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Sex and COVID-19: A Protective Role for Reproductive Steroids

Graziano Pinna [1,*]

Evidence shows coronavirus disease 2019 (COVID-19)-induced symptom severity and mortality is more frequent in men than in women, suggesting sex steroids may play a protective role. Female reproductive steroids, estrogen and progesterone, and its metabolite allopregnanolone, are anti-inflammatory, reshape competence of immune cells, stimulate antibody production, and promote proliferation and repair of respiratory epithelial cells, suggesting they may protect against COVID-19 symptoms.

COVID-19 Sexual Dimorphism and the Role of Reproductive Steroids

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly spreading pandemic characterized by a strong sex-bias, with male patients showing double the odds of requiring intensive care unit admission and higher mortality when compared with female patients [1]. This evidence points to female sex hormones as a possible explanation for the sexual dimorphism in COVID-19 symptom severity and mortality.

Estrogen, progesterone, and allopregnanolone are endogenous reproductive steroids, which are abundantly produced in the periphery by the adrenal glands and ovaries and *de novo* by the brain [2]. These steroids play important physiological roles by modulating inflammatory processes and behavior. Estradiol and progesterone exert peripheral and neuronal functions mediated by genomic influencing nuclear hormone receptors, are anti-inflammatory, reshape competence of immune cells, and stimulate antibody production. Allopregnanolone, instead, rapidly modulates $GABA_A$ receptors in neurons, which is associated with mood and cognition improvement, and affects immune-competent cells and induces potent anti-inflammatory functions. Allopregnanolone was recently approved by the US FDA as Zulresso™ for the treatment of postpartum depression [3].

Progesterone and Estradiol Anti-inflammatory Effects and Role in Immune Cell Competence

In addition to the well-known role of progesterone in reproduction, this steroid regulates important immunomodulatory functions, including reshaping the competence of immune cells and inducing potent anti-inflammatory actions. Progesterone plays a significant role in the maternal reproductive apparatus, for example, it induces immune adaptations and **immune tolerance** (see Glossary) that promote and sustain pregnancy.

There are several ways by which progesterone induces immunomodulatory effects. It stimulates T cell activation and plays a direct role on their differentiation. Progesterone can also modulate T cell receptor signaling, suppress cellular cytotoxicity, and may also block **degranulation** by influencing progesterone-induced blocking factor [4].

Intriguingly, progesterone binds to progesterone receptors in immune cells, including natural killer cells, T cells, macrophages, and dendritic cells, but can also bind non-immune cells, including epithelial and endothelial cells in the respiratory tracts where it alters cellular signaling/activity improving infections. In influenza A virus, progesterone administration decreased inflammation and promoted pulmonary repair after clearance of the viruses by increasing regulatory CD39+ Th17 cells and stimulating cytokine (TGF-β, IL-6, and IL-22) levels (Figure 1). IL-22, similarly to TGF-β, can stimulate the proliferation of epithelial cells and promote repair of the damaged alveolar epithelium. Progesterone signaling through progesterone receptors stimulates the epidermal growth factor amphiregulin, thereby promoting proliferation and respiratory epithelial cell repair [5]. In females, a faster recovery of the lung tissue may reduce the susceptibility to secondary bacterial infection, which is the primary cause of mortality after influenza virus infection. This finding is substantiated by studies showing that following influenza A virus infection, the histological density of pulmonary inflammation was decreased during pregnancy. Thus, pregnancy-associated pulmonary physiology may protect females during severe influenza. Combination of estradiol with progesterone showed the strongest protective effects in the lungs after the stimulation of the inflammatory cascade involving toll-like receptor 4 (TLR4) function in cultured alveolar macrophages [6].

Like progesterone, estrogens are strong immune regulatory agents. Estrogens regulate immune cell responses and promote anti-inflammatory and neuroprotective effects. Increase in circulating estrogen concentration affects progenitor and mature cells of both the innate and adaptive immune systems.

Glossary

Chemotaxis: the movement of an organism in response to chemical stimuli.
Degranulation: a cellular process of secretory vesicles by releasing antimicrobial cytotoxic molecules, which are called granules and are contained inside cells, including granulocytes (neutrophils, basophils, and eosinophils) as well as mast cells.
Immune tolerance: the state of immune system unresponsiveness to substances or tissues that potentially may induce an immune response.
Phagocytic activity: the activity of cells that protect the body by ingesting exogenous and potentially harmful bacteria and viruses, as well as dead cells.

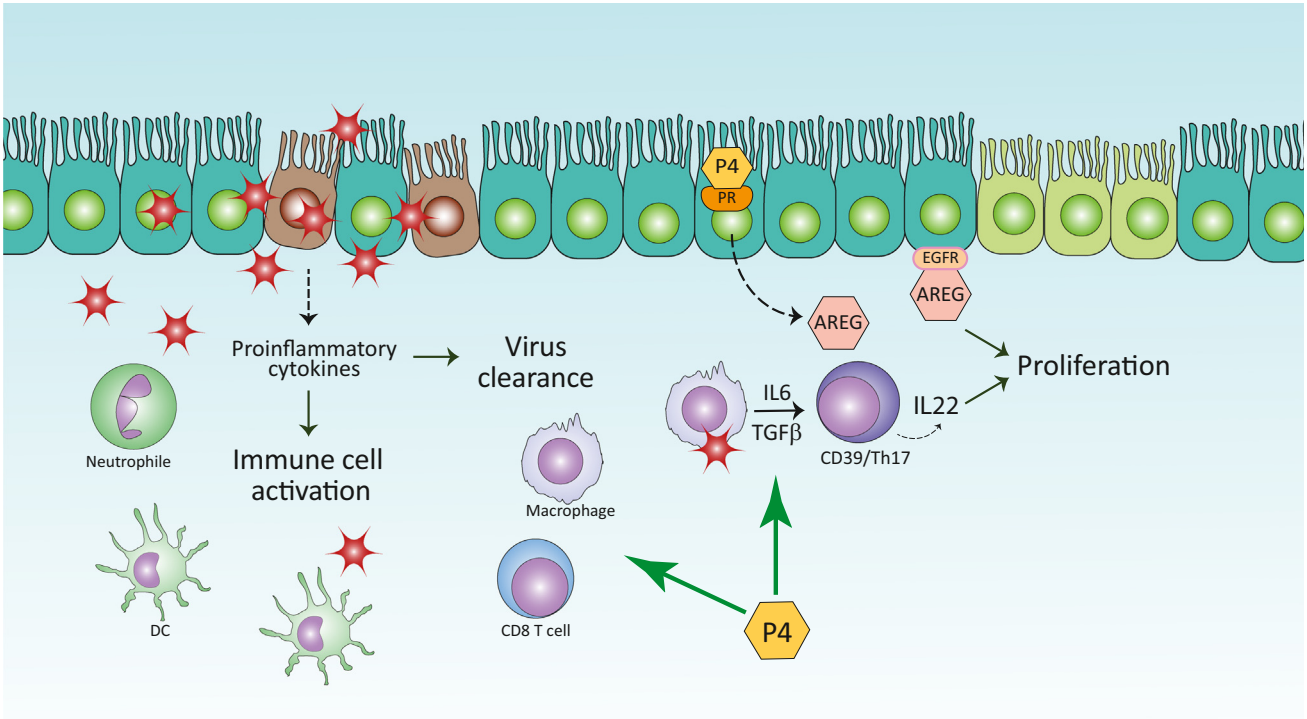

Figure 1. Schematic Representation of Progesterone (P4) Effects on Inflammation and Alveolar Respiratory Epithelium Repair Following Influenza Virus Infection. Respiratory epithelial cells produce proinflammatory cytokines following influenza virus infections and they can also activate immune cells, including macrophages and neutrophils, which initiate inflammatory processes. Together, these actions result in clearance of the virus. The damaged respiratory epithelium (brown epithelial cell) following viral infection is more vulnerable to secondary bacterial infection. However, physiological conditions (in women), or treatments that increase P4 levels, stimulate the production of cytokines (e.g., IL-6 and TGF-β) that, together with recruitment of CD39/Th17 regulatory cells, promote anti-inflammatory processes. By binding at progesterone receptors (PR), P4 can also stimulate amphiregulin (AREG), which is a growth factor that signals via the epidermal growth factor receptor (EGFR). AREG induces respiratory epithelial cell proliferation (light green cells). Likewise, Th17 cells, by producing IL-22, promote regeneration of epithelial cells. A repaired alveolar epithelium impedes the secondary bacterial infections that constitute the main cause of mortality after influenza virus infection [5].

In the innate immune system, estrogens regulate the number of cells and their functions. In neutrophils, estrogens regulate **chemotaxis**, infiltration, as well as the induction of cytokine-induced chemoattractants and cytokines (e.g., TNF-α, IL-6, IL-1β). In dendritic cells, they stimulate the differentiation and directly regulate expression of chemokines (e.g., IL-8) and cytokines (e.g., IL-6, IL-10). In macrophages, estrogens regulate chemotaxis, **phagocytic activity**, and the production of cytokines (e.g., IL-6, TNF-α). Estrogens influence the phenotype of T helper cells and have profound effects on B cell maturation, differentiation, activity, and survival. Estradiol also modulates cytokine secretion by CD4+ T cells [7].

Intriguingly, in a mild SARS-CoV-infected mouse model, mortality accounted for 90% of males and only 20% of females. In a more severe infection, all males died within 5 days, whereas 50% of females survived. Gonadectomy increased mortality rate in females but failed to change it in males, supporting a protective role of female reproductive steroids against SARS-CoV. Furthermore, sex bias to SARS-CoV outcomes increased through age, reflecting SARS-CoV observations in humans [8]. Estradiol decline during menopause reduces the number of B and T cells, while increasing production of proinflammatory cytokines. Intriguingly, during menopause, women are at higher risk for developing diseases. Thus, men and older females are generally less protected by estrogens.

SARS-CoV-2 replicates in the respiratory epithelium after gaining access through the angiotensin-converting enzyme 2 (ACE2) receptor (Figure 2). High estradiol concentrations decrease lung ACE2 expression, while lower levels increase it [9], which may explain the increased vulnerability to COVID-19 through sex and age biases. Furthermore, estradiol potently stimulates higher concentrations

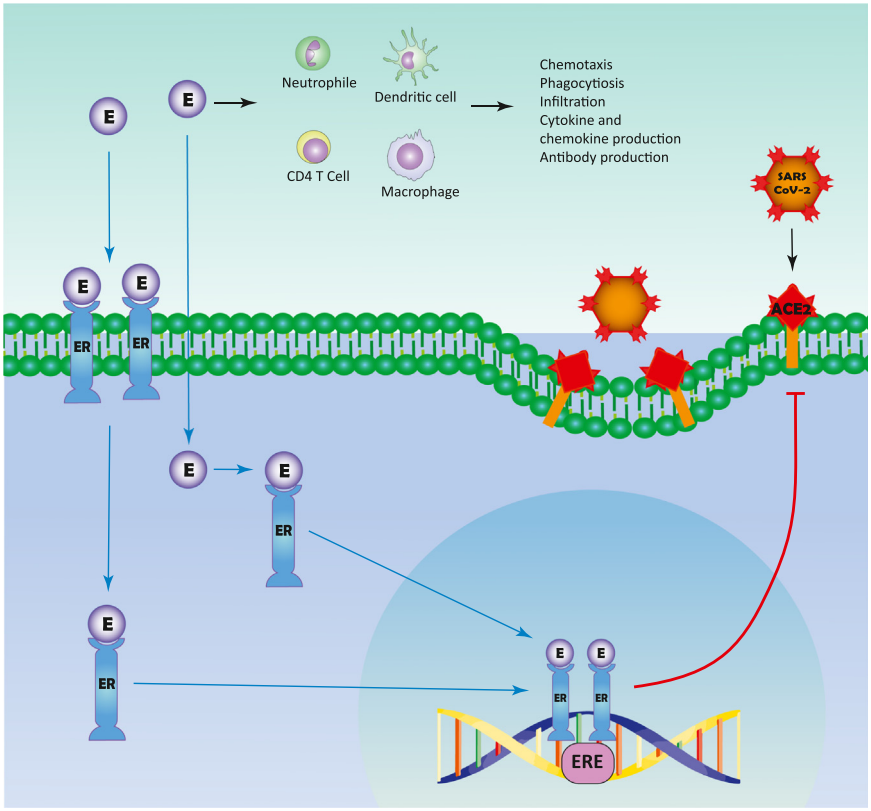

Figure 2. Schematic Representation of Estrogen Effects in Response to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. Estrogens (E) stimulate the differentiation of chemokines and cytokines, regulate chemotaxis and phagocytic activity, influence the phenotype of T helper cells, and affect B cell maturation, differentiation, activity, and survival. Estrogens can also stimulate higher antibody concentrations and increase the number of cells devoted to antibody production in response to viral infections. SARS-CoV-2 replicates in the respiratory epithelium after binding at the angiotensin-converting enzyme 2 (ACE2) receptor. Importantly, elevated estradiol levels decrease ACE2 expression (red arrow) in lung epithelium while lower concentrations stimulate ACE2 expression [9], which may explain why women are generally more resistant to coronavirus disease 2019 adverse outcomes.

of antibodies as well as cells involved in antibody production in response to viral infection.

Emerging Anti-inflammatory Role of Allopregnanolone by Repressing the TLR4 Pathway

Immune cells express receptors for several neurotransmitters, neuropeptides, and hormones. Allopregnanolone is a physiologically active progesterone derivative and a neurosteroid abundantly produced by the brain, where it acts by potently and positively modulating the inhibitory neurotransmission mediated by $GABA_A$ receptor [2]. $GABA_A$ receptor regulates emotional behavior and is a target of psychotropic drugs, including benzodiazepines. Allopregnanolone is an 'endogenous tranquillizer' that shows protective functions in neuropsychiatric disorders, including postpartum depression, post-traumatic stress disorder, alcohol use disorder, epilepsy, and Alzheimer's disease [10]. These pathophysiological conditions are characterized by enhanced proinflammatory signaling mediated by TLR4 activation in peripheral organs and brain. In recent studies conducted in macrophages and monocytes, allopregnanolone inhibited the binding of TLR4 with its specific ligand, lipopolysaccharide, which promoted proinflammatory cytokines and chemokines [11]. This effect was also demonstrated in brain, where TLR4 signal cascade has been demonstrated in neurons and glia. Allopregnanolone acted independently of $GABA_A$ receptors. Indeed, both the steroid precursor, pregnenolone, which is devoid of GABAergic actions, and allopregnanolone, but not the GABAergic tetrahydrodeoxycorticosterone (THDOC) blocked the entire TLR4 signaling pathway. Pregnenolone and allopregnanolone inhibited the activation step that involves TLR4 binding to the adaptor proteins MD2 in macrophages and MyD88 in brain. While TLR4 also binds a $GABA_A$ receptor subunit (alpha-2) in brain, this binding was not observed in macrophages [11].

Other studies have shown that pregnenolone and allopregnanolone inhibit TLR4 signals through enhancement of TLR4 degradation. Furthermore, progesterone and allopregnanolone, at equivalent efficacy, inhibited the TLR4 signaling pathway when activated by traumatic brain injury in rats. All of these effects were independent of $GABA_A$ receptors and suggest a direct action of allopregnanolone to inhibit TLR4-mediated proinflammatory signaling in the innate immune system and the brain. Since COVID-19 is characterized by excessive TLR4 signals in the lungs, marked by the overexpression of proinflammatory cytokines, including IL-6 and TNF-α and culminating with the cytokine storm, allopregnanolone may protect against COVID-19-induced inflammation.

Inhibition of proinflammatory processes following allopregnanolone blockade of TLR4 underlies a novel function in the regulation of periphery and brain immune response [11].

Progesterone, Estrogens, and Allopregnanolone May Protect against COVID-19

The summary earlier suggests that reproductive steroids may play a role in COVID-19 sex bias by explaining why more severe symptoms and higher mortality following SARS-CoV-2 infection are observed in men and older subjects. Generally, men and women who are exposed to other viral infections also show differences in prevalence and outcomes, with women being less susceptible than men, owing to a generally more efficient immune response. Indeed, women may be more protected than men during physiological conditions, including pregnancy or across the menstrual cycle when fluctuation of reproductive steroids warrants a stronger immune protection. Although, data are mixed and currently the Center for Disease Control and Prevention (CDC) reports that pregnant women may have a worse course[i], only a few studies evaluated COVID-19 symptom severity in late pregnancy (high hormone levels) and after delivery (low hormone levels). Observational studies have noted that some SARS-CoV-2-positive pregnant women with mild or absence of COVID-19 symptoms on admission to obstetrical service escalated symptoms severity immediately postpartum in coincidence with the drastic hormonal decrease following childbirth. Some women required unplanned intensive care unit admission [12–14]. Progesterone, estradiol, and allopregnanolone concentrations increase up to 100 times, mainly from the first to the second trimester, and then remain elevated until delivery. This progression is consistent with a hormonal protective role during pregnancy and postpartum pathophysiology, which, in one in nine women, is associated with perinatal psychopathology and sustained inflammation [3]. The CDC reports that on 22 October 2020, the mortality rate among SARC-CoV-2-positive pregnant women in the USA was 0.16% (44 total deaths for 27 566 cases) compared with 2.24% of the American female population[i], pointing to immunological and hormonal protective factors in lowering the risk of COVID-19-related deaths in pregnant women [15]. This protection may also be guaranteed during the administration of oral combinations of hormonal contraceptive or by treatment with hormone replacement therapy against hypoestrogenism in postmenopausal women. Nutrition may also play a role when diets are enriched with phytoestrogens (e.g., soy beans, lentils, oats) with the ability to bind directly to human estrogen receptors or that can be converted to estradiol by the microbiome.

There is currently no specific treatment available for COVID-19, however, the US FDA has approved the antiviral medicine remdesivir and the steroid dexamethasone following positive clinical trials showing faster recovery and higher survival rates. Thus, there is an urgent need to develop novel efficient treatments and to unveil biological risk factors to protect vulnerable subjects. Estrogens, progesterone, and its physiologically-active metabolite allopregnanolone are involved in multiple pharmacological effects, ranging from improvement of mood disorders to analgesic properties and improving cognitive deficits [2,3,10]. The anti-inflammatory action, the role in reshaping immuno-competence and increasing number of immune cells, and the stimulation of higher antibody concentrations against viral infections raise the hypothesis that these reproductive steroids may be beneficial to prevent or improve COVID-19 symptom severity and mortality. Clinical trials should test whether these hormones offer benefits in men and in postmenopausal women at risk of developing severe COVID-19 symptoms.

Acknowledgments

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Resources

[i]www.cdc.gov/coronavirus/2019-ncov/cases-updates/special-populations/pregnancy-data-on-covid-19.html

[1]The Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA

*Correspondence:
gpinna@uic.edu; graziano_pinna@yahoo.com (G. Pinna).

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