

Regulation of Macrophage Polarization and Wound Healing

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Abbreviations and Acronyms

A_{2A}R = adenosine A_{2A} receptor

FIZZ1 = found in inflammatory zone 1

IFN- γ = interferon gamma

IL = interleukin

IL-4R α = interleukin-4 receptor alpha

IRF5 = interferon regulatory factor 5

Jmjd3 = Jumonji domain containing-3

LPS = lipopolysaccharide

MR = mannose receptor

M1 M ϕ = classically activated M ϕ

M2 M ϕ = alternatively activated M ϕ

M ϕ = macrophage

(continued)

Background: Macrophages (M ϕ s) participate in wound healing by coordinating inflammatory and angiogenic processes. M ϕ s respond to environmental cues by adopting either “classically” activated (M1) proinflammatory or “alternatively” activated (M2a, M2b, M2c, M2d) wound healing phenotypes.

The Problem: M ϕ polarization is essential for wound healing and aberrations in this process are linked to several pathologies. It is important to elucidate molecular mechanisms underlying M ϕ polarization.

Basic/Clinical Science Advances: M ϕ s are categorized as proinflammatory (M1) or anti-inflammatory/wound healing (M2). M1 M ϕ s are observed in initial tissue damage responses, are induced by exogenous pathogen-associated molecular patterns or endogenous damage-associated molecular patterns, and exhibit increased phagocytosis and pro-inflammatory cytokine production, facilitating innate immunity and wound debridement. M2 M ϕ s predominate later in repair, express vascular endothelial growth factor, transforming growth factor beta, and interleukin 10 (IL-10), are activated by varied stimuli, assist in the resolution of inflammation, and promote tissue formation and remodeling. Recent work has characterized a novel “M2d” phenotype resulting from adenosine-dependent “switching” of M1 M ϕ s that exhibits a pattern of marker expression that is distinct from canonical IL-4/IL-13-dependent M2a M ϕ s. Recent studies have demonstrated important roles for specific transcriptional elements in M1 and M2a M ϕ polarization, notably members of the interferon regulatory factor family interferon regulatory factor 5 (IRF5) and IRF4, respectively. The role of these IRFs in M2d polarization and wound healing remains to be determined.

Clinical Care Relevance: Knowledge of microenvironmental signals and molecular mechanisms that mediate M ϕ polarization should permit their manipulation to regulate important physiological processes and resolve pathological conditions.

Conclusion: Proper M ϕ polarization is essential to effective wound healing, and distinct phenotypes, such as the angiogenic M2d M ϕ , may be of critical importance to this process. The IRF5 transcription factor has been shown to play a key role in M1 M ϕ activation and the Jumonji domain containing-3-IRF4 pathway has been implicated in M2 M ϕ activation.

BACKGROUND

AS IMMUNE EFFECTOR CELLS, the role of macrophages (M ϕ s) in inflammation and host defense is well characterized. Additionally, M ϕ s are integral in the promotion of proper

wound healing as well as the resolution of inflammation in response to pathogenic challenge or tissue damage. These diverse physiological functions stem from the remarkable plasticity of M ϕ s, which allows these

cells to dramatically change their form and function in response to local environmental signals.¹⁻³ Unstimulated M ϕ s are typically quiescent; stimulation of these cells, however, results in the development of markedly polarized phenotypes in response to molecular cues residing in the local microenvironment. Current classification of M ϕ s recognizes polarization into two distinct phenotypes, termed “classically” activated (M1) or “alternatively” activated (M2).² M1 M ϕ s are induced by recognition of pathogen-associated molecular patterns, such as bacterial lipopolysaccharides (LPS) and peptidoglycan, or damage-associated molecular patterns, such as released intracellular proteins and nucleic acids, as well as stimulation by the T-cell–secreted cytokine interferon gamma (IFN- γ). M1 M ϕ s represent a proinflammatory phenotype, exhibiting increased phagocytic and antigen processing activity as well as increased production of proinflammatory cytokines (*e.g.*, interleukin 1 [IL-1], IL-6, IL-12, and tumor necrosis factor alpha [TNF- α]) and oxidative metabolites (*e.g.*, nitric oxide and superoxide) to promote host defense and removal of damaged tissue. In contrast, M2 M ϕ s are induced by a variety of stimuli (*e.g.*, IL-4/IL-13, glucocorticoids) and represent a phenotype that is potentially important in the promotion of wound healing and tissue remodeling as well as the resolution of inflammation.¹⁻³

CLINICAL PROBLEM ADDRESSED

The remarkable plasticity of M ϕ s has important implications for clinical science. Proper M ϕ polarization is necessary in several important physiological processes including, but not limited to, wound healing, immune response, and nerve/muscle regeneration.¹⁻⁵ Thus, it is not surprising that aberrations in M ϕ po-

larization are associated with some of the pathology observed in defective wound healing, diabetes, muscular dystrophy, fibroproliferative diseases such as rheumatoid arthritis and liver and lung fibrosis, as well as tumor progression.^{1-4,6-8} Elucidating the specific microenvironmental signals that contribute to M ϕ polarization could potentially lead to methods for the pharmacological manipulation of M ϕ phenotypes to promote favorable processes (*e.g.*, wound healing) or inhibit pathologic processes (*e.g.*, fibroproliferative diseases and tumor growth).

RELEVANT BASIC SCIENCE CONTEXT

One of the hallmarks of M ϕ s is their remarkable plasticity, that is, the ability to alter their phenotype in response to different environmental stimuli. Two major categories of M ϕ s, those exhibiting proinflammatory and anti-inflammatory/wound healing phenotypes, are currently recognized and are termed M1 and M2, respectively.² Considerable research has sought to both identify the wide variety of signals that induce these phenotypes as well as characterize the molecular profiles of M1 and M2 M ϕ s, as outlined in Fig. 1.¹⁻³ However, as our knowledge of M ϕ polarization becomes more complex, it has emerged that there is a broader set of signals that induce distinct M ϕ phenotypes than the traditional M1/M2 classification accommodates. For instance, although IL-4/IL-13 signaling through the IL-4 receptor- α (IL-4R α) represents the prototypical M2 M ϕ activation pathway, recent research has demonstrated the presence of M ϕ s exhibiting M2-like characteristics even in the absence of this signaling.⁹ In addition, M2 M ϕ s induced by IL-4, although exhibiting reduced phagocytic activity, show markedly increased secretion of proinflammatory cytokines in response

Abbreviations and Acronyms (continued)

NF- κ B = nuclear factor- κ B
 TGF- β = transforming growth factor beta
 TLR = Toll-like receptor
 TNF- α = tumor necrosis factor alpha
 VEGF = vascular endothelial growth factor
 Ym1 = eosinophil chemotactic factor L

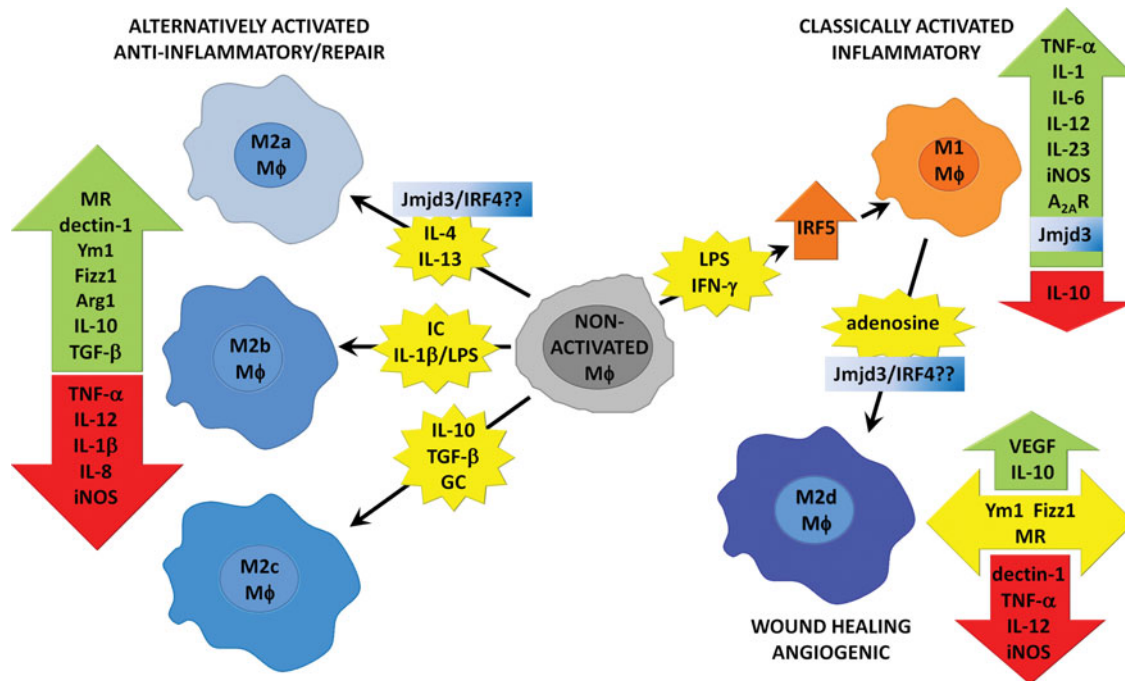


Figure 1. Pathways of M ϕ polarization. Nonactivated M ϕ s are polarized into distinct phenotypes by specific inducing agents and display typical changes in gene expression. Note that not all inducing agents are included and expression profiles for different M2 M ϕ subtypes can differ based on the nature of induction.

to LPS challenge.¹⁰ Finally, we recently defined a subtype of M2-like M ϕ s induced by costimulation of M ϕ s with Toll-like receptor (TLR) and adenosine A_{2A} receptor (A_{2A}R) agonists that display a distinct molecular signature. A_{2A}R stimulation by adenosine in the presence of TLR agonists switches M ϕ s

from a M1 phenotype into an angiogenic M2-like phenotype, which we have termed “M2d.”¹¹ The discovery of this and other novel M ϕ activation states underscores the importance of local extracellular signals in determining M ϕ function. Thus, a more complete understanding of the spatiotemporal changes in signaling molecules during wound healing and their effect on M ϕ function may allow for the enhancement of this critical process.

TARGET ARTICLES

1. Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Muller W, Roers A, and Eming SA: Differential roles of macrophages in diverse phases of skin repair. *J Immunol* 2010; **184**: 3964–3977.

2. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, and Udalova IA: IRF5 promotes inflammatory macrophage polarization and T_H1-T_H17 responses. *Nat Immunol* 2011; **12**: 231–238.

3. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, and Akira S: The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 2010; **11**: 936–944.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

Studies of the influence of M ϕ polarization on wound healing have traditionally utilized *in vitro* culture of M ϕ s and *in vivo* models of wound healing in mice as experimental models. *In vitro* culture represents a good model for the identification of M ϕ activation stimuli as well as elucidation of the molecular profiles of resultant M ϕ populations, because of the ability to precisely control the extracellular milieu. However, the lack of other cell types that would normally be present, as well as other physiological limitations, could obscure important *in vivo* changes that may occur in the local environment that could affect M ϕ polarization. *In vivo* models of wound healing certainly address some of these concerns and the use of mice allows for genetic manipulation to identify proteins and

other factors that may contribute to M ϕ polarization, but important limitations remain. In particular, significant species-specific differences in M ϕ protein expression have been demonstrated, indicating the need for further elucidation of M1/M2 M ϕ polarization in the human system.¹

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

M ϕ s are a population of immune cells that orchestrate a diverse array of functions including inflammation, tissue repair, and immune responses. This functional diversity is achieved by the remarkable heterogeneity of M ϕ s, which have the capacity to dramatically change their phenotype as a result of differentiated plasticity as well as local environmental cues. M ϕ s are generally classified as either classically (M1) or alternatively (M2) activated. M1 M ϕ s have a proinflammatory phenotype exhibiting increased phagocytic activity and secretion of proinflammatory cytokines that aid in the removal of pathogens and abnormal or damaged tissues. M2 M ϕ s have a polar opposite phenotype exhibiting high levels of anti-inflammatory cytokines and fibrogenic and angiogenic factors that serve to resolve inflammation and promote wound healing.^{1–3} Both M1 and M2 M ϕ s express distinct molecular markers as outlined in Fig. 1; however, further characterization of each phenotype has begun to demonstrate marked variability in M ϕ molecular profiles as well as the activating agents that induce them.^{1–3,11} Thus, M2 M ϕ s are now classified in three distinct subgroups, termed M2a, M2b, and M2c, based upon the inducing agent and molecular marker expression (Fig. 1).^{1,2} In this classification, the M2a subtype represents the prototypical IL-4/IL-13-dependent, IL-4R α -dependent M2 phenotype.¹ Our laboratory has characterized an additional M2 subtype, which, unlike previously described M2 M ϕ s, involves “switching” from an inflammatory M1 into an angiogenic M2 phenotype. This subtype, which we termed M2d, is induced by A_{2A}R signaling pursuant to initial stimulation by TLR agonists and is marked by decreased proinflammatory cytokine release concurrent with upregulation of traditional M2 cytokines such as IL-10 and the potent angiogenic molecule vascular endothelial growth factor (VEGF). We have recently characterized the phenotypic characteristics of this M2d M ϕ population in comparison to M2a M ϕ s. M2d M ϕ s express high levels of IL-10 and VEGF and low levels of TNF- α and IL-12 and do not show increased expression of eosinophil chemotactic factor L (Ym1), found in

inflammatory zone 1 (FIZZ1), mannose receptor (MR), or dectin.¹¹

Efforts to establish a more complete picture of M ϕ phenotypes have recently uncovered two transcriptional regulators that appear central to M1 and M2 M ϕ polarization. These regulators, interferon regulatory factor 5 (IRF5) and IRF4, are members of the interferon regulatory factor family and have been recently reported to play important roles in M1 and M2 M ϕ polarization, respectively. Krausgruber *et al.* demonstrated that IRF5 expression drives M1 polarization in M ϕ s, with IRF5 directly activating transcription of several M1 proinflammatory cytokines, such as IL-12 and IL-23, while repressing transcription of the anti-inflammatory cytokine IL-10, an established M2 cytokine. IRF5 is found at high levels in M1 M ϕ s, and its expression is induced by IFN- γ , LPS, or GM-CSF. Knockdown of IRF5 severely impairs the expression of inflammatory cytokines.¹² IRF5 represents a potentially important clinical target, as genetic polymorphisms in IRF5 have been linked to several autoimmune diseases, including rheumatoid arthritis and multiple sclerosis.^{13,14} Likewise, Satoh *et al.* have implicated IRF4 as a crucial mediator of M2 M ϕ polarization. IRF4 inhibits TLR signaling by interacting with MyD88, but the molecular mechanisms of M2 M ϕ induction by IRF4 remain unknown.^{15,16} An upstream effector of IRF4-induced M2 polarization, the histone demethylase Jumonji domain containing-3 (Jmjd3), was also identified in this study. Jmjd3 and IRF4 are critically involved in the IL-4-dependent induction of a subset of genes expressed by M2a M ϕ s (arginase 1, Ym1, FIZZ1, and MR), whereas regulation of iNOS is independent of this Jmjd3/IRF4 pathway.¹⁶ The roles of IRF4 and IRF5 in the adenosine-dependent induction of the M2d phenotype are not yet known; however, studies from our lab clearly indicate that this induction is independent of IL-4/IL-13 and STAT6, and switching to the M2d phenotype occurs unimpeded in M ϕ s from IL-4R α knockout mice. Interestingly, Jmjd3 expression is induced in M ϕ s by TLR signaling via nuclear factor- κ B (NF- κ B), and induction of the angiogenic M2d phenotype also requires TLR stimulation and NF- κ B signaling.^{17,18}

Wound healing is a complex and dynamic process, requiring the coordination of many different signals and cell types to promote effective scar tissue formation. The functional plasticity of M ϕ s in response to spatiotemporal changes in environmental signals underlies their ability to participate in diverse aspects of wound repair. Wound M ϕ s in the early stage of repair are more M1-like, when clearance of foreign/damaged matter is required,

but M2 M ϕ s predominate in later stages of repair in response to the need for new tissue formation.⁹ Lucas *et al.* have recently demonstrated the essential role of M ϕ s in the early and middle phases of wound repair, characterized by inflammation and granulation tissue formation, respectively. In these studies, transgenic mice expressing the human diphtheria toxin receptor under the control of the CD11b promoter (CD11b-DTR mice) were used to enable selective depletion of M ϕ s from skin wounds at different phases of repair by administration of diphtheria toxin. Wounds depleted of M ϕ s during either the early or middle stages of wound repair demonstrate markedly attenuated wound repair. Loss of M ϕ s in the early stage impairs induction of granulation tissue formation, myofibroblast differentiation, and angiogenesis. Interestingly, these changes correlate with the impairment of M2 M ϕ polarization, as evidenced by lack of Ym1/FIZZ1 expression and reduced secretion of transforming growth factor beta (TGF- β) and VEGF. Depletion of M ϕ s during the middle stage of wound repair resulted in similar pathologic alterations, with wounds displaying immature granulation tissue and impaired angiogenesis because of endothelial cell apoptosis. Likewise, this impaired healing was associated with the lack of the M2 cytokines TGF- β and VEGF, suggesting a role for M2 M ϕ polarization in this process.¹⁹ These studies again strongly confirm the classical observations of Leibovich and Ross (1975) concerning the roles of M ϕ s in wound repair.²⁰ With regard to M ϕ polarization, however, it should be noted that this study used prototypical M2a markers to identify polarized M ϕ phenotypes. Additional studies to analyze the presence and role of the M2d M ϕ population are required. The M2d population expresses elevated levels of A_{2A}Rs and A_{2B}Rs, but does not express elevated MR, Ym1, FIZZ1, or dectin. These studies are currently in progress.

INNOVATION

Our discovery of the novel M2d M ϕ subtype coupled with recent discoveries elucidating the essential role of M ϕ s in the early and middle stages of wound repair suggests an important role for adenosine-mediated “switching” in the regulation

TAKE-HOME MESSAGES

Basic science advances

- Local environmental cues influence the phagocytic and secretory behavior of M ϕ s to promote development of either an inflammatory M ϕ s phenotype (M1) or an anti-inflammatory/wound healing phenotype (M2).
- M2a M ϕ s are induced by IL-4R α -dependent activation and express MR, Ym1, and FIZZ1; M2d M ϕ s are induced by TLR/adenosine A_{2A} receptor-dependent activation, in an IL-4R α -independent manner, and do not express elevated MR, Ym1, and FIZZ1.
- M2 M ϕ s play an essential role in early and middle stages of wound repair. Depletion of M ϕ s during healing attenuates TGF- β and VEGF signaling and delays the formation and maturation of new tissue. Further studies of the roles of M2a and M2d M ϕ s are required.
- IRFs play critical roles in the polarization of M ϕ s. IRF5 promotes polarization of M ϕ s into the M1, proinflammatory phenotype, whereas IRF4 influences M2a M ϕ polarization in response to transcriptional regulation by Jmjd3 demethylation.

Clinical science advances

- Characterization of local environmental signals and subsequently induced M ϕ subpopulations that regulate inflammatory and tissue repair phases of wound healing provides insight into potential mechanisms for therapeutic modulation. Therapeutic modulation of M ϕ polarization presents novel opportunities for the treatment of conditions whose pathogenesis is linked to aberrant M ϕ activation.
- The identification of adenosine “switching” of M ϕ s from an M1 to M2d phenotype provides a novel paradigm for analysis of M ϕ polarization, underscoring the importance of signaling crosstalk in the complex processes of wound repair and disease pathogenesis.
- The identification of the roles of IRF4 and IRF5 in M1/M2 polarization of M ϕ s provides novel insights that should prove valuable for development of selective therapeutic modulation.

of angiogenesis during the repair process. Moreover, the identification of “adenosine switching” of M ϕ s highlights the importance of crosstalk among several signaling pathways in mediating M ϕ polarization. The extracellular milieu of wounds represents a “primordial soup” of signaling cues, and it is quite likely that several stimuli act on M ϕ s at any given time. Thus, it becomes important to characterize the spatial and temporal changes in the wound environment and determine the differential contributions of the stimuli present to M ϕ polarization. This could potentially enable the artificial manipulation of M ϕ s polarization to enhance normal physiological processes, such as wound repair, while combating pathological processes resulting from dysregulation of M ϕ function. Our experimental model, along with recent knowledge of the central roles of IRF4 and IRF5 in M ϕ polarization outlined above, presents a promising approach for analyzing relative contributions of disparate signals on M ϕ polarization.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Although significant progress has been made identifying factors underlying M ϕ s polarization, much work remains. It is important to remember that, despite traditional classification of M ϕ s as classically (M1) or alternatively (M2) activated, many novel phenotypes that do not fit the canonical molecular profile of these two groups have been identified. As our identification of the M2d phenotype has demonstrated, M ϕ polarization can be influenced by the concurrent stimulation of several different signaling pathways. The extracellular milieu in a healing wound comprises different palettes of signaling cues based upon spatial location within the wound, temporal location during the repair process, and the organism's surrounding environment. The study of M ϕ polarization at the transcriptional level, as observed with IRF4 and IRF5, should provide ways to observe the interplay of various signaling pathways on M ϕ polarization. Additional benefits may result from the fact that factors regulating transcription appear to influence clusters of genes expressed during polarization, potentially providing insight into the varied molecular profiles observed during polarization.

FUTURE DEVELOPMENTS OF INTEREST

Recent research has detailed the critical role of M2 M ϕ s in the early stages of wound healing. The

discovery of the ability of adenosine signaling in the presence of TLR stimulation to induce phenotypic switching from M1 into M2d M ϕ s, coupled with evidence documenting impaired healing and, most notably, crippled angiogenesis due to decreased VEGF signaling in the absence of M ϕ s, underscores the importance of evaluating the effect of TLR/A_{2A}R costimulation of M ϕ s on wound repair. Additionally, evaluation of the intracellular signaling pathways underlying M1 to M2 “switching” could aid in the development of therapeutics to regulate this switch, thereby allowing for the enrichment of certain M ϕ populations to promote enhancement of selective physiological processes to aid in the treatment of infection and disease.

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DISCLAIMER

The contents of this article are solely the responsibility of the author and do not necessarily represent the official views of the NIGMS or NIH.

AUTHOR DISCLOSURE AND GHOSTWRITING

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REFERENCES

- Martinez FO, Helming L, and Gordon S: Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 2009; **27**: 451–483.
- Martinez FO, Sica A, Mantovani A, and Locati M: Macrophage activation and polarization. *Front Biosci* 2008; **13**: 453–461.
- Zhang X and Mosser DM: Macrophage activation by endogenous danger signals. *J Pathol* 2008; **214**: 161–178.
- Villalta SA, Rinaldi C, Deng B, Liu G, Fedor B, and Tidball JG: Interleukin-10 reduces the pathology of mdx muscular dystrophy by deactivating M1 macrophages and modulating macrophage phenotype. *Hum Mol Genet* 2011; **20**: 790–805.
- Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, and Popovich PG: Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* 2009; **29**: 13435–13444.
- Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, Hainzl A, Schatz S, Qi Y, Schlecht A, Weiss JM, Wlaschek M, Sunderkötter C, and Scharffetter-Kochanek K: An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest* 2011; **121**: 985–997.
- Olefsky JM and Glass CK: Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 2010; **72**: 219–246.
- Wynn TA: Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; **214**: 199–210.
- Daley JM, Brancato SK, Thomay AA, Reichner JS, and Albina JE: The phenotype of murine wound macrophages. *J Leukoc Biol* 2010; **87**: 59–67.
- Varin A, Mukhopadhyay S, Herbein G, and Gordon S: Alternative activation of macrophages by IL-4 impairs phagocytosis of pathogens but potentiates microbial-induced signalling and cytokine secretion. *Blood* 2010; **115**: 353–362.
- Grinberg S, Hasko G, Wu D, and Leibovich SJ: Suppression of PLC β 2 by endotoxin plays a role in the adenosine A_{2A} receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype. *Am J Pathol* 2009; **175**: 2439–2453.
- Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, and Udalova IA: IRF5 promotes inflammatory macrophage polarization and T_H1-T_H17 responses. *Nat Immunol* 2011; **12**: 231–238.
- Dieguez-Gonzalez R, Calaza M, Perez-Pampin E, de la Serna AR, Fernandez-Gutierrez B, Castañeda S, Largo R, Joven B, Narvaez J, Navarro F, Marenco JL, Vicario JL, Blanco FJ, Fernandez-Lopez JC, Caliz R, Collado-Escobar MD, Carreño L, Lopez-Longo J, Cañete JD, Gomez-Reino JJ, and Gonzalez A: Association of interferon regulatory factor 5 haplotypes, similar to that found in systemic lupus erythematosus, in a large subgroup of patients with rheumatoid arthritis. *Arthritis Rheum* 2008; **58**: 1264–1274.
- Kristjansdottir G, Sandling JK, Bonetti A, Roos IM, Milani L, Wang C, Gustafsdottir SM, Sigurdsson S, Lundmark A, Tienari PJ, Koivisto K, Elovaara I, Pirttilä T, Reunanen M, Peltonen L, Saarela J, Hillert J, Olsson T, Landegren U, Alcina A, Fernández O, Leyva L, Guerrero M, Lucas M, Izquierdo G, Matesanz F, and Syvänen AC: Interferon regulatory factor 5 (IRF5) gene variants

- are associated with multiple sclerosis in three distinct populations. *J Med Genet* 2008; **45**: 362–369.
15. Negishi H, Ohba Y, Yanai H, Takaoka A, Honma K, Yui K, Matsuyama T, Taniguchi T, and Honda K: Negative regulation of Toll-like-receptor signaling by IRF-4. *Proc Natl Acad Sci U S A* 2005; **102**: 15989–15994.
 16. Satoh T, Takeuchi O, Vandenberg A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, and Akira S: The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 2010; **11**: 936–944.
 17. De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, and Natoli G: The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 2007; **130**: 1083–1094.
 18. Ramanathan M, Pinhal-Enfield G, Hao I, and Leibovich SJ: Synergistic up-regulation of vascular endothelial growth factor (VEGF) expression in macrophages by adenosine A_{2A} receptor agonists and endotoxin involves transcriptional regulation via the hypoxia response element in the VEGF promoter. *Mol Biol Cell* 2007; **18**: 14–23.
 19. Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Muller W, Roers A, and Eming SA: Differential roles of macrophages in diverse phases of skin repair. *J Immunol* 2010; **184**: 3964–3977.
 20. Leibovich SJ and Ross R: The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol* 1975; **78**: 71–100.