MAYO CLINIC

Immunomodulatory Effects of Sex Hormones: Requirements for Pregnancy and Relevance in Melanoma

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Abstract

Similarities between the pathologic progression of cancer and the physiologic process of placentation (eg, proliferation, invasion, and local/systemic tolerance) have been recognized for many years. Sex hormones such as human chorionic gonadotropin, estrogens, progesterone, and others contribute to induction of immunologic tolerance at the beginning of gestation. Sex hormones have been shown to play contributory roles in the growth of cancers such as breast cancer, prostrate cancer, endometrial cancer, and ovarian cancer, but their involvement as putative mediators of the immunologic escape of cancer is still being elucidated. Herein, we compare the emerging mechanism by which sex hormones modulate systemic immunity in pregnancy and their potentially similar role in cancer. To do this, we conducted a PubMed search using combinations of the following keywords: "immune regulation," "sex hormones," "pregnancy," "melanoma," and "cancer." We did not limit our search to specific publication dates. Mimicking the maternal immune response to pregnancy, especially in late gestation, might aid in design of better therapies to reconstitute endogenous antitumor immunity and improve survival.

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In 1948, Beard and Krebs acknowledged a striking similarity between a trophoblast and a tumor, publishing their observation titled "The Unitarian or Trophoblastic Thesis of Cancer."[1-3] Since then, these similarities have been extensively studied; many shared pathways and immunologic mediators have been identified.[4,5] The purpose of this review was to take an in-depth look at existing research describing the role of sex hormones in the potentially parallel settings of reproductive and tumor immunology, with a focus on metastatic melanoma. Although imperfectly understood, sex hormones are important regulators of the immune system in both pregnancy and cancer.[6,7] It is clear that they are involved in regulation and modification of the immune system to allow invasion, proliferation, and migration of tumor cells and trophoblasts.[5] It is possible that an organ system—level view of the process of placentation as well as melanoma progression could yield additional insights into potential therapeutic targets for hormone-based immune modulation.[8]

The complexities and redundancies involved in orchestration of the maternal response to pregnancy as well as the host response to cancer are increasingly appreciated.[4,9] Importantly, however, we and others have observed that neither pregnancy nor advanced cancers are static immunologic events.[10-12] Oscillations in systemic immunity between inflammation and tolerance seen in patients with metastatic melanoma have been documented and seem to follow a biologically predictable pattern. When tolerance seen in malignant melanoma is disrupted and brought back to a state of inflammation, patients have a much better prognosis than do those whose immune systems stay in an immunologically exhausted state. Pregnancy is also characterized by many hormonal fluctuations, although the time scale for these hormonal and immunologic changes may be measured in weeks as opposed to days[13] (Figure 1) Although it seems intuitive to consider the involvement of sex hormones interacting with the maternal immune system during pregnancy, it is less obvious but just as possible that such hormones alter systemic

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immunity in the setting of cancers such as melanoma. We describe the observations and experimental evidence supporting such involvement in the following sections.

CLINICAL EVIDENCE OF HORMONAL REGULATION OF MELANOMA

The skin is capable of producing many neuroendocrine mediators such as melanin, steroids, thyroid hormones, and sex hormones such as androgen, estrogen, and progestin to maintain homeostasis; any failure to communicate between the skin, endocrine, and immune system could result in deregulation and disease.[14,15] Both melanocytes and melanoma tumors produce pigment in the melanosome that protects the skin against damaging ultraviolet rays through positive regulation by hormones such as L-tyrosine and L-dihydroxyphenylalanine.[16,17] Although the interplay between sex hormones and the immune system in melanoma remains poorly understood, several clinical observations support the role of sex hormones in melanoma development. Melanomas that are responsive to estrogens are associated with the superficial spreading melanoma subtype, a type of tumor with a much better prognosis. In addition, estrogen exerts a proliferative effect on melanocytes and can lead to the development of hyperpigmentation in women using oral contraceptives or hormonal replacement therapy.[18] Whether hormonal contraceptives increase the risk of melanoma is a matter of ongoing debate. Koomen et al[19] have reported that high levels of estrogens increase a woman's risk for developing malignant melanoma, while Lens and Bataille[20] have not observed a relevant association. It may be no coincidence that melanoma is the most common form of cancer associated with pregnancy.[21] This is believed to be due to the trophoblasts' increased need for lymphangiogenesis, which the melanoma then uses to promote its own growth. Complementary to this hypothesis, demographic characteristics and incidence may also provide an explanation as to this phenomenon. In addition, pregnant women are more likely than their nonpregnant counterparts to be diagnosed with an invasive melanoma.

We have shown that aging in healthy individuals is associated with a T_H2 bias.[22] Women are more likely than men to develop melanoma before age 40, after which the diagnosis of melanoma is observed at a much higher rate in men.[23] However, women diagnosed with melanoma have a better prognosis than do men, and premenopausal women have higher survival rates than do postmenopausal women.[24,25] Interestingly, melanoma metastasizes at a much slower rate in women than in men, and the pattern of metastatic spread is also different, with more

ARTICLE HIGHLIGHTS

- Systemic immunity in metastatic melanoma (cancer) mimics the systemic immune response of early pregnancy.
- Sex hormones promote/suppress different T cell responses during pregnancy and in melanoma.
- Metastatic melanoma and early pregnancy promote a systemic state of Th2 dominant chronic inflammation.
- Near parturition, hormones play a role in the return to cytotoxicity (Th1) of maternal immunity and the promotion of labor.
- Understanding the mechanism that causes immunity to switch from Th2 to Th1 in pregnancy may help researchers better understand how to break tolerance and improve patient outcomes in advanced cancers.

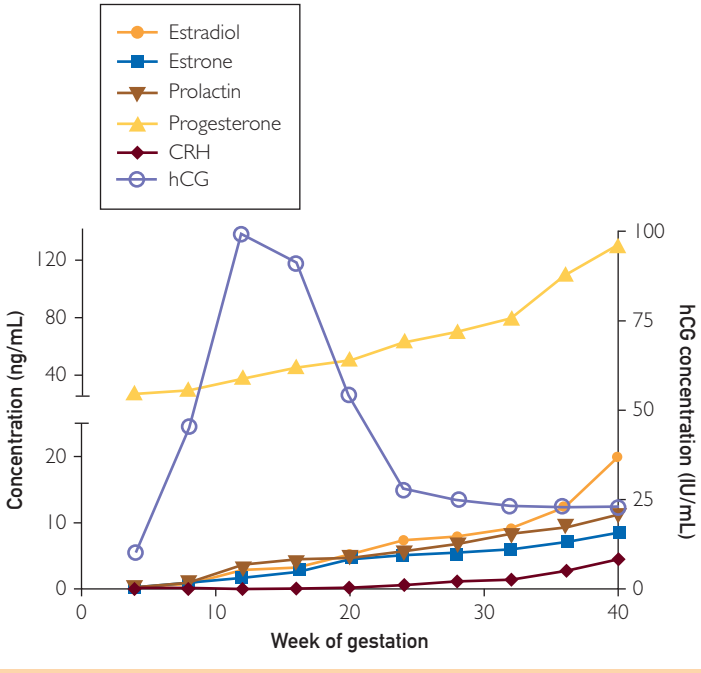

FIGURE 1. Hormonal changes (in weeks) important for the regulation of gestation in healthy pregnant women. CRH = corticotropin-releasing hormone; hCG = human chorionic gonadotropin.

locoregional recurrences observed in women.[26,27] Still, reasons behind these sex differences are yet to be elucidated. With an extensive amount of emerging data demonstrating the importance of sex hormones in immune function, in the remainder of the review we explore the mechanisms behind endocrine sex hormone—mediated immunomodulation. First, we highlight the potential involvement of hormones in tumor promotion, both via tolerance induction and as chronic inflammation and angiogenesis. Next, we describe hormones that may help improve antitumor immunity. Finally, we briefly discuss sexual dimorphism in immune responses, which may have implications for the development of personalized immunotherapy. A better understanding of immunologic switches that control tolerance, immune activation, and immune reconstitution, all of which can be studied using the different phases of pregnancy as a model, and could result in novel immunologic treatment strategies for melanoma and other malignancies.

PUTATIVE PROTUMOR/IMMUNE-SUPPRESSIVE HORMONES: HUMAN CHRONIC GONADOTROPIN, PROGESTERONE, PLACENTAL GROWTH FACTOR, AND RELAXIN

Human Chronic Gonadotropin

Soon after fertilization, the embryonic blastocyst begins secreting human chronic gonadotropin (hCG). Human chronic gonadotropin is a glycoprotein hormone primarily produced by trophoblasts that promotes many processes including implantation, recognition, differentiation, angiogenesis, and fetal-maternal homeostasis[28] (Figure 2). Levels of hCG continue to rise until the 11th week of gestation and then slowly decrease through the remainder of the pregnancy. The main purpose of hCG in pregnancy is to prevent degradation of the corpus luteum and stimulate progesterone production.[29] Human chronic gonadotropin also plays an important role in promoting immune suppression in the decidua by preventing

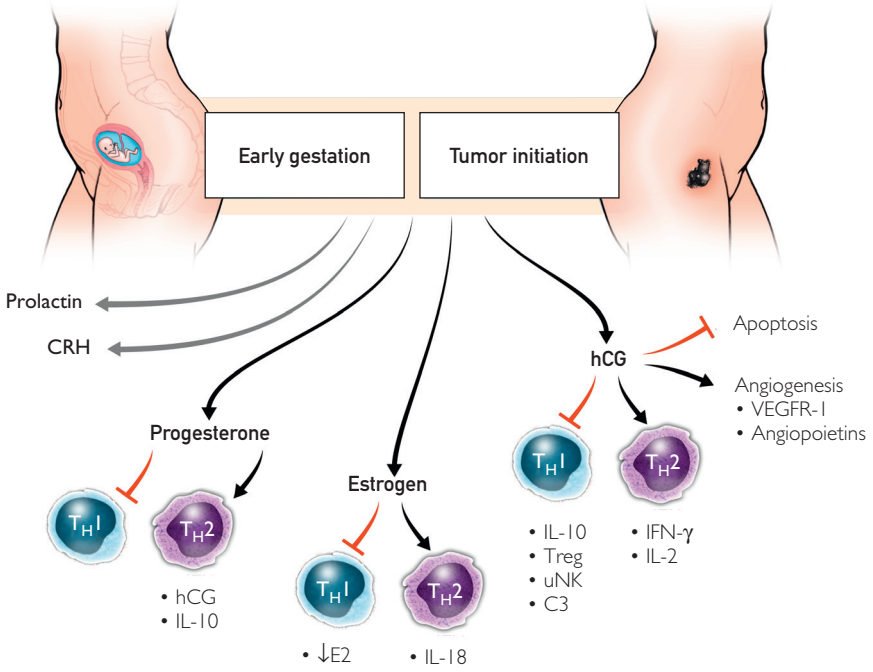

FIGURE 2. Effects of sex hormones during early pregnancy to promote implantation and fetal tolerance and the proposed role in initiation of cancer. CRH = corticotropin-releasing hormone; E2 = estradiol; hCG = human chorionic gonadotropin; IFN-γ = interferon gamma; Treg = regulatory T; uNK = uterine natural killer; VEGFR = vascular endothelial growth factor receptor.

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maternal macrophage phagocytosis to the invading trophoblast to establish immune tolerance.[30] The early trophoblast promotes fetal tolerance by secreting hCG, which acts as a powerful chemoattractant for regulatory T (Treg) cells to migrate to the placenta after fertilization.[31] Treg cells play an important role in maintaining self-tolerance and modulating tolerance to nonself antigen, such as those displayed by the fetus. Expression of complement component 3 was found to be up-regulated by hCG on stromal cells of the baboon endometrium postovulation, suggesting that hCG is also able to modulate the decidual environment during the preimplantation stage.[32,33]

Human chronic gonadotropin also regulates uterine natural killer (uNK) cells.[34] This uNK cell subset makes up approximately 70% of the lymphocyte population in the endometrium and plays an important role in maintaining and regulating the uterine spiral arteries during the first trimester.[35] Uterine natural killer cells stimulate decidual monocytes to secrete interferon gamma (IFN-γ), promoting Treg-cell proliferation through indoleamine 2,3-dioxygenase and transforming growth factor beta.[36] Interestingly, we have found uNK cells in the blood of patients with stage IV melanoma and they also positively correlated with transforming growth factor beta levels in plasma.[37] Human chronic gonadotropin drives a systemic response during pregnancy. Before in vitro fertilization, women given hCG had increased levels of anti-inflammatory IL-27 and IL-10 and reduced levels of pro-inflammatory IL-17, which resulted in an increase in the number of Treg cells and a more receptive uterine wall for implantation.[38] They also notice that hCG affected the maternal adaptive immune system, promoting a T_H2-differentiated state by activating T cells that produce IL-4 while inhibiting T cells that secrete IFN-γ.[38]

In addition, the formation of new blood vessels is also driven by hCG, which acts on proangiogenic molecules such as vascular endothelial growth factor (VEGF) receptor 1 and angiopoietins.[39] Soluble VEGF-C aids in immune tolerance by suppressing the cytotoxic activity of uNK cells at the fetal-maternal interface.[40] Human chronic gonadotropin enhances VEGF production by endometrial cells through paracrine feedback, further promoting blood vessel formation to the fetus.[41] IL-10, an inhibitor of inflammatory cytokines that is found at high levels near the beginning of pregnancy, induces the trophoblast to produce VEGF-C and stimulate placental angiogenesis.[42] Also, endocrine gland–derived VEGF has been implicated as a negative regulator of trophoblast invasion in the placenta, as high levels of endocrine gland–derived VEGF are seen in preeclampsia, and has recently been hypothesized to be regulated by hCG.[43] Human chronic gonadotropin prevents apoptosis of endometrial cells though the Fas-FasL pathway, which, in turn, drives maternal tolerance during early pregnancy.[44]

Human chronic gonadotropin plays very similar roles in the invasion and progression of cancer (melanoma). The hCG receptor was first discovered in trophoblastic neoplasms, which suggested that it has an important role in regulating growth and invasion not only in pregnancy but in cancer as well.[45] Several studies have found that many cancers, including bladder cancer, cervical cancer, lung cancer, pancreatic cancer, and colorectal cancer, can be diagnosed by high levels of hCG in the serum.[46,47] Human chronic gonadotropin mRNA has also been expressed in tumor cells from patients diagnosed with malignant melanoma and has been suggested for use as a biomarker for disease.[48] Others have shown that up to 60% of active neoplasia will express high levels of hCG in serum.[49] Antibodies against hCG have been detected during malignancy, but hCG levels are so high that the effect of these antibodies seems to be minimal, thereby allowing hCG to act as an autocrine growth factor to promote malignancy.[50] Tumor secretion of hCG prevents apoptosis, allowing the cancer to become more resistant and aggressive.[51] As a potent angiogenic factor, hCG secreted by the tumor stimulates sprout formation through vasodilation, maturation, and increased vessel permeability, thus promoting tumor growth.[52]

Progesterone

Initial production of progesterone in pregnancy is induced by hCG. Progesterone secretion by the placenta continues to increase throughout pregnancy, only slightly decreasing approximately 4 weeks before labor onset. Progesterone is a potent immunomodulator that establishes T_H2 bias in pregnancy by reducing the production of pro-inflammatory cytokines by macrophages in

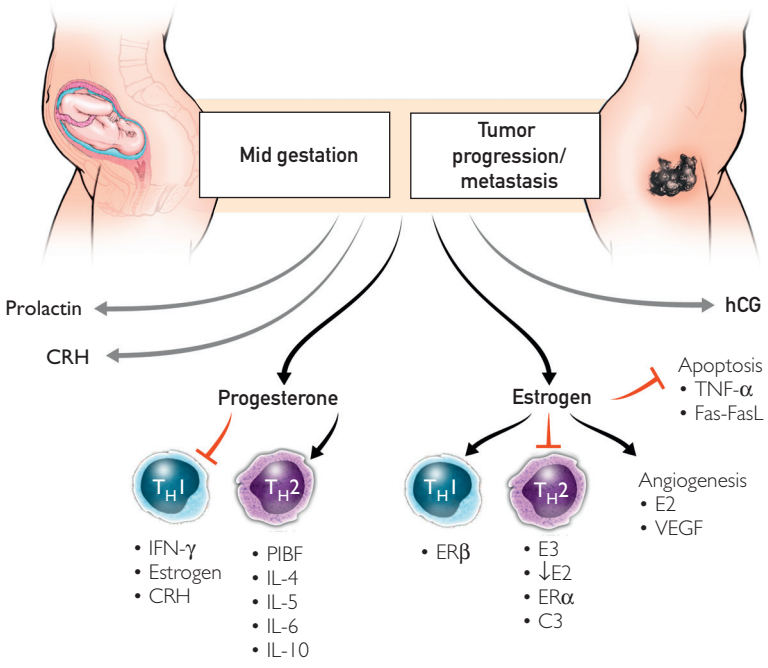

FIGURE 3. Maintenance and regulation of tolerance by sex hormones during midpregnancy and the proposed role of hormones in progression/metastasis of cancer. CRH = corticotropin-releasing hormone; C3 = complement component 3; ER = estrogen receptor; E2 = estradiol; E3 = estrone; hCG = human chorionic gonadotropin; IFN-γ = interferon gamma; PIBF = placental binding factor; TNF-α = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.

response to infectious stimuli, and altering cytokine secretion of T-cell subsets toward IL-10 production[53] (Figures 2 and 3). Furthermore, it induces secretion of chemokines such as CXCL10, CX3CL1, and CCL2 that localize T_H2-biased immune cells to the placenta and up-regulates nonclassical human leukocyte antigen-G.[54] Interestingly, most progesterone receptor (PR)-expressing decidual T cells express a γ/δ T-cell receptor.[55] This limits the number of ligands the T-cell receptor can recognize, which provides protection to the growing fetus. These T cells are also believed to be able to identify antigens presented by trophoblasts.[56] The PR comes in 2 main isoforms, PR-A and PR-B, which compete with one another for progesterone binding.[57] Progesterone receptor-B is expressed on myometrial cells during most of pregnancy and inhibits the expression of pro-inflammatory genes; however, PR-A is expressed during labor and it in turn promotes pro-inflammatory gene activation.[58] Progesterone receptor-A overexpression at the end of pregnancy also promotes the activation of estrogen by increasing the expression of estrogen receptor (ER) α.[59] A smaller, soluble PR isoform, PR-C, has been found to compete specifically with PR-B for progesterone binding, by binding directly to progesterone and inhibiting PR-B signaling near parturition, thereby promoting labor-associated myometrium changes and activating pro-inflammatory pathways.[60]

The immune effects of progesterone are exerted by inhibition of pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) through IκB kinase.[61] Effects of progesterone are also mediated by progesterone-induced blocking factor, which is expressed on lymphocytes in the decidua and is important in maintaining pregnancy. Women who suffer from spontaneous miscarriages or have high stress levels have been found to have low levels of progesterone-induced blocking factor, which was associated with pregnancy complications.[62] Natural killer (NK) cells in pregnancy are very sensitive to progesterone: low levels of

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progesterone are required to achieve inhibition, compared with 100-fold higher levels needed to achieve similar NK-cell inhibition in nonpregnant individuals.[63] In mice, progesterone activates a T_H2-biased immune response by inhibiting maturation of dendritic cells (DCs), which would more readily initiate a T_H1 response through the promotion of cytotoxic T-cell expansion.[64]

In cancer, progesterone modulates immune responses by inhibiting T-cell proliferation[65] and IFN-γ expression[66] while enhancing IL-4, IL-5, IL-6, and IL-10 production and promoting a humoral response.[67] Interestingly, human melanocytes can produce steroids de novo through the metabolism of progesterone or cholesterol.[68-70] Progesterone has been shown to inhibit the proliferation of human melanocytes by blocking the effects of estrogen.[71] The PR has been identified in the cytoplasm and nucleus of melanocytes by immunohistochemistry.[72] Melanoma cells that do not express the PR were still found to be regulated in the presence of progesterone, but are modulated through signal transduction versus transcription.[73] Progesterone-induced blocking factor has been suggested to play a role in cell cycle regulation and T_H2-biased immunity through the IL-4 receptor.[74] Progesterone-induced blocking factor mRNA is constitutively expressed in tumor cells and does not require the presence of the PR, which provides a mechanism for cancer to escape antitumor immune responses.[56] In vitro experiments using WM266-4 cells found that progesterone, estradiol 17b and dihydrotestosterone given together can inhibit tumor growth through the down-regulation of IL-8, which is a potent cytokine for inducing melanoma cell growth.[75]

Placental Growth Factor

We have previously described a VEGF-driven state of chronic inflammation in metastatic melanoma.[76] Placental growth factor (PlGF), a VEGF homologue, may play a similar role in perpetuating chronic inflammation. It plays a considerable role in embryogenesis, promoting both angiogenesis and vascularization to the fetus during inflammation.[77] It was discovered that Flt-1, a VEGF receptor, binds PlGF and mediates recruitment of monocytes.[78] Peripheral blood monocytes treated with PlGF showed increased expression of pro-inflammatory cytokines IL-1β and tumor necrosis factor alpha (TNF-α), and chemokines monocyte chemoattractant protein, IL-8, and macrophage inflammatory protein, suggesting that PlGF plays an important role in inducing inflammation.[79] Melanocytes and melanoma tumors are also known to secrete PlGF, which makes them weakly responsive to anti-VEGF therapy.[80] However, when PlGF was neutralized, even in tumors resistant to anti-VEGF therapy, the tumor could no longer signal through vascular endothelial growth factor receptor-1, inhibiting growth during a preclinical study.[81] Therefore, it has been hypothesized that PlGF plays an important role in allowing a tumor to become drug resistant.[82,83] Further studies need to be conducted to fully understand PlGF's role in inflammation and tumor progression.

Relaxin

The role of relaxin in pregnancy (induction of matrix metalloproteinases, extracellular matrix remodeling, labor), as well as induction of pro-inflammatory cytokines IL-6 and IL-8, has been well documented in rhesus monkeys.[84] Although relaxin has not been studied in melanoma to date, its role in carcinogenesis of breast and prostate cancer has been well established. Relaxin was implicated in tumor growth, cell invasion during pregnancy, and likely tumor invasion in carcinogenesis as well as in angiogenesis by the induction of VEGF.[85-87] Whether relaxin plays a role in melanoma development remains to be elucidated.

PUTATIVE ANTITUMOR/PRO-INFLAMMATORY HORMONES: CORTICOTROPIN-RELEASING HORMONE, PROLACTIN, AND VISFATIN

Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH) has been postulated to regulate the duration of gestation, with its levels being the highest during labor (Figure 4). It works directly on myometrial cells to facilitate the onset of labor, and it is tightly regulated by progesterone, estrogens, nitric oxide, IL-1β, and TNF-α.[60] Corticotropin-releasing hormone binding protein binds CRH and inactivates its ability to promote corticotropin production.[88] Corticotropin-releasing hormone binding protein levels decrease throughout pregnancy, allowing high levels of CRH to accumulate and promote labor. Other actions of CRH include regulation of

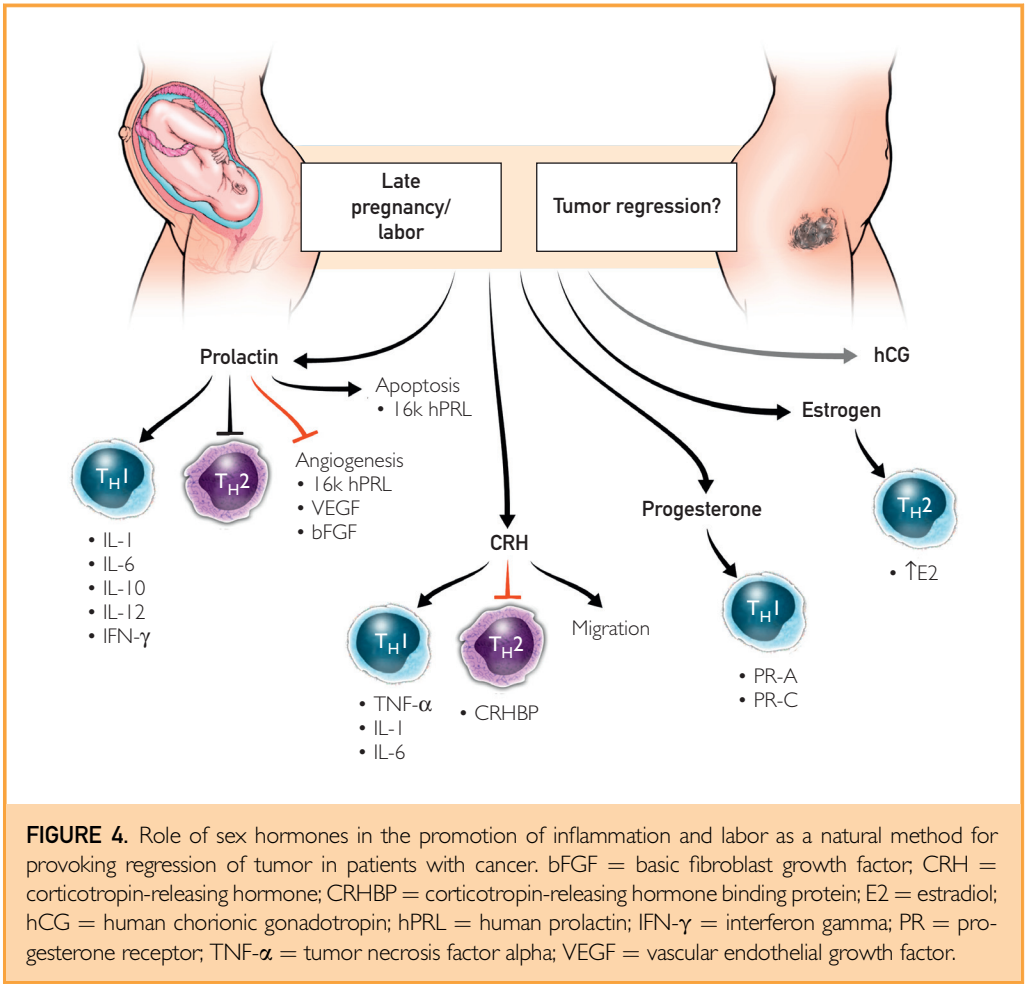

FIGURE 4. Role of sex hormones in the promotion of inflammation and labor as a natural method for provoking regression of tumor in patients with cancer. bFGF = basic fibroblast growth factor; CRH = corticotropin-releasing hormone; CRHBP = corticotropin-releasing hormone binding protein; E2 = estradiol; hCG = human chorionic gonadotropin; hPRL = human prolactin; IFN-γ = interferon gamma; PR = progesterone receptor; TNF-α = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.

fetal blood flow, placental prostaglandin and cortisol production, and uterine contractility.[89]

Corticotropin-releasing hormone regulates the hypothalamic-pituitary-adrenal (HPA) axis, which allows cells to respond to environmental stresses.[90] Melanocytes are known to secrete CRH, which permits them to produce cortisol and corticotropin to maintain skin homeostasis.[91] In cell culture, melanoma cells treated with CRH migrated further during stress, which was determined to be mediated by the extracellular signal-regulated kinase (ERK1/2) pathway.[92] Increases in the levels of CRH and proopiomelanocortin are strongly associated with malignant melanoma.[93] Another function of CRH in the skin is to act as a growth regulator by both promoting and suppressing cell proliferation. Corticotropin-releasing hormone receptor 1 controls the action of CRH and can promote cyclic adenosine monophosphate and inositol trisphosphate synthesis in dermal and epidermal cells.[94] Melanomas exclusively express corticotropin-releasing hormone receptor 1, which plays an important role in proliferation and has become an agonist target.[95] In a B16 mouse melanoma model, daily injections with CRH reduced tumor volume by 30% to 60% compared with that in control animals.[96] Research shows that CRH promotes the survival of melanocytes during starvation and prevents cell proliferation by inhibiting growth factor signaling through cyclic adenosine monophosphate and IP3 second messengers.[97] Tumor necrosis factor alpha, IL-1, and IL-6 stimulate the HPA axis to produce CRH, which drives a proinflammatory, T_H1-biased response.[98] When peripheral CRH acts on the HPA axis in the brain, it triggers a classical feedback mechanism that leads to the secretion of cortisol, a steroid hormone with anti-inflammatory

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effects.[99] Thus, CRH is a potential modulator of a T_H1-biased response and its role should be further studied in cancer.

Prolactin

Prolactin (PRL) is a polypeptide hormone secreted by the syncytiotrophoblast that reaches its highest levels during late pregnancy (Figure 4). It acts on corpus luteum cells in the ovary to stimulate the secretion of progesterone to maintain pregnancy and on epithelial cells of the mammary gland to initiate milk production.[100] The amount of PRL secreted is directly proportional to the size of the fetus, and it functions to provide energy for the mother and nutrients for the fetus. Through lipolysis and anti-insulin effects of PRL action, the maternal insulin level increases, providing free fatty acids and amino acids to the growing fetus.[101] Prolactin functions to increase β-islet cell proliferation, inhibit apoptosis, and cause β-islet cells to become more responsive to glucose.[102] Glucocorticoid expression of Rasd1 near the end of gestation changes insulin secretion during pregnancy through the inhibitory effects of PRL on Rasd1 transcription.[103] Activation of dopamine neurons suppresses PRL secretion through placental lactogens.[104] The N-terminal 16K PRL fragment had been determined to stop proliferation and migration of vascular endothelial cells and cause cell cycle arrest resulting in apoptosis.[105] High levels of 16K human PRL can be detected in serum and urine of women suffering from preeclampsia.[106]

In melanoma, PRL drives T_H1-biased immunity through the secretion of IL-1, IL-6, IL-10, IL-12, and IFN-γ by NK cells and B lymphocytes,[107,108] and plasma cell activation.[109] It increases T-cell proliferation[110] and decreases B-cell apoptosis.[111] Through animal studies, PRL's antitumor effect has been shown to promote tumor-specific macrophages through IFN-γ and IL-12, and CT26 tumor-bearing mice injected with recombinant human PRL and IL-15 had enhanced cytotoxic activity to the tumor, resulting in fewer lung metastasis and longer overall survival (OS).[112,113] However, PRL does not have an antitumor effect in all cancers. In breast cancer, high levels of PRL are associated with a greater risk for developing cancerous ERα+ tumors and is associated with much poorer outcomes for patients with these increased levels.[114] This is likely because PRL promotes mammary tumorigenesis independent of cyclin D1 activation.[115] This has not yet been observed in melanoma. Pro-inflammatory cytokines such as IL-1, IL-2, and IL-6 produced by both pituitary and extrapituitary cells have a stimulatory effect on PRL secretion, while IFN-γ inhibits its production.[116] Many immune cells express the PRL receptor including monocytes, macrophages, B and T cells, granulocytes, and NK cells.[117] Prolactin secretion from the anterior pituitary gland is inhibited by the dopamine D2 receptor, which is widely expressed on melanoma cells and plays a key role in inhibiting adenylyl cyclase, which is necessary for cellular signal transduction.[118,119] Prolactin also enhances the effect of immune cells, including $CD34^+$ stem cells, in the blood through the upregulation of major histocompatibility complex class II expression on antigen-presenting cells, T-cell clonal expansion, antibody production, increased cytotoxicity of NK cells, and microbe killing by macrophages.[120] In mouse melanoma models, 16K human PRL can block angiogenesis and inhibit tumor growth by activating NF-κB, causing tumor-infiltrating lymphocytes to access and destroy cancer cells.[121] This PRL peptide endogenously blocks Notch signaling, which greatly impairs the tumor's ability to vascularize.[122] 16K human PRL has also been shown to block angiogenesis through the inhibition of basic fibroblast growth factor and VEGF, making it a target for antitumor therapies.[123] Thus, better understanding of how PRL can promote cell-mediated immunity could help researchers design better ways to initiate tumor destruction.

Visfatin

Previously known as pre—B-cell colony-enhancing factor or nicotinamide phosphoribosyltransferase, visfatin is a visceral fat cytokine with an important role in the promotion of inflammation.[124-126] Visfatin levels are highest at the end of gestation and promote the secretion of IL-1β, IL-6, IL-8, cyclooxygenase 2, TNF-α, and prostaglandin E_2.[127,128] Interestingly, infection-associated preterm labor is concomitant with elevated levels of visfatin in maternal plasma.[129] These findings suggest that visfatin could be important for immune resolution back to a T_H1 state. However, the role of visfatin in melanoma is poorly understood. It appears that visfatin is more highly expressed in melanoma lesions than in benign

lesions.[130] Moreover, it seems that visfatin may promote melanoma cell growth in vitro. Namely, a study[131] using the melanoma Me45 cell line reported that visfatin increased the proliferation of these tumor cells. In another study,[132] neutrophils have been found to synthesize visfatin in response to inflammatory stimulus and inhibit apoptosis in vitro and in patients with sepsis. Thus, it remains possible that there could be an additional regulatory level of visfatin during pregnancy, which might be lost during tumorigenesis, and thereby promotes melanoma proliferation, but more studies need to be conducted to elucidate the role of visfatin in melanoma.

ESTROGEN: A DOUBLE-EDGED SWORD MODULATING T_H1/T_H2 IMMUNITY

Women have high levels of estrogen, which drop dramatically when they reach menopause. Estrogen modulates the immune response by inducing peripheral T cells to secrete pro-inflammatory cytokines IFN-γ and IL-2,[133] but also promotes tolerance by inducing IL-10 secretion[134] (Figures 3 and 4). Estradiol-17β (E2) at high concentrations induces a T_H1 response, whereas at low concentrations it biases the system toward immune tolerance.[135] Pro-inflammatory responses are regulated by E2 through NF-κB. However, estrone (E3) is detectable only in pregnant women because it is produced by the placenta and fetus. Estrone is important for reducing the production of pro-inflammatory cytokines in circulation by decreasing IκB degradation, which inhibits NF-κB activation and apoptosis (Figure 3).[136] Estrogens work through interaction with the ER to induce transcriptional regulation. Immune cells including DCs, NK cells, macrophages, and lymphocytes express ERs, signifying that this hormone modulates their function.[137] Indeed, the differentiation of DCs is regulated by E2 acting on ERα. Levels of E2 are the highest during the third trimester, when the number of immature DCs is high and actively presenting fetal antigen to the mother's immune system to begin labor.[138] Complement component 3, a protein that plays a central role in inducing inflammation in innate immunity, is increased by estrogen in oviductal epithelial cells.[139] Angiogenesis in the uterus is also driven by estrogen. Vascular endothelial growth factor mRNA levels increase in the endometrium in the presence of E2.[140] This increases placental blood flow and vasodilation, which are both characteristic of angiogenesis. Cytotrophoblasts, but not syncytiotrophoblasts, stimulate VEGF production directly, and this production is correlated to the levels of E2 in the serum.[141] Thus, estrogen stimulates VEGF production and blood vessel formation, which is essential for the establishment and maintenance of the fetus during pregnancy.

Women at risk for familial breast cancer have increased risk for developing melanoma and vice versa, suggesting that estrogens can promote tumorigenesis.[142] It has been known for many years that melanoma tumor cells express ERs.[143] Estrogens affect lymphocytes by initiating the secretion of IL-10, IL-12, and IFN-γ and inhibition of TNF-α,[144] stimulation of antibody production,[145] and the reduction of macrophage and DC apoptosis.[146,147] Estradiol-17β has been proven to inhibit melanoma growth by obstructing receptor binding of IL-8.[75] Chronic exposure to estrogens was suggested to increase NF-κB stimulation and induce pro-inflammatory responses by macrophages.[148] It has been shown that short-term and long-term inflammation leads to the up-regulation of ERα, but not ERβ, thus affecting the effects of estrogens on T cells, because T cells express ERα, while B cells express ERβ.[149] It was also reported that estrogen decreases apoptosis, as well as TNF-α production.[150] Estradiol-17β and estrone both have a strong affinity for binding ERα,[151] which is why ERα is commonly associated with tumor promotion. Yet, ERβ has been found on malignant melanoma cells that are negative for ERα.[152] Loss of ERβ expression correlated with increased invasiveness of the tumor,[153] suggesting that loss of ERβ expression increases malignant transformation in melanoma. The most important prognostic factors in melanoma are tumor thickness (Breslow depth) and invasive level (Clark level); as each increase, the patient's prognosis decreases.[154] Interestingly, increased ERβ expression is not found on nonmalignant cells surrounding the tumor, only on the tumor cells themselves.[155] Another role of ERβ is regulation of monocyte apoptosis through the Fas-FasL signaling pathway.[156] It has been shown that myeloid progenitor cells exposed to the granulocyte-macrophage colony-stimulating factor differentiate into immature DCs through the expression of ERα and increased levels of estrogens.[157,158]

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Recently, 2-methoxyestradiol, an estrogen derivative, has been determined to be a potent inhibitor of angiogenesis and melanoma growth in a mouse model.[159] Taken together, this indicates that estrogens can both promote and hinder tumor growth and monitoring ER expression could help clinicians determine the patient prognosis to the disease.

SEXUAL DIMORPHISM IN RESPONSE TO HORMONES

In this review, we have discussed existing data supporting a role for sex hormones and their ability to manipulate the immune system in both pregnancy and melanoma. Beyond these observations, several studies have identified sexual dimorphism with respect to inflammatory responses in various settings. For example, sex-based differences in vascular function have been described and show the development of early atherosclerotic lesions and plaques with increased production of inflammatory mediators (IL-10 and TNF-α) in women than in men.[160-162] That the state of pregnancy, and not a general state of tolerance in women, accounts for additional sex-based differences in immune function is supported by the fact that autoimmune diseases are more prevalent in women.[163] Moreover, autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematous are diagnosed more frequently in women and are strongly correlated with sex hormones.[164,165] Interestingly, some autoimmune diseases such as arthritis remit with pregnancy, but systemic lupus erythematous has been found to often worsen in severity during gestation.[164] Women also exhibit enhanced innate immune responses to infections,[166,167] not blunted ones, and survive episodes of severe sepsis to a greater degree than do men.[168] It is clear that sex plays a role in risk, severity, and prognosis of many diseases, including cancer, and research in this area has just begun. It may one day become important to consider the sex of the patient with cancer when personalizing cancer immunotherapies.

REVERSING TOLERANCE IN MELANOMA

To positively affect survival of patients with cancer, it appears critical to find a way to break immune tolerance to the malignancy. Insights into achieving this goal in metastatic melanoma may lie in well-recognized abnormalities of pregnancy (eg, spontaneous abortions). For example, serum taken from women suffering from recurrent spontaneous abortion have a cytokine profile indicative of a T_H1 response compared with healthy pregnant women.[169] To counter this, intravenous immunoglobin given to women, with reported previous recurrent miscarriages and/or suffering from recurrent miscarriages after in vitro fertilization, every 3 to 4 weeks starting from conception or 24 hours before embryo transfer showed a decrease in the number of NK cells and an implantation success rate of 92.5% compared with 25% for women not receiving intravenous immunoglobin.[170] Moreover, new immunotherapies for promoting tolerance to the fetus during gestation are being studied in animal models and could have important implications in melanoma. In mice, researchers have shown that blocking CD80 and CD86 enhances maternal tolerance, decreasing implantation failure through the increase in Treg cells and development of T_H2 cells.[171] The use of anti—cytotoxic T lymphocyte antigen 4 (CTLA4) antibody therapy with CD80/CD86 blockade has been found to regulate the T_H2/T_H1 balance in peripheral blood monocytes isolated from women with recurrent spontaneous abortion, which led to the design of an adenoviral CTLA4 antibody that improved pregnancy outcomes in a mouse model of spontaneous abortion.[172,173]

The opposite, immune-activating effect is desired in metastatic melanoma. Recent Food and Drug Administration approval of the anti-CTLA4 antibody, ipilimumab, was meant to initiate T-cell activation and tumor destruction by promoting cytotoxic capacity of naturally occurring tumor-specific T cells, thereby overcoming, in part, their state of immune tolerance.[174] Patients with metastatic melanoma treated with ipilimumab given alone or in combination with a peptide vaccine (gp100) exhibited a median overall survival OS time of approximately 10 months, an improvement over the 6.4-month OS observed in patients receiving gp100 vaccine alone.[175] As breakthrough as this drug is, it comes at a cost of significant immune-related adverse events in up to 15% of the patients.[176] Another new and promising antibody targets program death 1 (PD-1), an additional receptor

found on activated cells that is critical for immune regulation, which has been shown to promote a pro-inflammatory environment by IFN-γ and IL-2 secretion.[177] In clinical trials, this drug, after treatment with or without the anti-CTLA inhibitor, showed a high rate of sustained tumor regression and a median OS of 11 months in patients with metastatic melanoma.[178] This led to administering both ipilimumab and nivolumab (anti—PD-1) concurrently, resulting in 53% of the patients obtaining an 80% reduction in tumor volume.[179] Unfortunately, the reported increase in objective response rate was paralleled with a similar increase in severe toxicity. Other promising immunotherapies for patients with melanoma are also being studied. Antitumor immunity has been shown by using an antibody to CCR4, a marker found on immune-suppressive Treg cells, which when given to a patient with T-cell leukemia-lymphoma resulted in a $CD8^+$ T-cell response to the tumor.[180] Human epidermal growth factor receptor 2, an antibody commonly used to treat breast cancers, has recently shown positive effects on a number of melanoma cell lines and xenograft models.[181] Bevacizumab, an anti-VEGF antibody, given to patients with metastatic melanoma in combination with albumin-bound paclitaxel and carboplatin, resulted in an increase in $CD8^+$ lymphocytes but did not affect the T_H1/T_H2 ratio.[182]

Even though promising, the clinical successes of present day immunotherapeutic strategies for metastatic cancer fall short of their preclinical results. Many share the belief that the reason for this discrepancy in clinical translation is the result of tumor-driven immune tolerance of human cancer.[183] Another reason for this discrepancy is that malignant melanocytes use melanogenesis, which is a normal metabolic process that generates a local immunosuppressive environment through proopiomelanocortin-derived peptides and steroids.[17] It was discovered that inhibiting melanogenesis increases the potency of the immune system and chemotherapy against tumors.[184] Overcoming the tumor-induced modulation of systemic immunity to recover the ability of endogenously generated immune cells to effectively destroy the malignancy will be a considerable challenge. An interesting concept in pregnancy, which could lead to better understanding of malignancy, is the spontaneous return to cytotoxicity near the end of parturition.[138] Near parturition, an unknown event results in the reactivation of T_H1 maternal immunity and the initiation of labor (rejection). Characterizing this phenomenon by further studying the role that cytokines, cells, and hormones play could translate into different methods for breaking tolerance in patients with cancer, which could ultimately improve therapeutic efficacy and OS. The skin is a steroidogenic organ; it synthesizes its own steroids and sex hormones and can regulate local immune activities along with affecting the function of the epidermis.[185] Corticotrophin-releasing hormone has been found to block human melanoma cell proliferation in vitro[97] and could provide additional therapeutic value when paired with a targeted agent. As progesterone promotes a tolerant state in pregnancy, treating a stable melanoma patient with progesterone was found to cause proliferation of dormant micrometastases by tipping the immune system back to a T_H2 state.[186] B7-H1 (PD-L1) is a molecule expressed on antigen-presenting cells and contributes to tumor evasion and expansion of Treg cells.[187,188] In a B7-H1 knockout mouse model of melanoma, it was discovered that females had superior tumor growth resistance than did males due to estrogen-mediated suppression of Treg cells.[189] Clinically, in a phase I study of advanced cancers, including melanoma, patients treated with the PD-1 antibody reported a marked response rate (28%) that was durable in only those patients expressing PD-L1, making it a potential biomarker for anti—PD-1 treatment response.[190] However, the authors did not compare men and women to look at potential differences due to sex. Altogether, to improve outcomes for patients with melanoma treated with immunotherapeutics, we must more completely understand the process of normal pregnancy immunoregulation, specifically the return to cytotoxicity (T_H1 bias) at the end of gestation.

CONCLUSION

The dynamic maternal immune responses to normal pregnancy have evolved out of the need to support a semiallogenic fetus over the duration of the pregnancy, without relevant infectious or inflammatory impediment to the mother. In turn, the maternal immune system

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is tightly regulated by hormone release and cytokine action to protect the developing fetus. Cancers, including melanoma, appear to induce similar tolerogenic immune programs through various mechanisms, including paracrine secretion of sex hormones, to drive angiogenesis required for oxygen and nutrient supply, all the while evading immune attack in a manner similar to the process of placentation. A better understanding of the molecular switches involved in the induction and reversal of immune tolerance in the setting of pregnancy may help identify new methods for targeted immune modulation for patients with melanoma.

Abbreviations and Acronyms: **CRH** = corticotropin-releasing hormone; **CTLA4** = cytotoxic T lymphocyte antigen 4; **IFN-γ** = interferon gamma; **DC** = dendritic cell; **E2** = estradiol-17β; **ER** = estrogen receptor; **hCG** = human chronic gonadotropin; **HPA** = hypothalamic-pituitary-adrenal; **NF-κB** = nuclear factor kappa B; **NK** = natural killer; **OS** = overall survival; **PD-1** = program death 1; **PlGF** = placental growth factor; **PR** = progesterone receptor; **PRL** = prolactin; **TNF-α** = tumor necrosis factor alpha; **Treg** = regulatory T; **uNK** = uterine natural killer; **VEGF** = vascular endothelial growth factor

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