Macrophage Function and Polarization in Cardiovascular Disease
A Role of Estrogen Signaling?

Chiara Bolego, Andrea Cignarella, Bart Staels, Giulia Chinetti-Gbaguidi

Abstract—Macrophages are plastic and versatile cells adapting their function/phenotype to the microenvironment. Distinct macrophage subpopulations with different functions, including classically (M1) and (M2) activated macrophages, have been described. Reciprocal skewing of macrophage polarization between the M1 and M2 state is a process modulated by transcription factors, such as the nuclear peroxisome proliferator–activated receptors. However, whether the estrogen/estrogen receptor pathways control the balance between M1/M2 macrophages is only partially understood. Estrogen-dependent effects on the macrophage system may be regarded as potential targets of pharmacological approaches to protect postmenopausal women from the elevated risk of cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2013;33:1127-1134.)

Key Words: adipose tissue • cardiovascular disease • estrogen • macrophages • nuclear receptors

Although the cardiovascular risk profile worsens in postmenopausal women, the causative mechanisms are incompletely understood. Although the prominent role of nuclear receptors in the control of macrophage polarization in several disease conditions is well established, little is known on the contribution of estrogen-regulated pathways to this process. The macrophage polarization–estrogen connection is just beginning to be described and seems to be relevant in vivo under physiological and pathological conditions. In this review, we will discuss the mechanisms of macrophage phenotype specification and activation, as well as the role of estrogen and estrogen receptor (ER) pathways in macrophage polarization and function.

Macrophage Lineage Cells: Sentinels in Innate Immunity and Initiators of Atherogenesis

The inflammatory response occurs in tissues after exposure to a pathogen or other noxious substances. It usually has 2 components: an innate nonadaptive response and an adaptive (acquired or specific) immunologic response. The innate, nonadaptive response, which developed early in evolution, is the first line of defense. The adaptive immune response seemed much later in evolutionary terms and is found only in vertebrates. It provides the physical basis for our immunologic memory and is the second line of defense. The mononuclear phagocytic system comprising monocytes, macrophages, and dendritic cells is a critical effector and regulator of inflammation and the innate immune response.1,2

Monocytes are released from bone marrow into the circulation and migrate into most tissues of the body, including the arterial wall, where they differentiate into mature macrophages. Macrophages are involved in the initiation of atherosclerosis/formation of fatty streaks, as extensively discussed in recent reviews on the subject.3–5 The effects of estrogen and the role of ERs in these cells will be discussed below.

ER-Mediated Signaling

Estrogens exert most of their biological actions via ERs,6,7 which are prominent members of the nuclear receptor superfamily. The detection of the 2 nuclear isoforms, NR3A1 (ERα) and NR3A2 (ERβ), and their target genes has resulted in a better understanding of the mechanisms of estrogen action on vascular function.8 Although ERα and ERβ share a high degree of homology and ligand specificity, their target genes differ substantially. Experimental evidence indicates that targeted deletion of the ERα gene results in several abnormalities, including tissue inflammation and insulin resistance.6,7 Similar observations were made in human carriers of inactivating mutations in the ERα isoform.9 In contrast, the newly described G-protein–coupled ER-1 (GPER-1) mediates estrogen signals by modulating nonnuclear second messenger pathways.10 GPER-1 is a transmembrane molecule that mediates several rapid cellular effects of estrogens, including activation of the mitogen-activated protein kinase (MAPK) signaling cascade, cAMP formation, and intracellular calcium mobilization. GPER-1 is expressed in a wide variety of tissues.
Mechanisms of Inflammatory Cell Regulation by Estrogens

Estrogens exert profound effects on monocyte and macrophage immune function. 17β-Estradiol (E2) directly targets monocytes and inhibits their adhesion under flow conditions by decreasing adhesion molecule expression on the endothelial cell surface. 18 Systemic E2 administration attenuates both expression of inflammatory mediators and infiltration of leukocytes into balloon-injured carotid arteries of ovariectomized rats early after injury. 19 E2 induces a particularly robust modulatory effect on monocyte chemotaxis by reducing expression of monocyte chemoattractant protein-1 in murine macrophages. 20 E2 also decreases expression of CD16, a receptor mediating autoimmune disease symptoms, and cytokine production in monocytes through a genomic action, 21 thus suggesting a possible effect of E2 on distribution of monocyte subsets. With regards to inflammatory cytokine production, the literature is discordant with E2 enhancing or inhibiting secretion of tumor necrosis factor (TNF) and interleukin (IL)-1β likely related to the duration of estrogen exposure and experimental design, including the used cell models. 22 For instance, physiological endogenous estrogen levels and exogenous E2 promote the inflammatory status of thiglycolate-elicited peritoneal macrophages in mice, 23,24 whereas short-term in vivo exposure decreased expression of IL-1β and IL-6. 25 E2 can also elicit rapid decreases in cell-surface toll-like receptor-4 expression on macrophages via GPER-1. 25 It is conceivable that activation of MAPK by GPER-1, which can cause loss of ERα, represents a feedback inhibition loop for estrogen activation of macrophages.

Inhibitory effects of E2 on nuclear factor (NF)-κB activation were consistently found in several macrophage cell systems and are attributed to both genomic (regulation of let-7a and miR-125b micro-RNAs) and nongenomic (regulation of protein kinases MAPK and Akt) mechanisms. 26,27 Interestingly, E2 strongly inhibits activation of the NF-κB pathway and inflammatory cytokine production by human cord blood mononuclear cells exposed to microbial products, suggesting that maternal hormones are physiological regulators of neonatal immune responses. 28 Later in life, the production of cytokines by monocyte/macrophages is heavily influenced by the ovarian cycle and oral contraceptive use. 29,30 An age-relationship of estrogen-monocyte/macrophage number and function has long been identified, 31 which may have several implications for postmenopausal health. In vitro studies in human macrophages from older donors do not show significant effects of estrogens on the expression of proinflammatory mediators except an increase in C-reactive protein expression, which was positively correlated with the donors’ plasma small-dense low-density lipoprotein (LDL) concentration. 32

Several studies also indicate that E2 inhibits the cellular metabolism of LDL by macrophages and reduces cholesteryl ester content in women, but not male monocyte-derived macrophages 33 through inhibition of cholesterol uptake. This effect is attenuated in the presence of oxidized LDL, indicating that a proatherogenic lipoprotein milieu is an important variable in sex hormone modulation of cardiovascular disease. 34

Monocyte/macrophage functions are controlled by lymphocytes, 35 and estrogen modulation of these pathways has been found in humans and animals. 36–39 Also, lymphocytes are targets for estrogens and contain ERs. In particular, CD8 and regulatory T cells, but not effector T cells, express ERs. The inhibitory effects of E2 on T-cell activation are mediated through antigen-presenting cells, including monocyte-macrophages, not T cells. 40 GPER-1 activation elicits de novo production of IL-10 in T lymphocytes, suggesting that agonists of this ER isoform are effective in limiting inflammatory responses and reducing disease severity. 41 Furthermore, B cells via ERα and GPER-1 are sufficient to restore E2-mediated protection against experimental encephalomyelitis and reduce infiltration of inflammatory cells. 42,43

Estrogen Signaling and Monocyte–Macrophage Function

Expression of ERα is greater than ERβ in both monocytes and macrophages, whereas macrophages express higher levels of ERα and lower levels of ERβ than monocytes. 44 A significant increase in lipopolysaccharide-induced TNF release has been reported in ERα-deficient macrophages, 45 suggesting that ERα, but not ERβ, mediates the inhibitory effects of endogenous estrogen on proinflammatory cytokine production in innate immune responses. The ERα-dependent inhibitory effect of estrogen on levels of lipopolysaccharide-induced proinflammatory cytokines is unlikely to be mediated via induction of IL-10, which is significantly increased by both ERα and ERβ agonists. 46 ERβ transcript and protein are detected in primary monocyte-derived macrophages and are not regulated by E2 levels. 47 Studies on the role of ERβ in macrophage function are limited; ERβ is known to suppress CD16 expression 11 and partially reduces IL-6 and TNF-α production by splenic macrophages after trauma hemorrhage with no effect on the activation of MAPKs and NF-κB. 48 ERβ activation is decreased in monocyte-derived macrophages obtained from women on oral contraceptives compared with non-oral contraceptive users, 30 the implications of this are unknown.

Macrophage Polarization

Functional heterogeneity and plasticity are hallmarks of cells of the monocyte/macrophage lineage. In tissues, infiltrated mononuclear phagocytes respond to environmental signals (microbial products, damaged cells, and activated lymphocytes) and subsequently adapt distinct functional phenotypes. 49

In response to Th1 cytokines (IFNγ, TNF) or to lipopolysaccharide recognition, macrophages differentiate in a classically proinflammatory M1 phenotype, which produce...
high levels of IL-12 and IL-23\textsuperscript{50} and low levels of IL-10, secrete inflammatory cytokines (TNF, IL-6, and IL-1), and exhibit potent microbicidal properties, thus conferring a Th1-like response. The inflammatory response is, however, spatially and temporally counteracted by alternatively activated M2 macrophages that are stimulated by Th2 cytokines. Three major subclasses have been identified: IL-4 and IL-13 promote differentiation in M2a macrophages, immune complexes in combination with IL-1β or lipopolysaccharide drive the M2b subtype, whereas IL-10, transforming growth factor-β, or glucocorticoids induce the M2c state. M2 macrophages are characterized by high IL-10 and transforming growth factor-β (especially prominent in M2c macrophages) and low IL-12 production, high endocytic clearance capacities, protecting surrounding tissue from a detrimental immune response.\textsuperscript{51} More recently, a unique M4 macrophage phenotype has been reported, driven by the platelet factor-4 (CXCL4), characterized by reduced expression of CD163 and other scavenger receptors (CD36, SR-A), as well as by the absence of phagocytic activity.\textsuperscript{52,53} This classification represents the extremes of possible macrophage activation states and probably simplifies the in vivo situation where the full phenotypic spectrum between M1 and M2 can exist.\textsuperscript{54} An alternative classification of macrophage populations has been proposed on the basis of macrophage functions: classically activated macrophages, wound-healing macrophages, and immune complexes in combination with IL-1.\textsuperscript{55} These factors act on distinct subsets of the available enhancers. By contrast, SPI1 (PU.1), an ETS transcription factor, controls the global macrophage-specific enhancer repertoire, irrespective of the polarization state.\textsuperscript{55}

Transcriptional Regulation of Macrophage Polarization in Cardiovascular Disease

Classical and alternative macrophage polarization relies on the differential activation of transcription factors by distinct stimuli. This includes signal transducer and activator of transcription-1, interferon regulatory factor-5, and NF-κB for M1 polarization, whereas signal transducer and activator of transcription-6, interferon regulatory factor-4, and peroxisome proliferator-activated receptor (PPAR)γ regulate M2 macrophage polarization. These factors act on distinct subsets of the available enhancers. By contrast, SPI1 (PU.1), an ETS transcription factor, controls the global macrophage-specific enhancer repertoire, irrespective of the polarization state.\textsuperscript{55}

Among these transcription factors we will focus on nuclear receptors, particularly the PPARs and the liver X receptors (LXRα and LXRβ), which are highly expressed in macrophages where they control the inflammatory response and cholesterol metabolism.\textsuperscript{56} The first evidence for a role of the PPAR pathway in alternative macrophage polarization came from the observation that IL-4, an enhancer of alternative M2a macrophage polarization, induces PPARγ expression and activity by generating natural PPARγ ligands via the 12/15-lipoxygenase pathway.\textsuperscript{57} Furthermore, IL-4 enhances the interaction between PPARγ and signal transducer and activator of transcription-6 on promoters of PPARγ target genes.\textsuperscript{58} Stimulation of macrophages with IL-4 induces expression of the PPARγ-coactivator-1β and the genetic program of oxidative metabolism.\textsuperscript{59}

Data on the role of nuclear receptors in macrophage polarization in the context of atherosclerosis are scarce. In the Reversa mouse model (low density lipoprotein-receptor \textsuperscript{-/-}\textsuperscript{ApoB100/100}\textsuperscript{Mttp\textsuperscript{m4}}\textsuperscript{Mx1-Cre\textsuperscript{+/-}}, treatment with the PPARγ agonist pioglitazone during the phase of atherosclerosis regression significantly enhanced the expression of M2 markers in parallel with a reduction of plaque macrophage and lipid content.\textsuperscript{60}

In humans, PPARγ activation also promotes IL-4–induced M2 differentiation of primary monocytes resulting in a more pronounced anti-inflammatory phenotype.\textsuperscript{61} In vivo administration of pioglitazone increased the expression of the mannose receptor (MR, CD206), a marker of alternative differentiation, in peripheral blood mononuclear cells.\textsuperscript{61} Similarly, treatment of patients with type 2 diabetes mellitus with pioglitazone increases IL-10 and CD163 expression in monocytes, thus modulating the M1/M2 imbalance in these patients.\textsuperscript{62} By contrast, PPARα or PPARβ/δ activation does not affect IL-4–driven alternative macrophage differentiation of human monocytes.\textsuperscript{63}

Although the evidence for a direct action of LXR activation on macrophage polarization is modest, the LXR pathways are differentially active in different macrophage subsets. Ligand-activated LXRα markedly enhances basal and IL-4–induced arginase 1 (Arg1, marker of alternative polarization) mRNA and protein expression, as well as promoter activity in mouse macrophages, leading to a reduction of nitric oxide production.\textsuperscript{64} LXRα expression is reduced in human IL-4–polarized M2 macrophages.\textsuperscript{65} These macrophages are less competent to take up native and oxidized lipoproteins, likely because of the reduced expression of caveolin-1 and lectin-like oxidized LDL receptor (LOX-1), which provides a mechanism for the absence of cytoplasmic lipid accumulation. Simultaneously, these macrophages display lower cholesterol efflux capacities because of low expression of ATP binding cassette A1 and apolipoprotein E (apoE), both of which are LXRα target genes.\textsuperscript{66} By contrast, M2 macrophages express high levels of opsonins and receptors involved in phagocytosis resulting in high phagocytic activity, which is enhanced by activation of PPARγ.\textsuperscript{65} In vivo, a population of CD68+MR+ macrophages displaying this phenotype is enriched in areas of high cellularity surrounding the lipid core of human atherosclerotic plaques.\textsuperscript{65} Very recently, a subpopulation of CD68+MR+CD163+ alternative M2 macrophages has been detected in areas of hemorrhage in human atherosclerotic plaques. Such macrophages, induced in vitro by the hemoglobin/haptoglobin complex, produce anti-inflammatory factors and protect against lipid accumulation because of enhanced LXRα activity and cholesterol efflux.\textsuperscript{66} In line, heme-directed monocyte differentiation, which gives rise to the so-called Mhem macrophages,\textsuperscript{67} is characterized by the induction of activating transcription factor-1, heme oxygenase-1, and LXRβ, the latter inducing, in turn, the expression of both LXRα and ATP binding cassette A1. These macrophages, which are formed in adaptation to intraplaque hemorrhage, are protected from oxidative stress and are less prone to accumulate lipids and to transform into foam cells.\textsuperscript{57} Altogether these data illustrate the complex phenotype that atherosclerotic plaque macrophages can display when adapting to a specific environment.

A distinct macrophage phenotype, strikingly different from conventional M1 and M2 macrophages, has been reported in atherosclerotic lesions of low density lipoprotein-receptor-deficient
mice. This phenotype, called Mox, representing ≈30% of all macrophages in murine advanced atherosclerotic lesions, is induced by oxidized phospholipids and is characterized by high expression of heme oxygenase-1, sulforedoxin-1, and thioredoxin reductase-1, all redox-regulatory genes under the control of the Nrf2 (NF erythroid 2-like 2) transcription factor. Mox macrophages also present a decreased phagocytotic and chemotactic capacity.

Recent evidence also points to a role of the NR4A orphan nuclear receptors in macrophage polarization. Nur77/NR4A1 was identified as an early response gene induced by a variety of cellular signals and stresses, such as antigen receptor ligation, membrane depolarization, NGF stimulation, and cAMP. Deficiency of Nur77, already known to regulate the development of Ly6C− patrolling monocytes, changes macrophage polarization toward a proinflammatory M1 phenotype leading to an increase in atherosclerosis development in low density lipoprotein-receptor or apoE-deficient mice.71,72

An overview of transcription factors and intracellular receptors governing macrophage polarization states and function is depicted in Figure 1.

**Potential Role of E2/ER Signaling in Macrophage Polarization**

Although the role of several other nuclear receptors in the control of macrophage polarization is now well established, few data are available on the modulation of macrophage phenotype by the estrogen/ER pathways. Yet emerging evidence from animal and human studies outlines a role of estrogens in functional macrophage polarization.

Ovarian sex hormones have a profound impact on macrophage recruitment, toll-like receptor expression, and phagocytosis in female rodents. Preliminary data demonstrated a significant fall in alternatively activated patrolling Ly6C+/CX3CR1+ circulating monocytes in ERα-knockout mice compared with wild type, suggesting that ER-mediated pathways are involved in the development of inflammatory phenotypes in vivo. In addition, E2/ERα signaling leads to dendritic cell functional polarization through increased expression of the interferon regulatory factor-4 transcription factor, which is also involved in alternative activation of macrophages as described above. More recently, hematopoietic/myeloid-specific ERα deletion has been shown to impact on macrophage function in female mice. In particular, ERα is required for macrophage IL-4 responsiveness, including the induction of key transcription factors, markers of alternative activation, and transglutaminase 2 expression, a multifunctional atheroprotective enzyme, as well as maximal phagocytic capacity. ERα-deficient macrophages are refractory to IL-4–induced alternative activation (Figure 2). Although E2 is equally effective as IL-4 to induce IL-4 receptor expression, these effects were not observed in ERα-KO bone marrow–derived macrophages. Interestingly, ERβ is very minimally expressed in these cells and there is no compensatory increase in its expression in the absence of ERα. These defects in macrophage function with hematopoietic/myeloid-specific nuclear receptor deletion are paralleled by a decrease in the expression of other genes with estrogenic activity, such as PPARγ, IRF4, STAT6, and NR4A1, all of which are known to regulate macrophage phenotype.

Figure 1. Transcription factors and receptors controlling different macrophage polarization states and functions. Monocytes differentiate into macrophages, which are plastic and versatile cells adapting their phenotype/functions to environmental signals. Macrophage polarization is a process finely regulated by transcription factors which respond differently to the environmental stimuli. The M1 macrophage phenotype, which is driven by Th1 cytokines, is controlled by signal transducer and activator of transcription (STAT)-1, nuclear factor (NF)-κB, interferon regulatory factor (IRF)-5, and probably by the G-protein–coupled oestrogen receptor-1 (GPER1) receptor. In the presence of Th2 cytokines, such as interleukin (IL)-4 and IL-13, monocytes differentiate into M2a macrophages, which are characterized by activation of peroxisome proliferator–activated receptor (PPARγ), STAT6, IRF4, and decreased expression of liver X receptor (LXR)α. Estrogen receptor (ER)α and NR4A1 are also involved in the maintenance of this phenotype. Monocytes are also able to differentiate in the presence of heme, into Mhem macrophages. This phenotype involves the activating transcription factor (ATF)-1, LXRβ, and LXRα pathways. Finally, the Mox macrophages, driven by the oxidized phospholipids, are governed by the Nrf2 transcription factor. LPS indicates lipopolysaccharide.
lack of E2 inhibition of inducible NO synthase was evident in peritoneal macrophages isolated from PPARα-KO mice. However, IL-1Ra and LXRα transcript expression were significantly increased after estrogen withdrawal in human primary differentiated macrophages and THP-1 cells. Although IL-1Ra is a regulator of inflammation, LXRα is important for cholesterol homeostasis, further supporting the notion that an integrated nuclear receptor network governs macrophage activation and cholesterol homeostasis in the arterial wall.

Nuclear Receptors and Macrophage Polarization in Nonvascular Tissues

High-fat feeding and obesity induce an inflammatory reaction in adipose tissue and a shift of adipose tissue macrophages toward an M1 phenotype. PPAR isoforms are key players in this process. Disruption of PPARγ in myeloid cells impairs the alternative differentiation, an effect associated with the development of diet-induced obesity, insulin resistance, and glucose intolerance. It has been reported that PPARγ activation affects the M1/M2 balance in adipose tissue by promoting lipid redistribution from macrophages toward adipocytes, hence restoring a M2 phenotype by decreasing the number of M1 macrophages and enhancing the expression of M2 markers, which, in turns, causes inflammation and metabolic derangement in adipocytes and hepatocytes. 

Estrogens play an important physiological role with regard to body weight regulation, insulin sensitivity, and glucose utilization. Yet, the metabolic role of ERs and their contribution to local inflammatory processes in adipose tissue are only partially understood. E2 treatment enhances TNF, IL-6, and plasminogen activator inhibitor-1 mRNA expression induced by high-fat diet feeding in the liver and visceral adipose tissue of wild-type mice, but not of chimeric mice grafted with bone marrow cells from ERα-deficient mice, although the beneficial effect of the hormone on glucose tolerance is not altered. Expression of the M1 markers monocyte chemoattractant protein-1, TNF, and CD11c is elevated in ovariectomized mice fed a 60% high-fat diet. Subcutaneously, but not intracerebroventricularly administered E2 suppressed expression of TNF in white adipose tissue, whereas the number of CD11c-positive macrophages was almost equivalent between peripheral and central E2 administration.

Because macrophages play an important role in human adipose tissue inflammation, it would be important to dissect to what extent signaling through ERs may affect macrophage polarization and therewith inflammation and insulin resistance.

Conclusions and Perspectives

Although the prominent role of nuclear receptors in the control of macrophage polarization in several disease conditions is well established, we are just beginning to understand the contribution of estrogogenic pathways to this process. Estrogens seem to modulate known molecular determinants of the functional skewing of mononuclear phagocytes, such as interferon regulatory factor-4. The estrogen-regulated

Impaired alternative differentiation

Figure 2. Proposed mechanisms of how estrogen might affect macrophage polarization. Basal expression of interleukin (IL)-4 receptor (IL-4R) and IL-4-induced signal transducer and activator of transcription (STAT)-6 phosphorylation is decreased in estrogen receptor (ER)-α-deficient macrophages compared with wild type. As a result, ERα-deficient macrophages are less responsive to IL-4 as demonstrated by a reduced expression of alternative differentiation markers (Chi3L3, Retnla, transforming growth factor (TGF)-β), transcription factors involved in this process (peroxisome proliferator-activated receptor [PPAR]-γ and PPARγ-coactivator-1 [PGC1]-β) and fatty acid oxidation.

ERα deletion cause glucose intolerance and insulin resistance along with altered circulating adipokines, such as leptin, obesity, and increased atherosclerotic lesion area.

In humans, microarray analysis of CD14+ decidual macrophages identified a large number of regulated genes related to immunomodulation and tissue remodeling paralleling the expression profile of M2 polarized macrophages, with only few upregulated genes of classically activated M1 macrophage phenotype signature, suggesting that estrogens affect macrophage polarization during pregnancy. Preliminary in vitro data indicate that E2 enhanced the alternative phenotype (CD163+/CD206+) and blunted the effects of M1 polarization through multiple pathways, including activation of GPER-1. Very recently, analysis of alternative activation profile identified E2 as a novel regulator of mRNA and protein expression networks conserved in human and mouse macrophages.

Nuclear Receptor Cross-Talk in Macrophages

A cross-talk between ER and other nuclear receptors has been reported to operate in macrophages. E2 treatment inhibits NF-κB p65 nuclear localization, and thus NF-κB activation, and TNF levels in wild-type but not in PPARα-knockout mice. Upregulation and activation of inducible NO synthase, resulting in NO production, are significantly countered by E2 treatment in wild-type, but not in PPARα-KO mice. Accordingly,
M1/M2 switch and effect on inflammation may underlie the association of increased cardiovascular disease risk and hormonal status in women, and the association among estrogen/obesity/inflammation/metabolic syndrome. Further studies on functional nuclear receptor networks in macrophage polarization may reveal additional mechanisms of disease progression, and this knowledge can eventually lead to the identification of potential targets for the treatment of related disorders.

Disclosures

None.

References


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