cells secrete a large amount of CSF1 (Lin et al., 2001), leading to recruitment of macrophages in the tumor regions. High expression levels of CSF1 have been shown to correlate with poor prognosis in various cancers (Grobleswka et al., 2007; Mroczko et al., 2007; Zhu et al., 2008). CSF1-CSF1R inhibition is effective in reducing the number of macrophages in a mouse gastrointestinal stromal tumors model (Cohen et al., 2013) and enhances the antitumor effects of VEGF-targeted therapies (Priceman et al., 2010). Inhibition of CSF1-CSF1R also induces macrophages to be reprogrammed. These reprogrammed macrophages show an enhanced antigen presentation, thereby promoting antitumor T-cell responses in pancreatic cancer (Zhu et al., 2014). Androgen blocking therapy in combination with CSF1R inhibitors improves macrophage infiltration and enhances tumor disruption, thereby sustaining a more durable therapeutic response in a prostate cancer model (Escamilla et al., 2015). In addition, genetic deletion of CSF1 or inhibition of CSF1-CSF1R inhibits tumor progression in various tumor settings. Inhibitory molecules of CSF1R are now in clinical trials for cancer therapy (Ries et al., 2014). CSF1 also increases expression of Tie2 in tumor-associated macrophages. Tie2-expressing macrophages are thought to be involved in angiogenesis. Tumor cells also produce chemokine CCL2. CCR2 is highly expressed in circulating monocyte and inflammatory macrophages (Qian et al., 2011). CCL2 neutralization inhibits metastasis by reducing recruitment of monocytes. The CCL2-CCR2 interface is likely to be an attractive therapeutic target as cessation of CCL2 inhibition accelerates breast cancer metastasis by releasing monocytes from the bone marrow, enhancing cancer cell mobilization and promoting angiogenesis (Bonapace et al., 2014).

Cancer cells secrete a large amount of small vesicles, known as exosomes, compared with normal cells (Théry et al., 2002). Exosomes contain many proteins, microRNA, mRNA, and lipids derived from the parental cells. Exosomes also express the parental cell-derived surface adhesion molecules and specific surface markers. For example, ovarian cancer cell–derived exosomes specifically express epithelial cell adhesion molecules (Peng et al., 2011; Liang et al., 2013). The exosomes derived from cancer cells have been reported to show distinct features from those derived from normal cells. The tumor cell–secreted exosomes affect the behavior of macrophages within the tumor microenvironment (Ludwig and Giebel, 2012). Both exosomes secreted by normal cells and cancerous cell lines targeted macrophages, whereas only cancer-derived exosomes induced nuclear factor-κB activation in macrophages, resulting in the secretion of proinflammatory cytokines, such as IL-6, TNFα, and CCL2 through activation of TLR2 on macrophages (Chow et al., 2014). MicroRNAs within the cancer cell–derived exosomes trigger the TLR7/TLR8-mediated prometastatic inflammatory response (Valeri et al., 2014). In addition, cancer cell–derived exosomes activate distant macrophages. Because exosomes can be transferred to other cells located in both the peripheral region and distant area to modulate the immune system and facilitate tumor progression, exosomes can be a new potential therapeutic target for macrophage-targeted cancer therapy.

In vaccine strategies, tumor antigen-conjugated antibodies or dead tumor cells are treated to activate antitumor immune cells, such as natural killer T cells or cytotoxic T cells (Banchereau and Palucka, 2005). Tumor cell–associated antigens activate antigen-specific CD8 T cells in lymph nodes. These activated CD8 T cells elicit an antitumor immune response. In the activation of tumor-directed adaptive immune responses, macrophages function as antigen-presenting cells. CD169+ macrophages reside at the subcapsular sinus in the lymph node and take up dead tumor cells or tumor cell corpses, thereby crosspresenting tumor antigens to CD8+ T cells (Asano et al., 2011). In a human study, high levels of CD169+ macrophages are associated with a favorable clinical prognosis in colorectal carcinoma (Ohnishi et al., 2013). Thus, CD169+ sinmacrophages contribute to the generation and activation of tumor-specific T-cell immunity and seem to be an attractive antitumor approach for cancer therapy.

**Impact of Macrophages on the Development of Insulin Resistance and Type 2 Diabetes**

It is now generally accepted that low-grade inflammation and insulin resistance are two key components that are interwoven in individuals with obesity and type 2 diabetes. Rampant macrophage infiltration into peripheral tissues, such as adipose tissue and liver, are seen in animal models of obesity and type 2 diabetes as well as in obese human subjects (Weisberg et al., 2003; Xu et al., 2003; Coenen et al., 2007; Harman-Boehm et al., 2007; Nishimura et al., 2008; Ortega Martinez de Victoria et al., 2009). Indeed, adipose tissue contains bone marrow–derived macrophages, and the macrophage content tracks with the degree of obesity (Weisberg et al., 2003, 2006; Xu et al., 2003; Chen et al., 2005). Adipose tissue macrophages function in a paracrine and potentially...
endocrine fashion as a major source of proinflammatory cytokines and chemokines, including TNF-α, IL-1β, IL-6, and MCP-1, which can be released with the activation of resident macrophages (Olesky and Glass, 2010). Given that TNF-α promotes the proinflammatory signal cascade and impairs insulin signaling, this cytokine is considered to be a central player linking adipose tissue inflammation and insulin resistance (Weisberg et al., 2003; Xu et al., 2003). Increased macrophage IL-1β production is also closely associated with insulin resistance in obesity and type 2 diabetes. Treatment with neutralizing IL-1β antibody can improve glycemic control in diet-induced obese mice (Osborn et al., 2008). Furthermore, clinical evidence shows that blockade of IL-1β signaling by its receptor antagonist results in improved insulin sensitivity in patients with type 2 diabetes (Larsen et al., 2007). Subsequent study has shown the importance of FoxO1, a forkhead transcription factor that mediates insulin action on target gene expression, in regulating macrophage production of IL-1β in obesity and type 2 diabetes (Su et al., 2009).

MCP-1 has been found to be markedly increased in adipose tissue in obesity (Sartipy and Loskutoff, 2003; Weisberg et al., 2003; Xu et al., 2003). Adipose tissue–specific overexpression of MCP-1 in mice can increase macrophage infiltration into adipose tissue and insulin resistance, whereas disruption of MCP-1 dampens high-fat diet (HFD)–induced migration of macrophages into adipose tissues, thereby reducing adipose tissue inflammation and ameliorating insulin resistance (Kamei et al., 2006; Kanda et al., 2006; Tateya et al., 2010). CCR2 is the receptor for MCP-1, and mice with CCR2 disruption display reduced adipose tissue macrophage content and improved systemic insulin sensitivity relative to wild-type controls (Weisberg et al., 2006; Ito et al., 2008). In addition, short-term treatment with a pharmacological CCR2 antagonist leads to a lowered adipose tissue macrophage content and improved insulin sensitivity in mice with established obesity (Weisberg et al., 2006). Intriguingly, a recent study has demonstrated that mice receiving CCR2-deficient monocytes are protected from HFD-induced accumulation of macrophages in adipose tissue and the liver, whereas transplantation of intact monocytes into MCP-1 knockout mice on HFD does not cause infiltration of macrophages into the tissues (Oh et al., 2012). These findings all suggest that the MCP-1–CCR2 signaling pathway plays a key role in adipose tissue inflammation and insulin resistance, although there are conflicting reports showing that MCP-1–deficient mice on HFD exhibit no reduction in adipose tissue macrophages (Inouye et al., 2007; Kirk et al., 2008). In type 2 diabetic patients, circulating MCP-1 levels are elevated in comparison with normal subjects (Piemonti et al., 2003; Mine et al., 2006) and are correlated with homeostasis model assessment–insulin resistance (Kouyama et al., 2007). Collectively, inhibition of the MCP-1–CCR2 signaling pathway may provide the basis for the development of novel therapies for the insulin-resistance syndrome.

Resistin is a member of the cysteine-rich protein family termed resistin-like molecules and serves as a potential link between obesity and insulin resistance or type 2 diabetes (Kusminski et al., 2005). Whereas adipocyte-derived hyperresistinemia causes insulin resistance in rodents, circulating resistin in humans is mostly derived from macrophages rather than adipocytes (Kusminski et al., 2005). However, macrophage-derived human resistin has been shown to promote insulin resistance in HFD-fed mice that lack adipocyte-derived mouse resistin (Qatanani et al., 2009). Increased inflammation is accompanied by increased infiltration of macrophages, which, in turn, can promote resistin induction, leading to inflammation-induced insulin resistance (PARK et al., 2011). Finally, resistin has been demonstrated to stimulate the secretion of several proinflammatory cytokines and chemokines known to play a role in the induction of insulin resistance (Bokarewa et al., 2005). Thus, although the site of resistin production appears to differ between species, in humans, this adipokine, being predominantly expressed in macrophages, may contribute to the development of insulin resistance through organ inflammation, such as adipose tissue.

Although LPS promotes the generation of M1 macrophages via TLR4 and autocrine production of IFN-β (Chow et al., 1999), various lipids can also activate M1 macrophages via TLR4 in the setting of obesity. Circulating levels of free fatty acid (FFA) are often elevated in obesity, and macrophage FFAs released from adipocytes through lipolysis activate TLR4 signaling in adipocytes and macrophages (Shi et al., 2006). This concept has been reinforced by in vivo studies. TLR4-deficient mice are protected from lipid infusion–induced macrophage accumulation accompanied by insulin resistance (Shi et al., 2006), and loss-of-function mutation in TLR4 can prevent FFA-induced insulin resistance in HFD-fed mice (Tsukumo et al., 2007). Furthermore, it has been shown that mice with hematopoietic cell–specific TLR4 depletion become fully obese on an HFD but display attenuated obesity-related insulin resistance in adipose tissue and the liver (Saberi et al., 2009), suggesting the importance of innate immunity and hematopoietic-derived cells, particularly macrophages and Kupffer cells, in the induction of insulin resistance in obesity. Macrophages activated to M1 by FFAs through the TLR4 signaling pathway would secrete TNF-α, which could in turn increase lipolysis in neighboring adipocytes, leading to further production of FFAs. This paracrine loop involving FFAs and TNF-α between adipocytes and macrophages appears to establish a vicious cycle that further aggravates adipose tissue inflammation (Suganami et al., 2005).

Obesity has been found to lead to a shift in the polarization of adipose tissue macrophages from an M2-phenotypic state to the activation state that favors inflammation and insulin resistance (Lumeng et al., 2007). Interestingly, adipose tissue macrophages from CCR2 knockout mice are polarized to the M2 type even after obesity (Lumeng et al., 2007). This is in line with the observation that CCR2 knockout mice are protected from diet-induced insulin resistance (Weisberg et al., 2006). When M2 activation of macrophages is induced by IL-4 administration to mice, HFD-induced insulin resistance can be attenuated (Ricardo-Gonzalez et al., 2010). Moreover, it has been shown that inhibition of IL-10, which is secreted by M2 macrophages, with a neutralizing anti–IL-10 antibody or an antisense oligonucleotide against IL-10 enhances the impairment of insulin signaling in HFD-fed Swiss mice, suggesting a protective role of IL-10 against diet-induced insulin resistance (Cintra et al., 2008). Taken together, macrophage polarization toward the alternative M2 phenotype may play a preventive role in obesity-induced adipose tissue inflammation and insulin resistance.
The peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor family that is regulated by FFAs and their metabolites (Chawla et al., 2001) and is the target for insulin-sensitizing thiazolidinediones (TZDs), which are clinically used for type 2 diabetes (Consoli and Formoso, 2013). In mice on a chow diet, macrophage-specific depletion of PPARγ has been found to impair the maturation of M2 macrophages (Odegaard et al., 2007), implying that PPARγ deletion prevents polarization of the monocyte/macrophage to the M2 phenotype. In addition, macrophage-specific PPARγ-deficient mice exhibit glucose intolerance and insulin resistance in skeletal muscle and the liver under conditions of normal feeding (Hevener et al., 2007). Consistent with the finding that the PPARγ-deficient macrophage-induced impairment of the insulin action in L6 myoblasts is much greater after FFA treatment, the relative degree of insulin resistance could become more severe when the animals lacking PPARγ in macrophages are made with HFD feeding (Hevener et al., 2007). These findings suggest that the macrophage PPARγ is critical for the maturation of M2 activation and ultimately plays an essential role in the regulation of insulin sensitivity. The exacerbation of HFD-induced insulin resistance in animals lacking macrophage PPARγ was much less effectively reversed by TZD administration (Hevener et al., 2007). Thus, macrophages can be considered to be important target cells for the antidiabetic actions of this class of agents.

Insulin resistance is believed to be a key underlying factor driving atherosclerosis. Macrophages inherently have a functional insulin signaling pathway, and the development of insulin resistance in macrophages may worsen atherosclerosis. Thus, macrophage insulin resistance is indicated to potentially determine atherosclerotic responses in diabetes and the metabolic syndrome (Liang et al., 2007). Macrophage insulin resistance could promote endoplasmic reticulum stress-induced macrophage apoptosis due to different proapoptotic processes (Tabas et al., 2010). Increased apoptosis of insulin-resistant macrophages and defective phagocytic clearance or efferocytosis of the apoptotic macrophages may lead to the formation of vulnerable atherosclerotic plaques (Liang et al., 2007; Tabas et al., 2010).

In summary, macrophages orchestrate an inflammatory response that crucially contributes to the pathogenesis of insulin resistance and type 2 diabetes. M1/M2 macrophage polarization and switching hold the key to the regulation of insulin sensitivity. M1 macrophages promote insulin resistance by emission of proinflammatory cytokines, whereas M2 macrophages maintain insulin sensitivity through the secretion of anti-inflammatory cytokines (Fig. 2). Macrophage polarization toward the alternative state may be a novel and useful strategy for the treatment of obesity, insulin resistance, and type 2 diabetes.

**Macrophages/Microglia in Chronic Neurodegenerative Diseases of the CNS**

Microglia, the resident macrophages in the CNS, are currently regarded as members of the mononuclear phagocyte system and have a role in monitoring the brain for immune insults and invading pathogens. In the healthy CNS, the majority of microglia are found in the resting state and have a characteristic ramified morphology. Upon CNS injury, microglia are functionally activated and emerge as key players in propagating neuroinflammatory responses. Activated microglia, in common with macrophages, have the ability to secrete a multitude of immunomodulatory molecules, including proinflammatory cytokines and chemokines. In the inflamed CNS, blood-borne monocyte-derived macrophages that infiltrate the CNS are also evident in both the CNS barriers and parenchyma. Owing to the difficulty in discriminating between activated microglia and macrophages in humans, one may refer to these phagocytic cells collectively as macrophages/microglia.

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune, neurodegenerative disease of the CNS that starts as a relapsing-remitting disease in the majority of patients. The major pathologic hallmarks of MS are the formation of multiple demyelinated lesions in the white matter, which are associated with a breakdown in the blood-brain barrier and invasion of peripheral immune cells into brain tissue, causing inflammatory demyelination, although remyelination may occur following demyelination. Macrophages and activated microglia are...
abundantly present in demyelinating MS lesions (van der Valk and De Groot, 2000). A lot of evidence indicates that macrophages/microglia play a dual role in the pathogenesis of MS since they contribute to lesion formation and axonal damage but also support repair mechanisms (Kigerl et al., 2009; Rawji and Yong, 2013). The dual role of macrophages might be explained by the M1-M2 paradigm. However, M1-like macrophages/microglia-associated inflammation, which was previously thought to be harmful to neurons, causing neurodegeneration, is thought to be vital for CNS repair (Arnett et al., 2003; Foote and Blakemore, 2005). Furthermore, a recent study suggests that M2 macrophages in active MS lesions predominantly display M1 characteristics, a major subset of macrophages has an intermediate activation status (Vogel et al., 2013). The development of a therapeutic avenue with the ultimate goal to inhibit demyelination and increase remyelination, which is derived from a full understanding of when macrophage/microglia are detrimental or beneficial in the demyelination/remyelination process, would hold promise for treating MS. There are a number of currently approved therapies to treat MS, in which significant involvement of macrophage/microglia has been documented. Glatiramer acetate, IFN-β, fingolimod, metoxantrone, and dimethyl fumarate fall into this category (Rawji and Yong, 2013). Glatiramer acetate and fingolimod can lead to a shift in the microglial activation state toward anti-inflammatory activity (Jung et al., 2004; Weber et al., 2004; Hughes et al., 2008). IFN-β is implicated in the abrogation of macrophage/microglia activation (Hall et al., 1997). Metoxantrone can exert an inhibitory effect on macrophage migration into the CNS (Kopadze et al., 2006). Dimethyl fumarate has been reported to suppress macrophage infiltration to the lesion site (Kopadze et al., 2006). In analogy with MS, Alzheimer’s disease (AD) and Parkinson’s disease (PD) are both chronic neurodegenerative diseases in humans. In addition to an array of evidence for the presence of a variety of inflammatory mediators and mechanisms in the brain with AD, animal studies and epidemiologic studies have been informative with respect to the actual role of neuroinflammation in AD neurodegeneration (Rogers et al., 2007). It has been described that chronic neuroinflammation seen in AD is the major contributor to the overall suppression of neurogenesis in the subventricular zone and the subgranular zone of the hippocampal dentate gyrus (Monje et al., 2003; Deng et al., 2010). In AD, however, microglia could play a beneficial role in disease progression by clearing amyloid-β deposition (Simard et al., 2006; Takata et al., 2007). This suggests that microglial degeneration may reduce its phagocytosis and serve as a critical factor in the pathogenesis of AD. Animal models of PD using chemical toxins have shown the presence of activated microglia in the brain lesions (McGeer et al., 2003; Sugama et al., 2003; Walsh et al., 2011), indicating that neuroinflammation may play a pathogenic role in PD symptomatology. In PD patients, in vivo imaging using position emission tomography has confirmed that widespread microglial activation is associated with the pathologic process in PD (Gerhard et al., 2006). Microglia-induced chronic inflammation would contribute to the death of dopamine-producing neurons in the CNS, leading to the slow destructive process in PD. The precise role of neuroinflammation in the pathogenesis of PD remains unclear, but potential therapeutic interventions that target microglia may be in the options aimed at halting the demise of dopaminergic neurons before patients are diagnosed with PD (Long-Smith et al., 2009).

Concluding Remarks

A great deal of research has shed light on the pivotal role of macrophages in regulating inflammation and offered the view that they may therefore emerge as a potentially prime target for therapeutic intervention in clinical disorders involving chronic inflammation. Modulating the phenotypical and functional features of macrophages may be involved in the mechanisms of action of existing drugs. For instance, it has been recently proposed that the benefit of metformin, a drug widely used to treat type 2 diabetes, to decrease insulin resistance may result, at least in part, from modulating macrophage differentiation and polarization (Hattori et al., 2015). As stated above, TZDs are likely to identify macrophages as a potential therapeutic target for the treatment of type 2 diabetes (Charo, 2007). However, the diversity and plasticity of macrophages, including their subset behavior, are critically involved in determining the favorable or poor outcome of the disease. It is vital to devise therapeutic modalities targeting macrophages on a more strictly selective basis. We thus anticipate that macrophage-targeted strategies focused on the control of their pathogenic diversity without curbing the actually beneficial behavior hold promise for future management of the chronic inflammatory disorders that were covered in this review.

Authorship Contributions

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