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## Pathogenesis of Endometriosis: Roles of Retinoids and Inflammatory Pathways

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### Abstract

Endometriosis is a nonmalignant, but potentially metastatic, gynecological condition manifested by the extrauterine growth of inflammatory endometrial implants. Ten percent of reproductive-age women are affected and commonly suffer pelvic pain and/ or infertility. The theories of endometriosis histogenesis remain controversial, but retrograde menstruation and metaplasia each infer mechanisms that explain the immune cell responses observed around the ectopic lesions. Recent findings from our laboratories and others suggest that retinoic acid metabolism and action are fundamentally flawed in endometriotic tissues and even generically in women with endometriosis. The focus of our ongoing research is to develop medical therapies as adjuvants or alternatives to the surgical excision of these lesions. On the basis of concepts put forward in this review, we predict that the pharmacological actions and anticipated low side-effect profiles of retinoid supplementation might provide a new treatment option for the long-term management of this chronic and debilitating gynecological disease.

### Keywords

chemokine; cytokine; metaplasia; retinoic acid; retrograde menstruation

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Endometriosis is a common and ubiquitous gynecologic disorder, affecting up to 10% of reproductive-age women worldwide, which is defined by the presence of hormonally responsive, ectopic implants of endometrial mucosa dispersed in extrauterine locations. Based on data from the World Bank, it has been estimated that 176 million women affected by this condition suffer pelvic pain and/or infertility.<sup>1</sup> The direct and indirect costs associated with these cardinal symptoms, including diagnostic tests, medical and surgical treatment expenses, and lost productivity, have been estimated to approach \$12,000 per woman per year<sup>2</sup> and represent a global health burden. Beyond its economic impact, the physical and psychological tolls of endometriosis are onerous.<sup>3</sup> The immune system is intimately involved with mechanisms underlying the symptomatology of endometriosis.<sup>4,5</sup>

Our review focuses on the roles of inflammation and immune cell infiltration in the pathogenesis of this enigmatic disease process and summarizes evidence that supports fundamental defects in retinoid metabolism and action among women with endometriosis. Findings from our studies suggest a new line of investigation for developing potential endometriosis therapeutic or preventative agents.

The classical and neoclassical concepts of endometriosis etiology have been reviewed comprehensively<sup>6,7</sup> and will not be reiterated exhaustively here. However, among all the postulated etiologies, we wish to emphasize two popular hypotheses—“retrograde menstruation and implantation” and “metaplasia”—that appear to be particularly relevant to the theme of inflammation as a pathogenetic mechanism of endometriosis.

“Retrograde menstruation and implantation” refer to the process of menstrual regurgitation through the fallopian tubes. John Sampson, a Johns Hopkins gynecologist at the turn of the 20th century, postulated that the ovarian endometriosis implant was “acquired from the implantation of epithelium escaping from the tube during menstruation and its subsequent invasion of the ovary.”<sup>8</sup> This has become the dominant theory of peritoneal disease over the past century and has acquired more nuanced understanding with evidence that endometrial apoptosis is impeded in women with endometriosis,<sup>9</sup> and invasiveness<sup>10</sup> and neuroangiogenic properties are enhanced,<sup>11</sup> predisposing this subset of women to the establishment of adherent and viable satellite lesions.

The second hypothesis, “metaplasia,” is the process by which one committed cell type is converted into an alternative cell type. Coelomic mucosa typically gives rise to the peritoneum, pleura, and surface epithelium of the gonads. In endometriosis, a metaplastic phenomenon is postulated to occur as a result of transdifferentiation of specialized peritoneal mesothelial cells into endometrial mucosa, as attributed to Robert Meyer of Berlin in the early 1920s.<sup>12</sup>

It is fair to say that cellular concepts of “inflammation” were rudimentary at the time these hypotheses were first promulgated, but the writings of these perceptive pioneers indicate that they recognized the peculiar significance of the stroma at the invading phalanx of endometriosis implants.<sup>13</sup> The contributions of stromal cell–derived chemokines and the leukocytes they recruit have been a major focus of our studies in endometriosis.<sup>5,14</sup> It is now accepted that within metaplastic foci, differentiated cells commonly arise in the setting of chronic inflammation and they may be predisposed to neoplastic transformation; all of these phenomena occur in endometriosis.<sup>15</sup> Moreover, in contemporary views of metaplasia, the programming or recruitment and differentiation of intrinsic<sup>16</sup> or bone marrow–derived<sup>17</sup> stem cells, respectively, to ectopic sites is envisioned.

The presence of ectopic or metastatic rests of autologous, benign tissues is in fact quite rare in human biology, but two examples, not typically considered by students of endometriosis, may be informative. The first of these is Barrett esophagus, wherein gastroesophageal reflux of bile acids and other stomach contents triggers a progressive replacement of stratified squamous esophageal cells by ectopic foci of intestinal mucosa with mucin-containing goblet cells above the pyloric valve. Histological evidence of inflammation, particularly

neutrophil infiltration, is commonly observed in these lesions.<sup>18</sup> The degree of intestinal metaplasia and inflammation in human gastric mucosa specimens from 67 study participants were inversely correlated with the ability of the tissue to produce all-*trans* retinoic acid (RA).<sup>19</sup>

A second setting in which ectopic foci of autologous metaplastic “implants” occur is within the tracheobronchial mucosa, characteristically in response to chronic inflammation induced by cigarette smoking. These lesions also represent a clinically premalignant form of metaplasia, wherein reparative processes induce the substitution of respiratory epithelium by squamous cells. The density and cross-sectional area of microvessels within metaplastic lesions increase progressively as they manifest more neoplastic histological features.<sup>20</sup> In respiratory tract biopsies from smokers, squamous metaplasia, immune cell (CD45 +) infiltration, and a profile of proinflammatory cytokines very similar to those reported in endometriosis (e.g., TNF- $\alpha$ , IL-1 $\beta$ , and IL-6<sup>4</sup>) were all upregulated compared with biopsies from nonsmoking volunteers. In cell culture models, RA-deprived tracheobronchial epithelial cells also manifest squamous metaplasia.<sup>21,22</sup> It has been established that RA can attenuate clinical and experimental airway inflammation.<sup>23,24</sup>

As with endometriosis, where odds ratios for developing clear cell or endometrioid carcinomas of the ovary may be as high as threefold,<sup>15</sup> Barrett esophagus metaplasia<sup>25</sup> and squamous metaplasia of the lung<sup>26</sup> also are associated with increased carcinogenesis.

## Endometriosis: An Inflammatory Paradigm

In each of the disorders described above, immune cell recruitment and infiltration into the ectopic lesions are a consistent theme. In endometriosis, intralesional accumulation of leukocytes was initially recognized around the turn of the 20th century, shortly after Paul Ehrlich and Ilya Mechnikov were jointly awarded the 1908 Nobel Prize in Physiology or Medicine for their discovery of what are now recognized to be the adaptive and innate immune systems, respectively.<sup>27</sup> It was Meigs, then serving as director of gynecology at the Vincent Memorial Hospital in Massachusetts, who first described the microscopic infiltration of “endothelial leukocytes” into endometriosis implants, which he noted were associated with fibrosis and neoangiogenesis.<sup>28</sup> These prescient observations are currently viewed as fundamental principles underlying the cell biology of endometriosis, but his progressive ideas lay dormant for nearly 60 years.

It was not until 1980 that the seminal publication of Weed and Arquembourg<sup>29</sup> reinvigorated Meigs’ insights into leukocyte infiltration, suggesting there were multiple features of local autoimmune phenomena associated with ectopic endometriosis implants. In addition to intralesional lymphocyte accumulation, the authors provided immunohistochemical evidence of complement C3 deposition in ectopic and eutopic biopsies from affected women and postulated that endometriosis-associated infertility might be due to “the rejection of early implantation of embryos,”<sup>29</sup> a hypothesis that continues to have advocates to this day.<sup>30</sup>

Indeed, autoimmune disorders<sup>31</sup> and type 1 allergies, including immediate hypersensitivity,<sup>32</sup> have been increasingly associated with endometriosis. Many of the

chemokines and cytokines activated in lesions have been reviewed recently,<sup>33</sup> and several of the genes that regulate these immunoreactive proteins have single-nucleotide polymorphisms and copy variants affecting their expression.<sup>34</sup>

Abnormal innate cell-mediated immune responses, particularly those of macrophages and natural killer cells, appear to facilitate endometriotic lesion attachment and growth.<sup>35</sup> Accumulation of activated macrophages within the pelvic fluid of women with endometriosis is well established<sup>36,37</sup>; however, the potency of their scavenger function and phagocytotic potential appears to be inhibited.<sup>5</sup> As discussed in detail below, in a murine endometriosis model, peritoneal macrophage function can be partially rescued following RA supplementation.<sup>38</sup>

By the mid-1990s, investigators who questioned how these peritoneal macrophages were recruited into the pelvic fluid were rewarded by discovering chemokines that accumulate in the peritoneal fluid of subjects with endometriosis. Using bioassays<sup>39</sup> and newly developed enzyme-linked immunosorbent assays (ELISAs),<sup>40</sup> the concentrations of several of these activities were shown to be correlated directly with the extent of endometriosis as assessed by laparoscopic staging.<sup>4</sup>

In clinical endometriosis studies to date, misexpression of two major classes of chemokines has been identified; these are categorized based on their amino acid structure.<sup>33</sup> The largest class consists of the CC chemokines, named for conserved adjacent cysteine residues in the proteins' carboxyl termini. CC chemokines target monocytes, T cells, and eosinophils, and include MCP-1 (CCL2),<sup>41</sup> MIP-1 $\alpha$  (monocyte inflammatory protein-1 $\alpha$ , CCL3),<sup>42</sup> RANTES (CCL5),<sup>40</sup> and eotaxin (CCL11).<sup>43</sup> The second commonest class of chemokines is the CXC family, in which a single, variable amino acid is interposed between the two conserved cysteines. These chemokines predominantly attract monocytes and neutrophils and include growth regulated oncogene (GRO)- $\alpha$  (CXCL1),<sup>44</sup> epithelial cell-derived neutrophil-activating peptide (ENA)-78 (CXCL5),<sup>45</sup> IL-8 (CXCL8),<sup>46,47</sup> and stromal cell-derived factor (SDF)-1 (CXCL12).<sup>48</sup>

Different chemokines have different sites of synthesis in endometrial and endometriosis tissues.<sup>33</sup> RANTES protein and mRNA are mostly confined to the stromal compartment of endometriosis tissues.<sup>49</sup> By contrast, eotaxin, ENA-78, MCP-1, and IL-8 are predominantly epithelial.<sup>43,45,50,51</sup> As the highest concentrations of tissue-associated macrophages are found in the stromal compartment of endometriosis lesions, as well as endometrial hyperplasia and carcinoma,<sup>52</sup> we have concentrated on the role of RANTES in immune cell recruitment to the stroma of these lesions. In vitro, stromal cell cultures derived from endometriosis implants robustly synthesize RANTES mRNA and secrete protein when stimulated by proinflammatory cytokines, whereas epithelial cells synthesize neither transcripts nor protein encoded by this gene.<sup>53,54</sup> The transcription factor nuclear factor (NF)- $\kappa$ B is a critical regulator of RANTES gene and protein expression.<sup>4,55</sup>

## Deficiency States of Anti-Inflammatory Hormones and Autacoids

Clinicians and investigators have suspected for over 60 years that the action of progesterone on uterine function was dysfunctional in cases of endometriosis. Since the early days of

radioimmunoassay, the luteal rise in serum or pelvic fluid progesterone concentration was variably reported to be reduced<sup>56,57</sup> or delayed.<sup>58,59</sup> In more recent years, alterations in progesterone receptor isoform expression are increasingly recognized to modulate progesterone action.<sup>60</sup> The original observation by Attia et al,<sup>61</sup> that PR-B transcript levels were markedly reduced in endometriosis lesions, corroborated findings that progesterone-regulated endometrial genes were generally underexpressed in cases of endometriosis.<sup>62–64</sup> This concept was further supported by evidence that the PR-B promoter was hypermethylated<sup>65</sup> and other chromatin modifications occur that may account for reduced PR-B expression.<sup>66</sup> Interaction of the PR with Hic-5 also is attenuated as a result of reduced expression of the latter in endometrial tissue and stromal cells derived from women with endometriosis.<sup>67</sup> Moreover PR resistance also was shown to be manifested in baboons with surgically induced endometriosis.<sup>68</sup>

Excessive estrogen signaling has long been associated with endometriosis and constitutes a traditional target for medical therapies.<sup>69</sup> Increased estrogenic action in these lesions appears to be a consequence of altered expression of both its receptors, estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$ ,<sup>70,71</sup> and increased local hormone biosynthesis by aromatase, the CYP19A1 gene product.<sup>72</sup> Recent pharmaceutical developments have focused on the important role of estrogen receptor signaling in endometriosis. Two novel ER ligands, which bind preferentially to ER $\alpha$  and ER $\beta$ , respectively, are oxabicycloheptene sulfonate (OBHS) and chloroindazole (CLI). These compounds displayed dual suppression of proliferative and inflammatory activities and effectively prevented the establishment and progression of endometriotic lesions in a mouse model.<sup>73</sup> The selective estrogen receptor modulators, bazedoxifene<sup>74</sup> and ERB-041,<sup>75</sup> also were shown to suppress endometriotic lesion growth in rodents.

Another family of nuclear receptor proteins with anti-inflammatory activities is the peroxisome proliferator-activated receptor (PPAR)- $\gamma$ .<sup>76</sup> The actions of PPAR- $\gamma$ -ligand complexes affect endometriotic stromal cells,<sup>77–79</sup> as well as infiltrating macrophages,<sup>5</sup> and vascular endothelial cells.<sup>80,81</sup> PPAR proteins are obligate heterodimer partners with retinoic X receptors (RXR) in target cell nuclei.<sup>82</sup> Several naturally occurring, high-affinity ligands for PPAR- $\gamma$  have been identified, including the eicosanoid (9*S*,10*E*,12*Z*)-9-hydroxy-octadeca-10,12-dienoic acid (9-*S*)-HODE).<sup>82</sup> To date, we are unaware of published evidence of reduced circulating concentrations of endogenous PPAR- $\gamma$  ligands in cases of endometriosis. However, such findings have been described in the clinical setting of pregnancy complications.<sup>83</sup>

The salutary effects of synthetic PPAR- $\gamma$  agonists have been shown in rodent<sup>84,85</sup> and nonhuman primate<sup>86</sup> models of endometriosis. Given the remarkable potential, but significant side-effect profiles, of current PPAR- $\gamma$  pharmaceuticals (thiazolidinediones), a spectrum of natural compounds with high-affinity agonist activities have been screened. Among these are some familiar compounds—resveratrol, honokiol, and 6-hydroxydaidzein—but their safety and efficacy have not been tested rigorously in clinical trials.

## Retinoid Metabolism in Endometriosis

In addition to critical regulation by steroid hormones and other known ligands for nuclear receptors as mentioned above, studies have shown that retinoids also play fundamental roles in the normal maintenance of the endometrium and particularly with respect to endometriosis.<sup>87–89</sup> To this end, the action of RA, produced by metabolic conversion of retinol (ROL), has long been recognized as being necessary for endometrial cell differentiation and function.<sup>90,91</sup> This activity is mediated by the expression of nuclear and cytoplasmic retinoid receptors and localized RA synthesis within endometrial and endometriotic stromal cells<sup>92,93</sup> (Fig. 1). During the human menstrual cycle, expression of retinoid receptors and synthesis of RA are influenced by the changing patterns of ovarian steroid exposure. Among the numerous aspects of cell behavior pertinent to endometriosis and regulated by local RA production are matrix metalloproteinase (MMP) secretion, gap junctional intracellular communication, and the expression of a variety of cytokines involved in cell differentiation and immune regulation.<sup>94,95</sup> Some examples of such RA-regulated genes are IL-6, MCP-1, TNF- $\alpha$ , VEGF, connexin 43, various integrins, and fas ligand,<sup>96–99</sup> genes which are also known to be aberrantly expressed in endometriotic lesions.<sup>100</sup> Thus, several seemingly discordant features of endometriosis, including repression of apoptosis, increased growth and migration, inflammation, and enhanced invasive properties of intraperitoneally seeded endometrial cells, could be accounted for by dysregulation of RA synthesis.

The group of Serdar Bulun was among the first to investigate the role of retinoid action in endometriosis.<sup>101</sup> Those studies showed altered expression of several genes involved in retinoid biosynthesis and signaling in lesion cells from endometriosis patients, compared with normal eutopic endometrium from patients without endometriosis. Although their study did not measure RA levels directly, the results were consistent with decreased retinoid uptake, metabolism, and action within endometriotic lesions. Subsequent work by Pierzchalski et al<sup>102</sup> utilizing matched samples from subjects with endometriosis (i.e., lesions vs eutopic tissue from the same person) directly quantified RA levels and metabolic conversion of ROL to RA in stromal cells derived from the corresponding biopsies; these cells are the primary source of RA biosynthesis in endometrial tissue.<sup>103</sup> The studies confirmed that RA biosynthesis is impaired in ectopic endometriotic implants versus their normal eutopic counterpart. A major defect noted was the reduced expression of cellular retinol-binding protein type 1 (RBP1), an ROL chaperone protein that serves as the preferred substrate for retinol dehydrogenase enzymes and the rate-limiting step in RA biosynthesis.<sup>104</sup> Thus, reduced RBP1 results in significantly less efficient metabolism of ROL to retinal and its subsequent oxidation to RA. In addition to endometriosis, RBP1 has been shown to be aberrantly expressed in certain mammary, cervical, and ovarian cancers,<sup>105–109</sup> as well as some developmental diseases of the brain, bone, and skin.<sup>104,110</sup> These studies suggest that defects in *RBP1* gene expression in endometriotic stromal cells result in abnormal retinoid biosynthesis and could play a role in the etiology and/or progression of endometriosis.

Two possible scenarios to account for aberrant retinoid metabolism in ectopically growing endometrial cells have been suggested, based on the histogenic mechanisms we provided

above. (1) Among the cells that reach the peritoneal cavity via retrograde menstruation, those with intrinsically defective RA synthesis preferentially populate the ectopic sites because of downstream effects of reduced RA levels (e.g., proliferative effects on cell cycle dynamics, increased MMP production, immune cell activation, and proinflammatory cytokine synthesis). (2) A hypothesis in keeping with the metaplastic theory of etiopathogenesis posits that the peritoneal milieu provides environmental cues that induce defects in RA synthesis in metaplastic foci, as opposed to impaired retinoid metabolism being an intrinsic characteristic of the cells. Evidence from studies showing alterations in cytokine and MMP profiles in eutopic endometrium from some women with endometriosis supports the former possibility.<sup>30,111</sup> However, support for the latter hypothesis comes from the observation that oxidative stress and prostaglandin (PG)E<sub>2</sub>, known to be elevated in peritoneal fluid from patients with endometriosis,<sup>112</sup> can inhibit biosynthesis of RA<sup>109,113</sup> and induce transcriptional repression of *RBP1*.<sup>114,115</sup> Indeed, quite a rich literature supports a critical role for PGE<sub>2</sub> as a master regulator of endometriosis. It has been demonstrated to modify a variety of pathophysiological features of the disease, including cell proliferation, antiapoptosis, inflammation, and angiogenesis.<sup>116</sup> Using direct, metabolic labeling of purified endometriotic stromal cells with [<sup>3</sup>H]arachidonic acid in vitro, we could demonstrate that PGE<sub>2</sub>, along with PGF<sub>2</sub> $\alpha$ , is a major prostanoid product of these cells (Fig. 2).

It has also been reported that cellular RA-binding protein 2 (CRABP2), which delivers RA to RA receptor  $\alpha$  (RAR $\alpha$ ),<sup>117</sup> is reduced in endometriosis, potentially as a consequence of progesterone resistance.<sup>30,101,103</sup> Whereas it is unknown whether CRABP2 loss precedes RBP1 reduction, it is likely significant to the persistence of reduced RBP1 expression, because loss of CRABP2 function can result in heritable chromatin repression of multiple loci downstream of RAR $\alpha$ , including *RBP1*.<sup>114</sup>

## RA and Inflammation

The cause and effect relationship between impaired RA synthesis and the development of endometriosis is unknown. However, numerous papers indicate that the effects of RA on inflammatory processes suggest that reduced levels in endometriosis can promote some of the abnormal immunological changes that are thought to contribute to its etiology and/or progression. In model systems involving activated monocytes/macrophages, RA decreased proinflammatory cytokines while increasing anti-inflammatory proteins such as interleukin-(IL)10.<sup>118</sup> In a variety of cell types, RA has been shown to profoundly affect IL-6-driven events through down-regulation of IL-6 ligand and/or IL-6 receptor production.<sup>119,120</sup> One of the most striking in vivo demonstrations showing the ability of retinoids to alter IL-6 levels came from a trial of 13-cis RA (Accutane, Hoffmann-La Roche Ltd., Nutley, NJ) in patients with common variable immunodeficiency (CVI).<sup>119</sup> These patients have elevated levels of circulating IL-6 thought to be due to reduced sensitivity and failure of CVI B cells to mature in an IL-6-dependent fashion. The IL-6 concentrations in four of five CVI patients fell to the normal range while on Accutane treatment. This change in circulating IL-6 levels was thought to have resulted from direct effects of the retinoid on monocytes/macrophages, the main identified source for the IL-6 produced in CVI.<sup>121</sup> Endometriotic stromal cells also are a rich source of IL-6.<sup>122</sup> Studies demonstrated that RA suppresses IL-6 from human

endometrial cells through functional antagonism with the nuclear factor IL-6 binding site located in the IL-6 gene promoter.<sup>99</sup> In addition to altering the cytokine profile of activated macrophages, RA has been shown to upregulate the CD36 type-B scavenger receptor in cells of the monocyte lineage.<sup>123,124</sup> This receptor has been implicated in the uptake and degradation of apoptotic cells and other debris,<sup>125</sup> and is regulated by RA in human monocytes/macrophages by a novel mechanism of action that does not require cell adherence.<sup>123,124</sup> Thus, treatment of human monocytes/macrophages with RA results in markedly increased protein and mRNA levels of CD36 that occur in the absence of cellular adhesion and differentiation. This fact is important, as multiple studies have demonstrated that women with endometriosis show an increased number of nonadherent macrophages in their peritoneal cavity.<sup>36,37</sup> In addition to increasing the ability of peritoneal macrophages to “clear” ectopic endometrial cells, upregulation of CD36 would also cause an increased scavenging of oxidized lipoproteins (e.g., oxLDL) in peritoneal fluid, effecting a net reduction of reactive oxygen species (ROS).<sup>126,127</sup> ROS, and other consequences of oxidative stress in the peritoneal fluid of endometriosis patients, has been suggested to play an active role in exacerbating the growth of endometriotic lesions.<sup>128</sup> Thus, the failure of adequate RA biosynthesis to selectively upregulate CD36 expression in the monocyte/macrophage lineage would predict impaired scavenging function of peritoneal macrophages, allowing both the initiation and progressive growth of endometriotic implants. Furthermore, in primary murine astrocyte cultures, pretreatment with RA suppressed the production of chemokine (CCL2, CCL3, CCL5, CXCL1, and CXCL2) mRNAs and proteins in response to lipopolysaccharide endotoxin. Based on experiments using RAR and RXR inhibitors, it is hypothesized that both receptors are likely to be involved in RA’s anti-inflammatory effects.<sup>129</sup>

## Therapeutic Implications

Although it has yet to be determined that the abnormal immune functions associated with endometriosis are caused directly by impaired retinoid action, there is mounting evidence that treatment modalities that target the retinoid metabolic pathway may have therapeutic utility. An example of this possibility was shown by the ability of statins to reduce the number and size of lesions in animal models of endometriosis.<sup>130–132</sup> A mechanistic and genetic analysis of this effect indicated that statins modulate the expression of genes involved in the regulation of synthesis and actions of RA,<sup>133</sup> suggesting that this action may play a role in their therapeutic efficacy. To directly test this possibility, studies by our group utilizing an immunocompetent mouse model of endometriosis demonstrated that in vivo RA treatment suppressed the establishment and growth of ectopic peritoneal implants along with inhibiting peritoneal fluid accumulation of IL-6 and MCP-1.<sup>38</sup> In addition, RA treatment modulated the differentiated state of the murine peritoneal macrophages, as reflected by increased expression of CD38, CD11b, and F4/80. This observation may have important implications in terms of understanding the therapeutic mechanism of RA treatment. On a quantitative basis, both F4/80 and CD11b increase as monocytes differentiate to macrophages and with inflammatory reactions that are associated with mature macrophage function, such as phagocytosis.<sup>134–137</sup> The type II transmembrane glycoprotein, CD38, is widely recognized as a marker of lymphocyte and macrophage activation and

differentiation.<sup>138</sup> RA transcriptionally activates CD38 expression in immune cells via an RA response element located in the first intron of the CD38 gene.<sup>139</sup> In macrophages, RA induction of CD38 is associated with increased differentiated functions, including antigen presentation and cell adhesion.<sup>138,140</sup> Together, these findings suggest that RA-induced inhibition of endometriotic implants in the mouse model was due, at least in part, to suppression of IL-6 and MCP-1, and promotion of peritoneal macrophage differentiation. In a rat model of endometriosis, where lesions were induced by autotransplantation of uterine pieces into the peritoneal cavity,<sup>141</sup> the therapeutic effects of RA were compared with known antiangiogenic agents (bevacizumab and sorafenib). All three compounds induced a significant reduction in the size of the endometriotic implants. However, while the other two agents demonstrated antivascular effects that accompanied a reduction in endometriotic volumes (e.g., decreased VEGF and microvessel density), RA showed the most effective therapeutic benefits without affecting angiogenic parameters. The fact that host immune responses play a primary role on the growth of lesions in this model system<sup>142</sup> suggests an immunologically mediated mechanism of RA action. Interestingly, RA was the only compound to concomitantly promote an increase in primordial follicle number, indicating a favorable effect also on ovarian reserve, which has been documented to be impaired in cases of endometriosis.<sup>143</sup> Finally, a recent analysis of retinoid levels in the plasma and follicular fluid from women with endometriosis undergoing IVF showed a significantly lower mean concentration of RA, but not ROL, in both compartments compared with similar infertile subjects without endometriosis (women with unexplained, tubal, or male factor infertility).<sup>144</sup> Although RA levels were within the reported normal concentration range,<sup>145,146</sup> these data support the hypothesis that women whose overall retinoid metabolism is in the low normal range of activity are at higher risk for endometriosis. Such a possibility may help us gain insight into two quintessential questions: (1) Why do only some women develop endometriosis, in spite of the fact that retrograde menstruation seeds the peritoneal cavity with endometrial cells in almost all women?<sup>147</sup> (2) What are the mechanisms that predispose women with endometriosis to peritoneal inflammation and immune cell dysfunction?

## Conclusion and Future Directions

While the etiology of endometriosis remains obscure, scientific and clinical contributions over the past 95 years have progressively illuminated its pathophysiology. Surgery, particularly laparoscopic excision and bipolar electrocauterization of pelvic implants, remains a mainstay for the treatment of pain and infertility symptoms in affected women. With our advancing understanding of the cell biology of endometriosis, medical therapeutics are increasingly evolving from strategies that focus on the suppression of the hypothalamic–pituitary–ovarian endocrine axis<sup>148</sup> to approaches that target alternative pathways, for example, oxidative stress and inflammation.<sup>149</sup> As we have summarized in this review, activation of retinoid signaling in endometriosis tissues is predicted to have several salutary effects on the resident cells within these lesions. We propose that adjuvant medical therapies should be developed based on these concepts and predict that their pharmacological actions and anticipated low side-effect profiles will provide women with endometriosis more

treatment options for the long-term management of a chronic and debilitating gynecologic disease.

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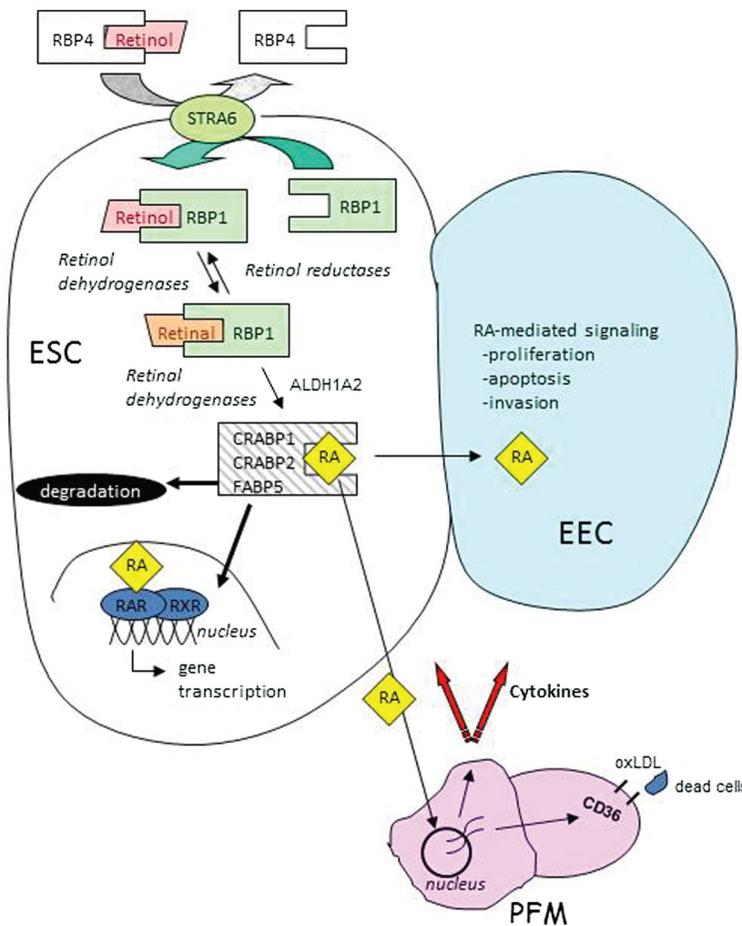
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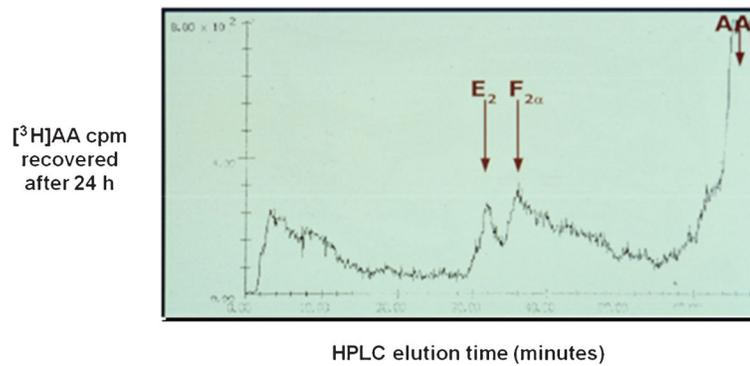
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**Fig. 1.** Endometrial vitamin A (retinol) metabolism and retinoic acid (RA) signaling. Uptake of retinol (ROL) bound with circulating plasma “retinol binding protein 4” (RBP4) is controlled by the membrane receptor, “stimulated by retinoic acid 6” (STRA6). Intracellular chaperone “cellular ROL-binding protein type 1” (RBP1) physically interacts with STRA6 to pick up ROL, protect ROL from nonspecific metabolism, and deliver it to retinol dehydrogenase enzymes which reversibly catalyze conversion of ROL to retinal. RBP1 then chaperones retinal-to-retinal dehydrogenases (ALDH1A2) which irreversibly convert retinal to RA. RA is then chaperoned by a distinct set of RA-binding proteins (CRABP1, CRABP2, FABP5), which are known to be expressed in endometrial stromal cells (ESC).<sup>101,103</sup> Once formed, RA can be: (1) transported to the nucleus of the RA biosynthesizing ESC where it binds to nuclear receptors (RAR) and initiates gene transcription; (2) transported to adjacent epithelial cells (EEC) or secreted into the microenvironment to affect gene transcription in other cells such as peritoneal fluid macrophages (PFM); or (3) degraded. Genes known to be affected in PFM include various proinflammatory cytokines (IL-6, MCP-1, TNF $\alpha$ ), which are downregulated by RA and the CD36 type-B scavenger receptor which is upregulated by RA. The consequence of this activity in PFM is a reduction in the inflammatory and oxidative status of the peritoneal environment and increased clearance of ectopic endometrial cells.



**Fig. 2.** Endometriotic stromal cells effectively metabolize [<sup>3</sup>H]arachidonic acid to PGE<sub>2</sub> and PGF<sub>2α</sub> in vitro. Metabolic labeling of endometriotic stromal cell prostanoid production was performed as described by de Groot et al.<sup>150</sup> Briefly, the cultures were incubated with 3 nM [<sup>3</sup>H] arachidonic acid (AA) for 24 hours and the spent media were extracted with chloroform:methanol:acetic acid (180:20:1) and subjected to high-performance liquid chromatography using a 3.9 × 150 mm Nova-Pack C18 reverse phase column on a Waters Model 204 liquid chromatograph. Unlabeled PGE<sub>2</sub> and PGF<sub>2α</sub> standards eluted at 32 and 38 minutes, respectively, under these conditions. Radioactive counts per minute (cpm) were detected with a Radiometer FLO/ONE-β Model A250 detector.