Journal of Steroid Biochemistry & Molecular Biology 120 (2010) 105–115

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Sex steroids as inflammatory regulators☆

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ARTICLE INFO

Article history:
Received 30 September 2009
Received in revised form
15 December 2009
Accepted 23 December 2009

Keywords:
Sex steroids
Inflammation
Atherosclerosis
Wound healing

ABSTRACT

It is becoming increasingly clear that endogenous sex steroids are key players in a range of inflammatory contexts. Androgens and estrogens have been shown to have a profound influence on the function of inflammatory cells including macrophages and on the secretion and activation of a range of plasma-borne inflammatory mediators. The menopause and polymorphisms in estrogen receptor genes have separately been shown to affect the incidence of a range of inflammatory disorders. Sex steroids themselves have been shown to be protective in certain conditions; harmful in others. This review will summarize their documented effects on inflammatory processes, with particular focus on two areas that have received much recent attention: the antiatherosclerotic properties of estrogens in females and the wound healing effects of sex steroids.

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1. Introduction

Today the subject of ever-increasing interest, the influence of sex hormones on immune and inflammatory processes was initially implied by a series of clinical observations. It is well recognized that females have a more active immune system than do males, which may underpin the greater reported incidence of surgical site infections, but less common occurrence of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, in the latter [1,2]. It is also the case that females more rapidly reject skin allografts than do males [3], but suffer lower rates of certain tumors, including colorectal [4], renal cell [5] and liver [6] carcinomas.

☆ Article from the special issue on Steroids: modulators of inflammation and immunity.

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doi:10.1016/j.jsbmb.2009.12.015

Table 1
Expression of sex steroid receptors in inflammatory and immune cells.

Cell type	Origin	Species	Steroid hormone receptor [Ref.]				
			AR	PR-A	PR-B	ER-α	ER-β
Neutrophil	Bone marrow	Mouse	[175][a,b]				
Neutrophil	Peripheral blood	Human				[176][b]	[176][b]
Monocyte	Peripheral blood	Human				[177][a,b]	[177][a,b]
Macrophage	Peripheral blood	Human	[178][a]				
Macrophage	Synovium	Human	[179][b]				
Macrophage	Peritoneal fluid	Human		[180][a,b,c]	[180][a]		
Macrophage	Peripheral blood	Human				[177][a,b]	[177][a,b]
Macrophage	Peritoneal cavity	Mouse				[164][b]	[164][b]
Macrophage	IC-21[d]	Mouse	[14][b,e]				
Eosinophil	Peritoneal cavity	Rat				[42][b,c]	
Mast cell	HMC-1[d]	Human		[46][b]	[46][b]		
Mast cell	HMC-1[d]	Human				[52][a]	
Mast cell	Bone marrow	Mouse				[52][a]	
Mast cell	Nasal mucosa	Human		[181][b,c]	[181][b,c]		
Neutrophil	Peripheral blood	Human		[16][a,f]	[16][a,f]		
Eosinophil	Peripheral blood	Human		[16][a,f]	[16][a,f]		
Mast cell	HMC-1[d]	Human					[52][a,f]
Mast cell	Bone marrow	Mouse					[52][a,f]
Dendritic cell	Bone marrow	Rat		[182][a,b,c]			
Dendritic cell	Bone marrow	Mouse				[183][b]	[183][b]
NK cell	Peripheral blood	Human		[184][b]	[184][b]		
NK cell	Spleen	Mouse				[185][b]	[185][b]
B cell	Spleen	Mouse	[186][a,b]			[186][a,b]	
T cell	Thymus	Mouse	[15][b]				
T cell	Spleen	Mouse	[15][b,e]				
T cell	Peripheral blood	Human		[187][a,b,e]	[187][a,b,e]		
T cell	Peripheral blood	Human				[188][a]	
Dendritic cell	Bone marrow	Mouse	[183][b,f]				
B cell	Spleen	Mouse					[186][a,b,f]

[a] mRNA.
[b] Protein.
[c] Individual receptor isoforms not identified.
[d] Cell line.
[e] Membrane-associated receptor.
[f] Expression not detectable.

On the basis of these and other observations it was hypothesized that endogenous sex steroids may influence immune functions. Indeed, it has been reported that androgens suppress both humoral and cell-mediated immune responses [7], while estrogens enhance at least the humoral response [8]. Moreover, sex steroids have been directly implicated in several common pathological conditions with inflammatory components, including asthma [9] and atherosclerosis [10,11].

Much is now known about the roles of androgens and estrogens in immunity/inflammation (for a detailed review of the latter, refer to [12]), as well as how age-linked changes in circulating hormone levels – menopause in females; the more gradual decline in serum testosterone levels in males [13] – impact upon local and systemic inflammation.

This review will specifically focus on the roles of sex steroids in inflammation. It will initially describe some of the known effects of androgens, estrogens and progesterone on inflammatory and endothelial cell function and plasma inflammatory cascades, before outlining the critical influence of these same sex hormones and the menopausal transition on inflammatory diseases. Finally, it will evaluate the involvement of sex steroids in inflammation in two specific clinical contexts: atherosclerosis and cutaneous wound healing.

2. Sex steroids and inflammatory cell function

Inflammatory and immune cells including macrophages, neutrophils and mast cells express steroid hormone receptors (Table 1). This indirectly suggests the potential influence of sex steroids on inflammatory cell function and, indeed, they have been shown to influence the activation and chemotaxis of inflammatory cells and their secretion of a range of inflammatory factors. Much of the research in this area has focussed on the regulation of monocyte/macrophage function; effects of sex steroids on eosinophils/basophils/mast cells remain underexplored.

In addition to the well-characterized classical steroid hormone receptors, which effect/inhibit transcription following ligand binding, plasma membrane-bound receptors that perform nongenomic functions have also been identified. Such receptors on macrophages [14] and T cells [15] stimulate Ca^{2+} ion fluxes following androgen binding. The ultimate outcome of such signaling events remains, in most cases, to be established

2.1. Neutrophils

Peripheral blood neutrophils were reported not to express progesterone receptor (PR)-A or PR-B mRNA [16]. Nonetheless, neutrophil apoptosis was shown to be reduced by progesterone and estradiol (the response to which was blocked by co-treatment with the estrogen receptor (ER) antagonist ICI 182,780), but not the testosterone metabolite (and potent androgen) 5α-dihydrotestosterone (DHT) [17]. Neutrophil activation, assessed through superoxide release, was reportedly reduced by testosterone [18].

Consensus on the effects of other sex steroids has not been reached: separate studies describe stimulatory [17,19] and inhibitory [18,20] effects of estrogens and progesterone on the release of reactive oxygen species (ROS) by neutrophils.

Estradiol was further shown to drive neutrophil production of nitric oxide (NO) and thereby to reduce surface expression of CD18

(and hence neutrophil adhesiveness) [21], and to restrain platelet activation in a coculture model [22].

2.2. Monocytes

Progesterone has pleiotropic effects upon cultured human monocytes. It stimulates their production of ROS [23] and, in lipopolysaccharide (LPS)-activated cells, secretion of tumor necrosis factor (TNF)-α [24]. In monocytes stimulated with heat-killed *Escherichia coli*, progesterone increased the secretion of interleukin (IL)-1β and the neutrophil chemoattractant IL-8 while depressing TNF-α secretion [25]. Along with estradiol and testosterone, it also reduced the secretion of IL-6 from LPS-activated cells [24]. Progesterone and estradiol additionally reduced monocyte secretion of IL-1α and IL-1β [26]; testosterone, prostaglandin E2 [27].

Several additional estrogenic responses have been documented. Estradiol reduced monocyte secretion of IL-8 [28] and inhibited the chemotaxis of monocytes to monocyte chemotactic protein (MCP)-1 [29]. These responses were, respectively, blocked by the ER antagonists ICI 182,780 and clomifene; the latter is seemingly realized through downregulation of surface expression of the MCP-1 receptor CCR2 [30]. Estradiol also inhibited adhesion of monocytes to endothelial cells by blocking activation of the G protein Rac1 in the former [31].

2.3. Macrophages

Sex steroids have been reported to influence various macrophage activities. DHT reduced secretion of TNF-α from LPS-treated splenic macrophages, while inducing secretion of both TNF-α and IL-6 from Kupffer cells [32]. Testosterone reportedly increased macrophage production of ROS [33]. Progesterone, meanwhile, was shown to limit the production of NO by LPS-activated alveolar macrophages [34] and bone marrow-derived macrophages (BMDM) activated with a combination of interferon (IFN)-γ and LPS [35]. It also stimulated the secretion of TNF-α, IL-1β and the chemokine macrophage inflammatory protein (MIP)-2 from unactivated peritoneal macrophages [36] while estradiol suppressed the production of these three inflammatory mediators [36]. In a mouse macrophage cell line, estradiol blocked LPS's induction of its own receptor (TLR4), and attenuated cell activation, as assessed morphologically [37]. And in LPS-activated splenic macrophages, it reduced the secretion of IL-1α, IL-6 and TNF-α, and activation of the inflammation-inducing transcription factor NF-κB [38]. The effects of estradiol on macrophage NO production appear to be cell source-specific [34,39].

Thus, it appears that, *in vitro*, estrogens inhibit macrophages' inflammatory functions. It should be noted, however, that chronic exposure of peritoneal macrophages to estradiol *in vivo* was shown to increase their subsequent secretion of IL-1β and IL-6 and activation of NF-κB following *ex vivo* exposure to LPS [40]. These responses may stem from estrogenic induction of cell surface TLR4 expression [41].

2.4. Eosinophils

Cell mediators of anti-parasitic and allergic responses, eosinophils were shown to be negative for PR mRNA [16]. By contrast, eosinophils do possess binding sites for estrogens [42] and are estrogen-responsive: estradiol treatment increases eosinophil adhesiveness [42] and degranulation [43,44].

2.5. Mast cells

While Mayo et al. provided indirect evidence that testosterone stimulates mast cell degranulation [45], others reported that progesterone inhibits chemotactic migration of mast cells to MIP-2, at least in part by downregulating surface expression of the chemokine receptor CXCR4 [46]. Moreover, progesterone has been shown to inhibit mast cell proliferation [47] and to, respectively, inhibit and stimulate mast cell secretion of histamine [48] and the platelet aggregation factor 5-hydroxytryptamine (5-HT) [49]. Estradiol, meanwhile, has been shown to stimulate mast cell degranulation [50] and to increase secretion of histamine and 5-HT by mast cells treated with the degranulator 48/80 [51].

Uncertainty surrounds the role of estradiol in IgE-induced degranulation, although Zaitsu et al. recently reported IgE-induced secretion of the granule-derived protein β-hexosaminidase by HMC-1 human mast cells to be increased by estradiol, acting through ER-α [52]. By contrast, estradiol pretreatment blocked PMA-induced secretion of IL-6 and TNF-α by HMC-1 cells [53].

2.6. Basophils

Although little is known of the effects of sex steroids upon basophil function, estradiol was, in one study, shown to enhance the secretion of histamine by human basophils treated with IgE [54].

2.7. Platelets

Platelets are sex steroid-responsive and express androgen receptor (AR) [55] and ER-β protein [55,56], but not ER-α [55]. Treatment with DHT was shown to enhance platelet aggregation in *in vivo* [57] and *in vitro* [58] models, while intramuscular injection of testosterone increased platelet densities of the receptor to thromboxane A2 (a key mediator of platelet activation and aggregation) in a group of 16 young males (aged 21–35 years) [59].

Estradiol and progesterone, by contrast, inhibited platelet aggregation in an *ex vivo* model [60]. This they achieved by stimulating endothelial production of NO [60] and other factors that suppress platelet secretion of 5-HT [61].

3. Endothelial cells

Adhesion of inflammatory cells to the vascular endothelium is a key process in inflammatory reactions and one that is modulated by sex steroids. Human umbilical vein endothelial cells (HUVEC), routinely used in *in vitro* assays, express ER-β [62,63] and PR-A protein [63], but not PR-B [63]. Whether or not HUVEC express ER-α protein is a matter of contention: while some were able to detect it immunocytochemically (albeit at much lower levels than ER-β) [62], others could not [63].

Though little studied, progesterone was shown to abrogate induction of vascular cell adhesion molecule (VCAM)-1 in TNF-α-treated HUVEC [64].

The roles of estrogens in endothelial physiology have been more intensively investigated. Estradiol was shown to reduce leukocyte diapedesis in rat aorta *in vivo*, a response dependent on NO biosynthesis [65], and subsequently reported to reduce *ex vivo* leukocyte rolling flux, adhesion and emigration in rat mesenteric venules [66]. Furthermore, it reduced aortic expression of the genes encoding acute phase proteins, including serum amyloid A3 and lipocalin 2, in *ex vivo* experiments [67].

Estrogens have variously been shown to reduce HUVEC surface expression of the adhesion molecules intercellular cell adhesion molecule (ICAM)-1 and E-selectin [68]; HUVEC VCAM-1 mRNA and protein levels [69]; and secretion of the chemokines IL-8 by HUVEC [70] and MCP-1 by HUVEC [70] and coronary artery endothelial cells [71]. Perhaps as a consequence, adhesion of monocytes to LPS- [67] and IL-1β-activated human aortic endothelial cells (AEC) [72] *in vitro* is reduced in response to estrogen treatment. Disruption

of the CD40–CD40L interaction, meanwhile, underpins estrogenic inhibition of neutrophil adhesion to IFN-γ-stimulated porcine AEC [73], while conflicting data describe inhibition [70] and stimulation [74] by estrogens of leukocyte adhesion to TNF-α-activated HUVEC.

Uncertainty surrounds the effects of androgens on endothelial cell function. In one study, DHT was shown to induce VCAM-1 mRNA and protein expression in IL-1β-stimulated HUVEC (which express AR protein) [75]. The authors further described increased adhesion of peripheral blood monocytes to HUVEC pretreated with DHT [76]. In a third study, however, preincubation with DHT blocked the induction of VCAM-1 and ICAM-1 protein by TNF-α in HUVEC and additionally reduced HUVEC secretion of MCP-1 and IL-6 and expression of TLR-4 mRNA [77]. Interestingly, both sets of investigators invoked altered NF-κB activity in their proposed mechanisms [75,77].

4. Plasma inflammatory cascades

Though the co-involvement of sex steroids and complement/kinin system proteins in the biology and pathology of reproductive organs has received a certain amount of attention, the effects of sex steroids on plasma-borne inflammatory and immune mediators have been little investigated. Nonetheless, a handful of studies have sought to determine whether sex steroids influence plasma inflammatory cascades.

In one such study, preincubation of isolated human coronary arteries with estradiol for 30 min enhanced their relaxation in response to subsequent exposure to bradykinin [78]. By contrast, there is evidence that androgens inhibit the actions of bradykinin on endothelial cells [79]. Acute administration of estradiol to postmenopausal women, meanwhile, reportedly influenced blood levels of the coagulation factors plasminogen activator inhibitor (PAI)-1 and tissue-type plasminogen activator (t-PA) antigen, and vascular release of active t-PA [80].

In male myocardial infarction patients and healthy controls, circulating levels of testosterone and PAI-1 were negatively correlated [81], while in postmenopausal women serum PAI-1 and t-PA antigen levels were decreased by estrogen therapy [82].

Estrogens increase liver expression [83] and plasma secretion [84] of factor XII mRNA and protein, respectively, and, when infused intraperitoneally, respectively, increase and decrease plasma levels of prothrombin and antithrombin III [85]. Estradiol also limited the induction of α2-macrogloblin (a regulator of both plasmin and thrombin) in ovariectomized rats following injury [86]. Effects on plasma levels of fibrinogen [87–89] and plasminogen [88] have also been reported.

Collectively, the available data suggest that estrogens may act to promote coagulation. Indeed, this has been demonstrated [90]. Furthermore, 1 month's estrogen treatment reduced systemic fibrinolysis in a randomized trial performed on postmenopausal women [80].

5. Menopause and HRT

Menopause and hormone replacement therapy (HRT) influence the occurrence and progression of a number of inflammatory conditions.

The incidence of coronary heart disease (CHD) increases markedly in postmenopausal women and estrogen-based HRT may protect some individuals from developing CHD (reviewed in [91]). However, it is worth remembering that the Women's Health Initiative placebo-controlled trial of combination estrogen-progestin HRT reported increased risk of CHD, as well as stroke and pulmonary embolism [92], though data from a parallel study revealed that the use of conjugated equine estrogens alone does not increase the risk of CHD [93].

A second, population-based study – the Million Women study – has revealed further insights into the effects of HRT on women's health [94–96].

HRT may have beneficial consequences in relation to other inflammatory conditions. In one study, postmenopausal women prescribed conjugated equine estrogens (in a quarter of cases in combination with the progestin medroxyprogesterone) as a group displayed reduced rates of inflammatory bowel disease relative to women not taking HRT [97]. By contrast, incidence of asthma declines following menopause in women who have never received replacement hormones and was increased in a group of women who had taken HRT (conjugated estrogens with or without progesterone) for 10 or more years [98].

Changes in various inflammatory parameters coincide with menopause. For example, leukocyte surface expression of CD11b and adhesion to endothelial cells was recently shown to be increased in a group of 13 postmenopausal women relative to premenopausal controls ($n = 6$) [99]. Circulating levels of inflammatory factors including MCP-1 [100], IL-β [100], IL-10 [101] and TNF-α [101] have variously been shown to increase as a result of menopause.

Two months of HRT (conjugated estrogen plus medroxyprogesterone), commencing 1.6 ± 1 years after menopause, reversed a number of these changes [101]. Elsewhere, 12 months of transdermal 17β-estradiol reduced serum levels of the inflammatory mediators IL-8 and MIP-1β in a group of 28 postmenopausal women who had undergone hysterectomy (average age 50 years) [102]. In a further study, 12 weeks' treatment with 17β-estradiol in combination with the progestin norethindrone acetate reduced mean serum levels of the soluble forms of ICAM-1 [103] and VCAM-1 [103] in nonhysterectomized postmenopausal women ($n = 29$, average age 53.4 years).

It is uncertain whether or not IL-6 levels vary as a factor of menopause [104,105]. Circulating levels of the inflammatory marker C-reactive protein (CRP) seemingly do not [100,104], though they have been positively correlated with serum estradiol levels [106]. Moreover, in a group of long-term HRT users, oral conjugated equine estrogens, but not transdermal 17β-estradiol, increased serum CRP levels following a period of withdrawal from HRT [107]. This last finding was confirmed by a separate cross-sectional study of 975 women (age 40–59 years) [108].

Genetic analyses have provided further insights into the roles of estrogens in menopause-associated inflammation. In a group of 68 postmenopausal women, polymorphisms in the ER-α-encoding *ESR1* gene were correlated with serum levels of soluble VCAM-1 [109]. In a second study of 82 postmenopausal women, particular *ESR1* alleles were linked to the incidence of the inflammatory condition aortic valve sclerosis [110].

6. Inflammatory disorders

Endogenous sex steroids have been implicated in the pathology of, and protection against, a limited number of inflammatory disorders (Table 2). Elevated serum androgen levels are associated with incidence of acne vulgaris in adult women [111,112], in whom androgen levels have further been correlated with disease severity [112]. In estrogen dermatitis [113] and autoimmune progesterone dermatitis [114], meanwhile, symptoms fluctuate as a factor of the menstrual cycle.

Elsewhere, testosterone and estrogens seemingly protect males and females, respectively, from developing atherosclerosis (reviewed in [10,11]). Progesterone may protect females against asthma [9] and testosterone males against rheumatoid arthritis (RA) [115]. Estrogens may, by contrast, contribute to the etiology of RA [116] and system lupus erythematosus [117], which are far more prevalent in females than in males.

Table 2
Inflammatory disorders in which sex steroids have been implicated.

Condition	Androgens		Progesterone	Estrogens
	Testosterone	DHT		Estradiol/estriol/estrone
Acne vulgaris	[111][a]	[112][a]		
APD[b]			[114][a]	
Estrogen dermatitis				[113][a]
Atherosclerosis	[10][c,d]			[11,130][a,d]
Asthma			[9][a,d]	
Osteoarthritis				[119][a]
Rheumatoid arthritis	[115][c,d]			[116]
Pancreatitis				[118]
Multiple sclerosis				[120][a,d]
SLE[e]				[117][a]

[a] Specifically in women.
[b] Autoimmune progesterone dermatitis.
[c] Specifically in men.
[d] Protective effect.
[e] Systemic lupus erythematosus.

Estrogen-based HRT is a risk factor for pancreatitis in postmenopausal women with preexisiting hypertriglyceridemia [118], but has shown promise in preventing osteoarthritis in older women (age >65 years) [reviewed in 119]. Estriol, meanwhile, is being trialled for the treatment of multiple sclerosis [120].

7. Study area 1: estrogens and atherosclerosis

Though atherosclerosis has been much publicized as a disease of cholesterol metabolism, it has recently been acknowledged to be also an inflammatory condition [121,122]. In areas of blood vessels where low density lipoprotein (LDL) has accumulated, endothelial cells express monocyte chemokines and adhesion molecules. Attracted monocytes migrate to the subendothelial space and differentiate into macrophages, which generate reactive oxygen species (ROS). The ROS in turn oxidize LDL. Macrophages ingest thus-oxidized LDL and, as a result, transform into foam cells [123,124]. Ultimately, macrophage-derived foam cells are thought to contribute to the expansion and rupturing of atherosclerotic plaques [125], and consequent thrombosis and stenosis.

The link between estrogen status and atherosclerosis has been the subject of more than five decades of research. Early menopause has been associated with increased prevalence and severity of atherosclerosis [126], while a number of investigators have probed relevant effects of estrogens on plasma lipid balance and vascular tone (reviewed in [11]).

Animal models have been useful in evaluating the influence of estrogens on atherosclerosis. In ovariectomized monkeys on a moderately atherogenic diet, estrogen replacement reduced coronary atherosclerosis [127,128]. Interestingly, in a rabbit model of atherosclerosis, estradiol was shown to reduce intimal plaque size in females but not males [129].

Collectively, these animal data suggest that estrogens may offer some form of atheroprotection. However, clinical findings are not so conclusive. One placebo-controlled study found that 17β-estradiol slowed the progressive narrowing of the carotid artery *intima-media* in a group of healthy postmenopausal women (average age 62.2 years) NOT taking lipid-lowering medication or HRT [130]. However, a second published in the New England Journal of Medicine in 2000 [131] reported that conjugated equine estrogen (alone or with medroxyprogesterone) failed to halt the progression of pre-existing coronary atherosclerosis.

It is now accepted that estrogens may offer some protection during the early stages of atherogenesis and that the precise timing of hormonal intervention may determine its effectiveness.

Attempts to identify the receptor target of estrogen's atheroprotective effects in animal models have identified ER-α as being the primary mediator [132]. Moreover, in a group of premenopausal women who died from coronary heart disease or unrelated causes, ER-α protein expression was more evident in normal coronary arteries than in atherosclerotic ones [133]. By contrast, a recent study found ER-β to be the predominant ER isoform expressed in the most severely affected regions of atherosclerotic coronary arteries, with intimal ER-β protein levels being positively correlated with plaque area [134].

Other studies have identified genetic and epigenetic changes that may be important. Methylation of the *ESR1* and (ER-β-encoding) *ESR2* genes is increased in coronary artery plaques relative to healthy tissue [135,136], while *ESR1* gene polymorphisms were associated with disease severity in a group of males above the age of 53 [137].

Several investigators have sought to establish a mechanistic basis for estrogen's atheroprotective effects. Recent studies in rabbits revealed that estradiol reduces vascular ROS production by shifting the balance between ROS-generating and ROS-scavenging enzymes [138], suggesting that its atheroprotective action has an anti-oxidative dimension (Fig. 1). It seems not to involve NO (a putative atheroprotective agent itself), since inhibition of NO synthase does not block estrogenic atheroprotection [139].

By contrast, it may involve effects on inflammatory cell recruitment: in rabbits, aortic levels of MCP-1 were (1) increased through either ovariectomy or enrichment of the diet with cholesterol and (2) reduced in ovariectomized (OVX) animals subjected to systemic estrogen replacement [140]. There is evidence also that estradiol prevents hypercholesterolemic induction of monocyte surface expression of the IL-8 receptor CXCR2 in OVX rats [141].

Furthermore, administration of estradiol prevents the increased aortic adhesion of monocytes that occurs in cholesterol-fed rabbits as a result of ovariectomy, at least in part by reducing local VCAM-1 levels [142].

Elsewhere, data from a pair of *in vitro* studies suggest that estradiol may slow the formation of foam cells from macrophages [143,144].

Though estrogenic repression of local IL-6 production has been proposed as a further inflammatory mechanism [145], knocking out the *IL6* gene did not prevent estradiol from reducing aortic lesional areas in genetically apolipoprotein E-deficient mice [146].

Since they are frequently included in HRT formulations (to prevent the development of endometrial hyperplasia in postmenopausal women with intact uteri [147]), it has been necessary to determine the effects of progesterone/progestins on estrogenic inhibition of atherosclerosis. Animal studies have produced conflicting results, some reporting antagonism of estrogenic protection by progesterone [129] and the progestins medroxyprogesterone

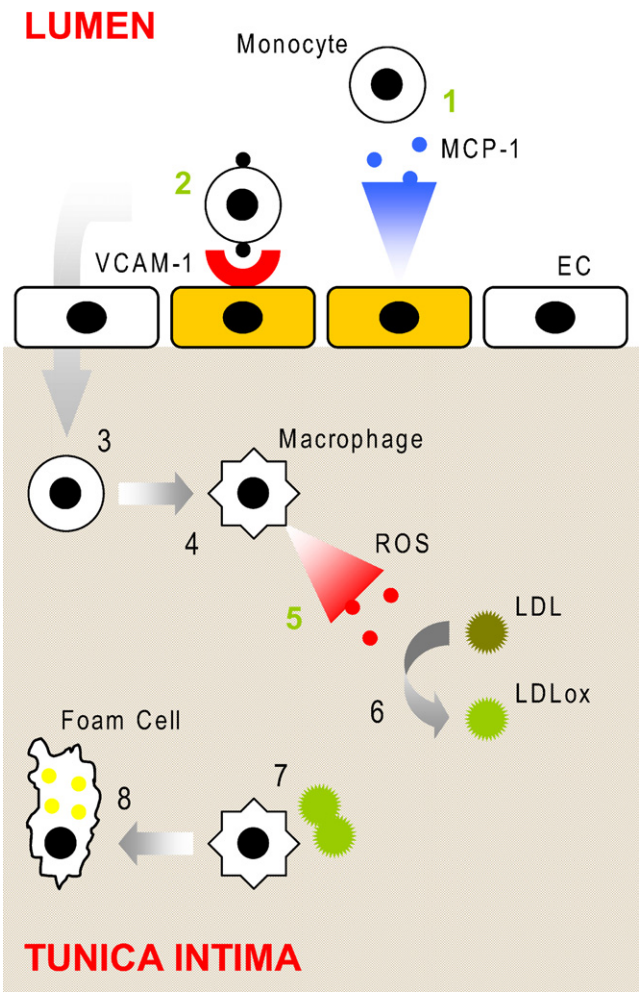

Fig. 1. Estrogen influences the early stages of atherosclerosis. Endothelial cell (EC) secretion of MCP-1 chemoattracts monocytes (1), which adhere to endothelial cells that have up-regulated leukocyte adhesion molecules such as VCAM-1 (2). Adherent monocytes transmigrate to the subendothelial space (3) and differentiate into macrophages (4). Macrophages secrete ROS (5), which stimulate the oxidation of LDL (6). Macrophages phagocytose oxidized LDL (LDLox) (7) and transdifferentiate into lipid-filled foam cells (8). Steps highlighted in green are subject to estrogenic inhibition.

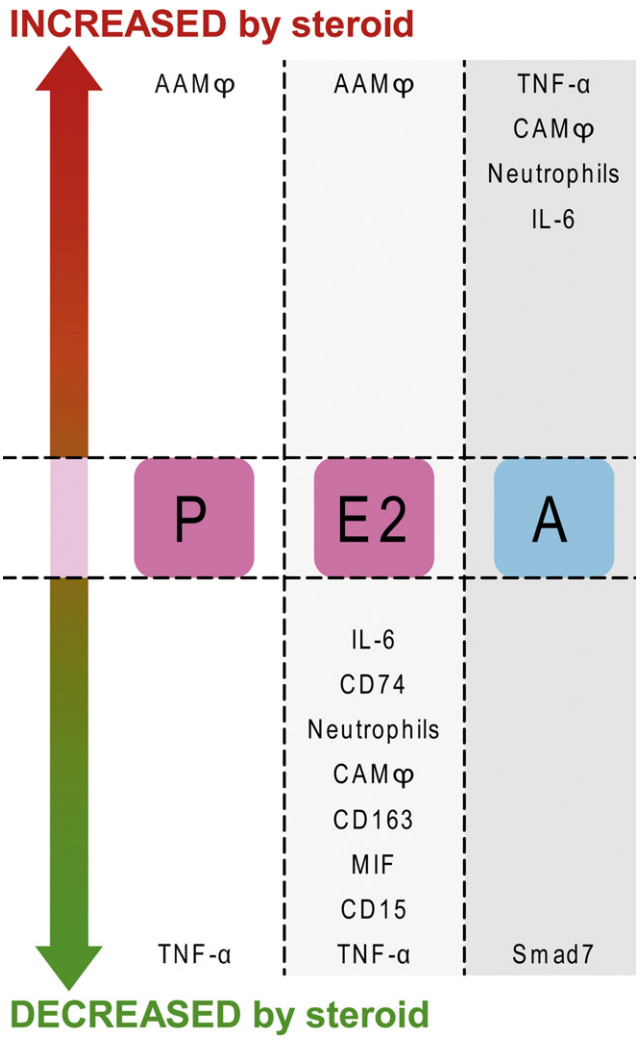

Fig. 2. Effects of sex steroids on wound inflammation. Both progesterone (P) and estradiol (E2) increase wound numbers of alternatively-activated macrophages (AAMφ). Estradiol additionally reduces local classically-activated macrophage (CAMφ), neutrophil and CD15- and CD163-positive cell populations, and levels of several key inflammatory mediators. Androgens (A) increase wound inflammation, as measured by inflammatory cell numbers and inflammatory cytokine levels.

acetate [128] and levonorgestrel [148]; another, enhancement by norethindrone acetate [149]; still others, no effect [127,150]. In a cross-sectional study involving 353 HRT users (average age 70.5 years), meanwhile, no differences in carotid wall thickness or stenosis were detected between individuals taking estrogen alone or estrogen plus progestin [151].

8. Study area 2: sex steroids in wound inflammation

Skin wounding initiates a series of overlapping events, with an acute inflammatory response, in which chemoattracted neutrophils and macrophages phagocytose contaminating microorganisms and tissue debris, preceding repair [152]. Excessive and unresolved inflammation delays healing and risks the development of a nonhealing ulcer.

Observed sex differences in wound inflammatory profiles in elderly humans [153] and young mice [154] suggested the potential influence of endogenous sex steroids. A decade of intensive investigation has revealed their critical involvement in wound inflammation (Fig. 2), with implications for both the use of HRT and the treatment of problem wounds.

8.1. Progesterone

Little is known about the effects of progesterone on wound-associated inflammation. Preliminary studies reported increased wound vascular permeability in progesterone-treated rabbits [155]. This response is suggestive of increased inflammation, a conclusion supported by the subsequent observation of increased local accumulation of inflammatory cells in Teflon cylinders implanted into OVX rats treated with progesterone [156]. More recently, however, accelerated healing in OVX mice treated systemically with progesterone was accompanied by respective increases and reductions in the wound populations of Ym1-positive alternatively-activated macrophages and TNF-α-positive cells [157]. Clearly the roles of progesterone in wound inflammatory responses merit further investigation.

8.2. Estrogens

Since Ashcroft et al. in 1997 established a link between the menopause and delayed healing of acute wounds in elderly women [158], there has been a concerted effort to describe and explain the roles of estrogens in wound inflammation. Acceleration of healing

in elderly females by topically-applied estradiol was accompanied by a reduction in wound CD15-positive neutrophil numbers [159]. *In vitro* treatment with estradiol, meanwhile, decreased neutrophil chemotaxis to fMLP and surface expression of the adhesion molecule L-selectin [159].

Estrogen replacement in rodents markedly reverses the increase in wound inflammation that occurs as a consequence of ovariectomy, decreasing overall local numbers of neutrophils [160,161], CD163-positive leukocytes [160] and macrophages (while expanding the alternatively-activated macrophage population) [157,160,161], and reducing wound levels of IL-6 [161], TNF-α [157,161] and macrophage migration inhibitory factor (MIF) [160,161], as well as the putative MIF receptor CD74 [160,162].

Studies employing MIF null mice have yielded yet more insights. A multifunctional protein whose secretion from LPS-activated mouse macrophages [163,164] and human monocytes [165] estradiol has been shown to reduce, MIF is tightly regulated by estrogens during wound healing. Excessive local MIF production contributes to delayed healing and increased wound inflammation in OVX mice [164], while local estrogen treatment has been shown to reduce wound MIF levels in elderly subjects [165].

In MIF null animals ovariectomy has minimal effect on wound repair or wound-associated inflammation [164,165].

A microarray-based study revealed the full extent of MIF's contribution to delayed healing in OVX mice: over 90% of genes identified as being regulated by estrogen during wound healing were also subject to control by MIF [165].

The connection between estrogens and wound inflammation is reinforced by genetic studies that established a link between polymorphisms in the *ESR2* gene and incidence of venous ulceration, a condition characterized by unresolved inflammation [166,167].

8.3. Androgens

There is growing evidence to suggest that endogenous androgens act to exacerbate wound inflammation. Wound inflammatory cells express AR protein [153] and accelerated healing in castrated (CSX) rodents is characterized by reduced local levels of TNF-α and wound numbers of macrophages [153,168]. Androgenic induction of TNF-α mRNA expression in peritoneal macrophages *in* vitro was partially blocked by co-treatment with the AR antagonist flutamide [153]. Accelerated healing induced by systemic administration of flutamide itself was accompanied by reduced wound TNF-α mRNA expression and NF-κB activation [153].

The ability of androgens to influence healing, and of testosterone to increase the secretion of MIF by BMDM, were shown to depend on the transforming growth factor (TGF)-β-activated transcription factor Smad3 [163], a documented inhibitor of wound healing [169], as were reductions in wound numbers of macrophages and neutrophils and levels of TNF-α in CSX mice [168]. Castration itself increased wound levels of Smad7, an inhibitor of TGF-β signaling and dampener of wound inflammation [168,170].

Targeted prevention of DHT biosynthesis in rats using an inhibitor of 5α-reductase, the enzyme responsible for metabolising testosterone to DHT, accelerated healing, reduced wound infiltration by macrophages and neutrophils, and decreased wound levels of IL-6 [168]. This suggests that some of testosterone's inflammatory effects require its prior conversion to DHT. Others seemingly do not: both testosterone and DHT were shown to increase macrophage expression of IL-6 mRNA [168]. Castration was previously found to protect against potentially harmful wound induction of the same cytokine in mice subjected to trauma-hemorrhage [171].

Elsewhere, improved granulation of diabetic foot ulcers in 3 male patients (aged 48, 73 and 77 years) who received a single injection of testosterone undecanoate (1000 mg) was accompanied by reduced blood leukocyte counts [172].

9. Conclusions

It is well established that age-associated changes in systemic sex steroid levels have profound effects on human health. Now it is clear also that the abrupt cessation of ovarian estrogen biosynthesis at menopause impacts greatly upon systemic markers of inflammation, inflammation associated with acute skin wounds, and the incidence and progression of specific inflammatory conditions. Notably, estrogens are antiatherosclerotic in females, while androgens fulfil a similar role in males. Age-related declines in the gonadal production of these sex hormones directly contribute to disease progression.

At the cellular level, estrogens and androgens influence such processes as chemotaxis and activation, as well as the secretion of inflammatory cytokines and adhesive interactions between inflammatory cells and the endothelium.

Some areas have received more attention than others. While hormonal modulation of monocyte/macrophage function has been pretty thoroughly investigated, the effects of sex steroids on other inflammatory cells including basophils and eosinophils remain underexplored. In the case of the latter, reported sex differences in the prevalence and intensity of a range of mammalian parasitic infections [173] suggest the potential influence of sex hormones.

Hormone replacement strategies have shown promise in the treatment of early-stage atherosclerosis [11] and promotion of wound healing in the elderly [148], but have been hamstrung by unacceptable side-effects [92]. Plant phytoestrogens or selective estrogen receptor modulators (SERMs), synthetic compounds that exert different effects (e.g. agonist/antagonist) in different tissues, may provide the solution. While the SERMs tamoxifen and raloxifene were shown (like estradiol) to speed healing and reduce associated inflammation in OVX mice [160], soy phytoestrogens effectively blocked the proliferative effects of estradiol in the endometrium and mammary gland of primates [174].

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Glossary

Stenosis: pathological narrowing of a blood vessel
Tunica intima: innermost layer of an artery/vein, comprising the endothelium and underlying connective/elastic tissue
Tunica media: middle layer of an artery/vein, in arteries composed of muscle fibres and/not elastic tissue