Nuclear receptor control of opposing macrophage phenotypes in cardiovascular disease

Ryan A. Frieler1,2, Saiprasad Ramnarayanan1, Richard M. Mortensen1,2,3

1Department of Molecular and Integrative Physiology, 2Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, 3Internal Medicine, Metabolism, Endocrinology, and Diabetes Division, University of Michigan Medical School, Ann Arbor, MI 48109

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1. ABSTRACT

Macrophages have important physiological roles and display a high degree of heterogeneous phenotypes in response to a variety of stimuli. In particular, the spectrum of alternatively activated macrophages has been a focus because many lines of evidence indicate a cardioprotective role for this macrophage phenotype. This phenotype is controlled in part by opposing nuclear transcription factors including the PPARs that stimulate alternative activation and the recently recognized role of the mineralocorticoid receptor in stimulating classically activated macrophages. This review highlights some of the recent findings involving alternatively activated macrophages and these nuclear receptors in cardiovascular disease.

2. INTRODUCTION

Macrophages have important roles in both innate and adaptive immune responses and can be found in nearly all tissues. They are critical in normal physiology and carry out functions like phagocytosis of cellular debris and apoptotic and necrotic cells, wound healing, fibrotic responses, and providing host defense against invading pathogens. Macrophages exhibit significant functional heterogeneity, and numerous macrophage responses can exist depending on the type of activation program elicited by different environmental stimuli or chemical signals. The importance of macrophage plasticity is evident by the wide range of phenotypes that can be generated in response to different diseases or microbial insults. Differing
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Initially, two major macrophage phenotypes were divided by the mechanisms of activation, classical activation by Th1 cytokines (called M1), and alternative activation by Th2 cytokines (M2)(1). Classically activated macrophages (CAM) can be induced by IFN-γ and endotoxin stimulation, which results in the expression and secretion of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, MIP1α, as well as iNOS and reactive oxygen species. IL-4 and IL-13 are cytokines that induce alternatively activated macrophages (AAM) and result in the expression of Arg1, Ym1, IL-10, mannose receptor, and others(2-4). Classically activated macrophages are present in type 1 immune responses and can promote inflammation and result in tissue damage, whereas alternative activation is generally associated with responses to parasitic infection and resolution of inflammation facilitating the wound healing response. Heme oxygenase-1 (HO-1) has emerged as an additional marker for a macrophage phenotype which falls within the spectrum of alternative activation (Figure 1). In addition, macrophage activation by toll-like receptors and scavenging receptors induces an innate, pro-inflammatory response, where as stimulation by IL-10 or TGF-β results in macrophage deactivation.

However, this classification has proved to be inadequate to describe the array of alternative macrophage phenotypes, although it is still useful. There can be a high degree of variation depending on the stimuli, and the *in vivo* cytokine milieu is much more complex than *in vitro* activation of macrophages with simply IL-4 or IL-13. Some classification schemes based on *in vitro* stimuli have been proposed, but have not gained wide acceptance likely because the *in vivo* phenotypes do not correspond well(5, 6). Others have taken the approach to name macrophages based on the expression of a particular marker (e.g. Mox, a type of alternatively activated macrophage expressing heme oxygenase). However, the functions of the different phenotypes are poorly understood and the markers used to identify them often do not have clear functional significance in the phenotype.

Many diseases have inflammatory components in which macrophage recruitment and infiltration occurs, and many studies have demonstrated that macrophage phenotypes have an important role and can significantly affect the pathophysiology of disease. In most cases, the Arg1, Ym1, IL-10 expressing alternative macrophages have protective effects (Table 1); however there are some diseases like pulmonary fibrosis and cancer where these macrophage phenotypes have been shown to in fact exacerbate pathogenesis. In contrast, prolonged classical activation is typically thought to have a detrimental role during most diseases (Table 2). However, it is difficult to fully understand their role because very few studies fully characterize the macrophage phenotype and rather just examine inflammatory markers. In this review, we will discuss the regulation of macrophage activation and polarization with an emphasis on nuclear receptors, and the effects of macrophage polarization in cardiovascular diseases.

## 3. NUCLEAR RECEPTOR CONTROL OF MACROPHAGE ACTIVATION

Over half of the nuclear receptor superfamily is expressed in macrophages, and many nuclear receptors have important roles in regulating macrophage activation and function(7). It has become apparent that many of the

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**Figure 1.** Classical and alternatively activated macrophage phenotypes. A.) Classical activation occurs in response to IFN-γ and LPS/TNF-α stimuli and results in the expression of pro-inflammatory mediators TNF-α, IL-1β, IL-6 and a respiratory burst generating ROS. A range of innate immune responses can occur via signaling through TLRs and scavenging receptors. B) Alternative activation involves a spectrum of macrophage phenotypes differing in gene expression profiles and activation of anti-inflammatory and wound healing mechanisms. Although there is significant overlap, major macrophage phenotypes include wound healing, HO-1+ and deactivation.
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### Table 1. Regulation and phenotypes of alternatively activated macrophages

<table>
<thead>
<tr>
<th>Wound Healing</th>
<th>Inducer/Regulator</th>
<th>Expression Profile</th>
<th>Disease Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>IL-4, IL-13, PPAR-gamma, PPAR-delta, MR KO/Antagonist</td>
<td>Increased: *Arg1, Ym1, Fizz1, MRC1, Mgl, CD163, MHC II, IL-10, HO-1</td>
<td>Protection: cardiac remodeling(14, 50), atherosclerosis(56, 57), DIO(12), liver fibrosis(110)</td>
</tr>
<tr>
<td></td>
<td>HO-1+</td>
<td>Decreased: *IL-1β, TNF-α, IL-6</td>
<td>Exacerbation: pulmonary hypertension(94), pulmonary fibrosis(113, 114)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inducer/Regulator</th>
<th>Expression Profile</th>
<th>Disease Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL, Hemin, Protoporphyrins</td>
<td>Increased: *HO-1, IL-10, CO, Biliverdin</td>
<td>Protection: atherosclerosis(78, 88, 89), pulmonary hypertension(94), renal injury(82, 115), EAE(86), pulmonary inflammation / fibrosis(118)</td>
</tr>
<tr>
<td></td>
<td>Decreased: *IL-6, TNF-α, MCP1, ROS, oxLDL uptake, TLR, SR-A</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deactivation</th>
<th>Inducer/Regulator</th>
<th>Expression Profile</th>
<th>Disease Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>TLRs, Immune complexes, IL-10, Glucocorticoids, TGF-β</td>
<td>Increased: *IL-10, TGF-β, HO-1</td>
<td>Protection: hypersensitivity reaction(8), pulmonary inflammation/asthma(119), autoimmune diseases</td>
</tr>
</tbody>
</table>

Abbreviations: DIO – diet induced obesity; EAE – experimental autoimmune encephalomyelitis; MI – myocardial infarction; *Some inducers/regulators result in expression of only some of the markers in the expression profile and/or may be specific to particular tissues or disease phenotypes.

### Table 2. Classical macrophage activation

<table>
<thead>
<tr>
<th>Classical Activation</th>
<th>Inducer/Regulator</th>
<th>Expression Profile</th>
<th>Disease Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPS, IFN-γ, TLRs</td>
<td>TNF-α, IL-1β, IL-6, IL-12, ROS, iNOS, MMPs</td>
<td>Protection: pathogen clearance(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exacerbation: MI/cardiac remodeling(14, 50), atherosclerosis, stroke(120)</td>
</tr>
</tbody>
</table>

nuclear receptors orchestrate the macrophage inflammatory response through regulation of inflammatory pathways and by regulating the expression of inflammatory mediators. The glucocorticoid receptor (GR) is one of the most extensively studied nuclear receptors in regards to inflammation and macrophage function, and pharmacological modulation of GR can suppress inflammatory pathways and alter the macrophage phenotype(8). GR activation by glucocorticoids increases the production of anti-inflammatory cytokines, IL-10 and TGF-β, and down-regulates MHC-II resulting in macrophage deactivation, also considered to be a regulatory macrophage.

3.1. Regulation of macrophage activation by PPARs

Several of the peroxisome-proliferator activated receptors (PPARs) have been shown to affect macrophage activation and polarization(9, 10). Previous studies have demonstrated that PPAR-γ activation has anti-inflammatory properties in numerous cell types including macrophages(11, 12). The activation of PPAR-γ is a negative regulator of monocyte and macrophage activation and suppresses the production of pro-inflammatory cytokines like TNF-α and IL-1β. Furthermore, it has been shown to induce an alternatively activated macrophage phenotype(10, 13). PPAR-γ is a positive regulator of some alternatively activated macrophage markers such as Arg1 and mannose receptor although it differs significantly from many of the markers induced by IL-4(14). PPAR-γ activation can also induce HO-1 expression and increase IL-10 expression indicating that this phenotype exhibits characteristics of different mechanisms of alternative activation(15). Studies using myeloid PPAR-γ knockout mice showed that PPAR-γ regulates alternative activation in vivo and is important in maintaining glucose tolerance and improving insulin resistance during diet-induced obesity(10, 16).

In addition to regulating alternative activation, PPAR-γ has also been shown to regulate the phagocytic capacity of macrophages. Both PPAR-γ agonists and myeloid PPAR-γ knockout inhibit macrophage phagocytosis of apoptotic cells(17). This is thought to be due to a direct suppression of genes involved in the phagocytic process including the established PPAR-γ regulated scavenging receptor CD36, which is upregulated in AAM(18). Alternately, in alveolar macrophages, PPAR-γ activation with PGJ2 enhances phagocytosis of neutrophils in a CD36 dependent manner(19).

PPAR-δ also regulates the macrophage phenotype and is important for maintaining glucose homeostasis(9, 20) as well as phagocytic function of macrophages(21). Myeloid PPAR-δ deletion suppresses alternative markers Mgl and Mrc2 and enhances IL-6, TNF-α, and MCP1 in macrophages co-cultured with adipocytes; this is associated with impaired insulin sensitivity. PPAR-α activation in macrophages also has anti-inflammatory properties with several similar mechanisms including NF-κB and AP-1 pathway inhibition(22) although one report has indicated no effect in inducing alternative activation(23).

Activation of PPAR-α has anti-inflammatory activity in macrophages that is similar to PPAR-γ(24). However, PPAR-α agonists have not been directly shown to specifically increase AAM phenotype or to alter macrophage polarization.
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3.2. Mineralocorticoid receptor activates proinflammatory macrophage function

Contrary to many other nuclear receptors, mineralocorticoid receptor activation has a pro-inflammatory effect in macrophages and enhances classical macrophage activation(14). The MR agonist aldosterone enhances LPS-induced expression of classical macrophage markers TNF-α, RANTES, and IL-12; this response is blocked by the MR antagonist eplerenone. In addition, inhibition with MR antagonists also suppressed LPS-induced classical markers (IL-12, RANTES, MCP1) in macrophages cultured in normal serum without the addition of aldosterone. Furthermore, treatment with MR antagonists results in a shift towards the alternative macrophage phenotype with increased expression of Arg1, Ym1, and mannose receptor; MR antagonists also suppressed the pro-fibrotic Pai1 and increased the anti-fibrotic marker Htra1. This alternatively activated phenotype was also present in macrophages isolated from mice with myeloid-specific MR deletion.

Not surprisingly, activation or inhibition of nuclear receptors has a significant role during the pathogenesis of many types of cardiovascular disease, and the role that nuclear receptors have in regulating inflammation has been exploited to modulate the inflammatory response during cardiovascular diseases.

3.3. Interaction of nuclear receptors and cytokines

Since cytokines are powerful stimuli for myeloid phenotypes and polarization, the interaction of these nuclear receptors with polarizing cytokines is also critical in determining phenotype. IL-4 can synergize with PPAR-γ agonists and MR antagonists to promote AAM activation(14). Since IL-13 uses the same receptor as IL-4, it is likely that it too can cooperate to enhance the phenotype. Other interleukins, such as IL-33, are known to affect macrophage polarization, however, their interaction with nuclear receptors remains to be determined(25, 26).

4. ALTERNATIVELY ACTIVATED MACROPHAGE PHENOTYPES IN CARDIOVASCULAR DISEASE

4.1. Cardiac inflammation, fibrosis and hypertrophy

Immune cells are present during the inflammatory response to cardiac hypertrophy and fibrosis; however the impact of infiltrating macrophages and their functional phenotypes is often underappreciated due to the lack of understanding of how different modes of macrophage polarization influence pathophysiology.

Aldosterone has pro-inflammatory effects in numerous cell types and MR antagonists exert cardioprotection even in the absence of mineralocorticoid excess. MR is expressed in immune cells including macrophages, and activation of MR can influence the expression and secretion of inflammatory cytokines, as well as alter oxidative status through the generation of reactive oxygen species. MR activation by aldosterone increases the production of \( \text{H}_2\text{O}_2 \) in blood mononuclear cells(27), and increases the production of peroxides and superoxide anion in isolated peritoneal macrophages(28). Conversely, the MR antagonist spironolactone suppressed the expression of LPS-induced pro-inflammatory cytokines TNF-α, IL-6, and IFN-γ in isolated blood mononuclear cells(29).

Several studies have demonstrated a clinical benefit of MR antagonists without altering blood pressure(30, 31) and numerous reports have shown that MR blockage ameliorates cardiac inflammation, fibrosis and hypertrophy in animal models(32, 33). MR antagonists can also suppress matrix metalloproteinase expression and activity(34). Usher et al. recently identified myeloid cells as critical targets for MR antagonists during cardiac fibrosis and hypertrophy. We demonstrated a novel role of MR in regulating macrophage polarization and showed that MR activation with aldosterone induces classical activation where as either MR antagonism or deletion results in alternative activation. Furthermore, myeloid MR knockout mice were protected from L-NAME/Angiotensin-II induced cardiac fibrosis and hypertrophy. This protection was associated with enhanced expression of alternatively activated macrophage markers and suppression of classical markers. A study by Rickard et al. also provided evidence that MR activation in myeloid cells is important in altering the fibrotic response to DOCA(35). Myeloid MR knockout resulted in mild suppression of cardiac fibrosis induced by DOCA/salt, but did not significantly alter collagen deposition or other markers of fibrosis during DOCA/salt treatment.

Similarly, the thiazolidinedione (TZD) class of PPAR-γ agonists has significant cardiovascular effects independent of their insulin sensitizing actions. Clinical trials have shown that TZDs can reduce blood pressure, alter lipid profiles, induce significant effects in the vasculature, and suppress inflammation(11, 36-41).

TZDs have beneficial effects during cardiovascular remodeling and suppress pro-inflammatory classical macrophage markers TNF-α, IL-6, TGF-β, and MCP1 during myocardial infarction-induced heart failure(42). This suppression is associated with a reduction in functional deficit as determined by an improvement in left ventricular systolic function. TZDs have been shown to have anti-inflammatory and antifibrotic effects during Ang-II induced cardiac hypertrophy and fibrosis(43, 44). PPAR-γ activation in macrophages results in alternative action as mentioned above, and myeloid PPAR-γ has been shown to be an important target for the TZD pioglitazone. Myeloid PPAR-γ knockout eliminates the anti-fibrotic actions and osteopontin suppressing effects of pioglitazone(43). Although it is clear that PPAR-γ modulates the macrophage phenotype during cardiac fibrosis and remodeling, it is unknown whether PPAR-γ activation alters the expression of alternative activation markers such as Arg1 and Ym1.

However, like many of the nuclear receptors, PPAR-γ is present in many cell types and has a wide array of pleiotropic effects. Cardiomyocyte-specific PPAR-γ knockout and overexpression studies have shown that cardiomyocyte PPAR-γ also has a role in the pathophysiology of cardiac fibrosis and remodeling(45, 46). Furthermore, some findings have shown that TZDs
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can in fact cause congestive heart failure, likely through renal PPAR-gamma activation(47-49). The contribution of PPAR-γ in different cell types and the pleiotropic effects during cardiovascular disease are still not well understood.

AAMs are also found in the healing myocardium following myocardial infarction. Microarray analysis and immunocytochemical analysis indicates that classical activation predominates during early inflammation, however AAMs expressing Arg1 and Ym1 are found during later stages(50). Furthermore, Hsd1-/- mice display increased cardiac Ym1 expression, which is associated with improved cardiac function(51). Both TZDs and MR antagonists reduce cardiovascular remodeling following experimental myocardial infarction(42, 52-54), however it is not known whether these drugs alter the polarization of macrophages in these models and whether this is a mechanism of cardioprotection.

4.2. Atherosclerosis

Leukocyte infiltration into atherosclerotic lesions has a critical role in development and progression of atherosclerosis. Macrophages are regarded as major effectors in the pathogenesis of atherosclerosis and are derived from infiltrating inflammatory monocytes, thought to be of the Ly-6Chi subset. Upon monocyte differentiation into macrophages, they uptake oxidized lipids forming foam cells and releasing a variety of pro-inflammatory molecules. There is a diverse range of macrophage phenotypes present during atherogenesis and both classically activated and alternatively activated macrophages are present in atherosclerotic lesions. Arg1 expressing alternatively activated macrophages are present in early lesions in the ApoE knockout mice(55), and it has been proposed that early alternative activation serves as a reparative function. In fact, ApoE deficient bone marrow derived macrophages exhibit enhanced IL-4-induced M2 polarization.

Other studies have shown that alternative activation and macrophage emigration is increased during regression of atherosclerosis. Feig et al. demonstrated that transplantation of atherosclerotic aortas from ApoE-/- mice into HDL-normalized wild type mice resulted in plaque regression(56). This was associated with suppression of inflammatory markers TNF-α, MCP-1, ICAM-1, and VCAM-1. Conversely, the gene expression of several markers of alternative activation including Arg1, Fizz1, and mannose receptor were increased in CD68+ cells. In another study, reversal of hyperlipidemia by microsomal triglyceride transfer protein inactivation also results in atherosclerosis regression in LDLR-/-, Apob100-/- mice(57). Similarly, atherosclerosis regression was associated with decreased CD68+ macrophages in atherosclerotic lesions and a reduction in pro-inflammatory markers of classical activation. The gene expression of alternatively activated markers Arg1, mannose receptor, and Fizz1 were increased during plaque regression.

PPARγ expression is present in atherosclerotic lesions, and PPARγ agonists such as the TZDs have anti-inflammatory and anti-atherogenic effects in models of atherosclerosis(58, 59). Myeloid-specific deletion of PPARγ in atherosclerosis results in exacerbation of atherogenesis(60). The polarization of macrophages in these lesions has not been studied in detail, although it is likely that this could be an important mechanism for protective effects TZDs during atherosclerosis. Interestingly, the addition of pioglitazone further enhances the alternative macrophage polarization seen during plaque regression by hyperlipidemia reversal(57).

Similarly, MR antagonists also have anti-atherosclerotic effects during models of atherosclerosis(28, 61-63). Although the cell type-specific effects are unknown, macrophages may be a likely target for these drugs given the critical role of macrophages during atherogenesis and the M2 polarizing effects of MR antagonists.

4.3. Stroke

Macrophages are part of a robust inflammatory response that ensues following an ischemic insult to the brain. Circulating monocytes infiltrate the ischemic brain and contribute to the detrimental effects of inflammation following stroke. Numerous studies have demonstrated that inhibition of leukocyte recruitment and suppression of inflammation positively impact neurological outcome(64-68). However, the role of macrophage activation during neuroinflammation is unknown. We have recently reported a role for myeloid MR in regulating inflammation during ischemic stroke(69). Myeloid-specific deletion of MR resulted in a reduction in infarct volume following ischemia-reperfusion. The MR antagonists spironolactone and eplerenone exhibit neuroprotection during models of stroke, thus myeloid cells are major targets for these drugs(70, 71). Furthermore, myeloid MR knockout was associated with a reduction in activated macrophages and microglia and markers of classical activation were suppressed. Moreover, preservation of alternative macrophage markers was observed. It is likely that alternatively activated macrophage phenotypes exert neuroprotection during stroke.

Interleukin-4 knockout mice have altered inflammatory responses to a variety of stimuli and have diminished Th2 responses and reduced alternative activation. Xiong et al. reported that IL-4 knockout mice have increased cerebral infarcts and impaired neurological function(72). Importantly, IL-4 knockout mice have increased macrophage and microglia recruitment and an increase in the Th1/Th2 ratio. This supports a hypothesis for a protective role of alternatively activated macrophages during stroke. In addition, microglia also adopt classical and alternative polarizations and it is unknown whether the microglia phenotypes can be altered to produce neurological benefit.

5. HEME OXYGENASE-1 IN ALTERNATIVELY ACTIVATED MACROPHAGES

Heme oxygenase-1 is an inducible enzyme that catalyzes the breakdown of heme and has antioxidant, immunomodulatory, and antiapoptotic effects(73).
Induction of HO-1 in macrophages suppresses the secretion of LPS induced, inflammatory molecules IL-6, MCP1, and TNF-α(74). Furthermore, HO-1 is induced by IL-10, and HO-1 is necessary for IL-10 mediated suppression of LPS-induced TNF-α production(75). HO-1 increases IL-10 expression indicating a positive feedback mechanism and a phenotypic overlap with macrophage deactivation mechanisms(76, 77). A subset of macrophages which expresses heme oxygenase-1, sulforedoxin-1, and thioredoxin reductase has been identified in atheromatous lesions and named Mox(78). While different from the IL-4 induced AAM phenotype, it is likely within the spectrum of AAM rather than a distinct subset. Other reports suggest that HO-1 is important in alternative activation and that HO-1 upregulation overlaps with AAM markers such as CD206(79, 80). HO-1 expressing macrophages have been reported to have a protective phenotype and the expression of HO-1 in macrophages has been shown to be beneficial during acute kidney injury, HPH, atherosclerosis and other diseases(81-85). Additionally, conditional knockout of HO-1 in myeloid cells results in an altered immune response and exacerbates diseases like EAE and pulmonary hypertension(86).

5.1. Atherosclerosis

Numerous studies have indicated that HO-1 has a protective role during atherosclerosis. Induction of HO-1 with hemin or overexpression using gene delivery techniques resulted in a reduction in lesion size during models of atherosclerosis(87, 88). Furthermore, both cobalt protoporphyrin IX and adenoviral-mediated HO-1 gene delivery decrease lipid content and prevent plaque destabilization(89). Alternatively, HO-1 knockout or inhibition with metalloporphyrins exacerbates atherosclerotic lesion development and results in increased lipid accumulation, secretion of pro-inflammatory cytokines and plaque destabilization(87, 89-91). HO-1 expression in atheromatous lesions is largely co-localized with macrophage markers(89, 92) and there is evidence in support of a protective role for the HO-1+ AAM phenotype.

Oxidized lipids are abundant in atheromatous lesions and are a likely inducer of the HO-1 positive macrophage phenotype during the pathogenesis of atherosclerosis. Kadl et al. showed that the HO-1 positive macrophage gene expression profile (Mox) is mediated by Nrf2 transcriptional activity and can be induced by stimulation with oxidized phospholipid. These findings are consistent with data showing that HO-1 deficient macrophages treated with oXLDL have increased lipid accumulation and foam cell formation, as well as increased pro-inflammatory cytokine secretion and ROS production. HO-1 is typically cytoprotective and although the exact contribution of these macrophages during atherosclerosis remains unclear they are thought to have a protective function through mechanisms mentioned above.

5.2. Alveolar macrophages and pulmonary hypertension

Alveolar macrophages found in the lung parenchyma undergo alternative activation in many types of lung diseases including asthma, airway inflammation, and pulmonary fibrosis. These alternatively activated macrophages express high levels of Arg1, Ym1, Fizz1 during disease development. Unlike many other diseases, Th2 inflammatory responses and alternative activation in the lung are thought to have an important, but detrimental role during lung inflammation. In pulmonary fibrosis, macrophage Arg1 is thought to enhance the fibrotic response by generating collagen precursors. However, studies using Arg1 deficient bone marrow chimeras have demonstrated that bone marrow cells, likely macrophages, are the primary source of Arg1 but are not necessary for collagen deposition during allergic airway inflammation(93).

Recent evidence indicates that alternative macrophages are also present during hypoxia-induced pulmonary hypertension and that they contribute to the pathogenesis of disease. Vergadi and colleagues reported that hypoxia increases the expression of Arg1, Ym1, and Fizz1 in alveolar macrophages and induces alternative macrophage polarization(94). Furthermore, alternatively activated macrophages are found during early stages of hypoxia-induced pulmonary hypertension and are associated with increased right ventricular systolic pressure.

Several studies have shown that HO-1 and CO, a byproduct of HO-1 activity, are protective against hypoxia-induced pulmonary hypertension. HO-1 deficient mice have exacerbated right ventricular dilation whereas HO-1 enhancement and CO reverses pulmonary hypertension(83, 95-97). To determine the role of macrophage HO-1 during pulmonary hypertension, Vergadi et al. generated myeloid-specific HO-1 overexpressing transgenic mice(94). Overexpression of HO-1 suppressed Arg1 and Ym1 alternative markers and resulted in a sustained increase in IL-10 expression during hypoxia-induced pulmonary hypertension. It is hypothesized that the IL-10 surge during early stages is critical for the protective phenotype. The role of IL-10 in the lung is variable depending on the type of disease and it appears that IL-10 mediated protection is important during pulmonary hypertension; however IL-10 has been shown to have both anti-inflammatory, but also profibrotic effects during lung fibrosis(98).

6. STRATEGIES TO STUDY MACROPHAGE POLARIZATION IN DISEASE

Several transgenic and gene knockout technologies have been employed to study IL-4/IL-13 signaling mechanisms in inflammation including IL-4 KO, IL-13 KO, IL-4Ra KO, and Stat6 KO(99-103). Since Th2 responses are commonly elicited by parasitic infection and allergic reactions, IL-4/IL-13 knockout models have been largely used to study these types of diseases. However, these models may be useful to delineate cardioprotective effects of alternative macrophage phenotypes in cardiovascular diseases. IL-4 has been implicated in stroke, and IL-4 knockout models have been used to study the importance of IL-4 signaling and the Th2 response (discussed above).

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Strategies have also been used to study the importance of HO-1 in inflammation, and HO-1 knockouts have been used to study the inflammatory effects in numerous disease models. Hemin and protoporphyrins have been used to induce HO-1 activity and have protective effects in various models of cardiovascular disease. Furthermore, myeloid HO-1 overexpressing transgenic mice and adenoviral-mediated gene transfer have also been used to study HO-1 in various disease models.

7. SUMMARY AND PERSPECTIVES

There are three areas that remain largely unknown, 1) then mechanisms controlling macrophage polarization phenotype including the interaction of cytokines with nuclear receptors and the activation mechanisms of nuclear receptors, 2) the function of these different phenotypes and the changing composition of polarized phenotypes during disease formation and progression and finally 3) the critical genes that convey the functional phenotype, not just the marker-defined phenotype.

Mechanisms controlling macrophage phenotype have focused on the inhibition of the pro-inflammatory phenotype that is mainly controlled by NF-κB. The PPARs have been studied most extensively and proposed mechanisms include stabilization of repressor complexes by PPAR-γ(104) and sequestration of Bc6 by PPAR-δ(105). However, there is a need to study the mechanisms of induction of the AAM genes that are increased in expression. Although there appears to be a reciprocal relationship between the CAM and AAM, the wide variety of AAM phenotypes shows that there is specific regulation with each manipulation. The possible mechanisms include relief of inhibition of expression by removal of factors such as the PPARs or even suppression of NF-κB as well as direct stimulation by nuclear factors of AAM genes. Similarly, it is unknown if MR directly binds to the promoters of the pro-inflammatory genes or acts through a more indirect mechanism.

The mechanism controlling the activity of these nuclear receptors has also been problematic. The use of pharmacologic agents that are agonists or antagonists of the PPARs has greatly aided the identification of their role. However, although it is generally agreed that the endogenous ligands are lipid derivatives and several have been identified(12, 106), the endogenous physiologic ligands have remained unknown in most circumstances. As a result, except in these experimental systems, the real activity of the nuclear receptors can not be determined.

Even in the case of MR, where the ligands are well known hormones, the physiologic ligands are unclear. Both glucocorticoids and mineralocorticoids bind with near identical affinity, with glucocorticoids circulating at 100-times higher concentrations. In many systems, both classes are activating(107, 108) and the mechanism of glucocorticoid inactivation by the enzyme 11βHSD2 evolved to allow MR to respond to aldosterone. In other cases their activity is not identical(109) or 11βHSD2 is not present (as in macrophages) so the presumption is that MR is mostly occupied by glucocorticoids. This raises the question of how the MR activity is modulated in the macrophage.

The function of these cells within the disease process is only in its infancy. In most studies, the kinetics and the changing environment and population of cells are ignored by looking only at a single time point. The critical process may be remote from the time point analyzed. Macrophages with different phenotypes play different roles during the evolution of disease and response to injury. Initially, production of inflammatory mediators that increase accumulation of immune cells are necessary with subsequent phagocytosis of necrotic cells and debris. The initial response then subsides and is replaced by healing and in some cases abnormal fibrosis. This transition is still incompletely understood but involves different macrophage phenotypes at different times. The initial response is more CAM mediated whereas the healing and fibrosis is more AAM mediated. Because of the sequential involvement, effects early in this cascade can dramatically alter the later steps and eventually the outcome. Without understanding the progression in the pathophysiology, the conclusions about the process will be unreliable. While this will require considerable investment, it is critical to advancing the field.

Understanding the role of monocyte/macrophage lineage in the dynamic disease initiation and progression, is also critically dependent on understanding the genes that are functioning to alter phenotype. We currently have markers with little understanding of the important phenotype. Even arginase, which was an early recognized marker of AAM, can be beneficial in liver fibrosis(110), or detrimental(111) depending on the system. Therefore, specific functions need to be identified for the genes in AAM that contribute to the beneficial (or detrimental) effects in CV disease.

Initially, investigators will have to rely on markers to identify the polarization cell types. Then by kinetic correlation with the functional changes in the lesions occurring with the presence of the subtypes, testable hypotheses about the function can be generated. These studies can be performed using different methods of producing or altering AAM, including studies such as IL-4KO, PPAR agonists or KO, and MR agonists and KO. By comparing the expression profile of AAM subtypes with the functional changes in disease, specific genes that are critical to the beneficial effects can be identified. Ultimately, the ability to pharmacologically manipulate macrophages may be understood as an important part of both current therapies (as we define the mechanisms of drugs) and the development of new therapeutic strategies.

8. ACKNOWLEDGEMENTS

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thiazolidinediones, 11βHSD: 11-β-hydroxysteroid dehydrogenase

Key Words: Macrophage, Mineralocorticoid receptor, Glucocorticoid receptor, Alternative activation, PPAR, Review

Send correspondence to: Richard M. Mortensen, Department of Molecular and Integrative Physiology, University of Michigan Medical School, 1301 E. Catherine St., 7641 Medical Science II, Ann Arbor, MI, 48109, Tel: 734 763-2021, Fax: 734 936-8813, E-mail: rmort@umich.edu

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