Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes

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Severe outcomes and death from the novel coronavirus disease 2019 (COVID-19) appear to be characterized by an exaggerated immune response with hypercytokinemia leading to inflammatory infiltration of the lungs and acute respiratory distress syndrome. Risk of severe COVID-19 outcomes is consistently lower in women than men worldwide, suggesting that female biological sex is instrumental in protection. This mini-review discusses the immunomodulatory and anti-inflammatory actions of high physiological concentrations of the steroids 17β-estradiol (E2) and progesterone (P4). We review how E2 and P4 favor a state of decreased innate immune inflammatory response while enhancing immune tolerance and antibody production. We discuss how the combination of E2 and P4 may improve the immune dysregulation that leads to the COVID-19 cytokine storm. It is intended to stimulate novel consideration of the biological forces that are protective in women compared to men, and to therapeutically harness these factors to mitigate COVID-19 morbidity and mortality. (*Endocrinology* 161: 1–8, 2020)

Key Words: estrogen, progesterone, immunomodulation, cytokine storm, COVID-19, sex difference.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing the novel coronavirus disease–2019 (COVID-19) pandemic that has infected more than 5 million people and killed more than 350 000 worldwide. The systematic investigation of clinically approved drugs is a priority to improve disease outcomes and invest resources to go to full-scale production. The search for an effective therapy is ongoing actively but is currently limited in success. Perhaps we should look outside the box and consider what is hidden in plain sight such as the biological reasons why women are relatively protected from COVID-19 compared to men.

This review highlights experimental evidence that the steroid hormones 17β-estradiol (E2) and progesterone (P4), at high physiological concentrations, are powerful immunomodulators and argues that acute steroid therapy with the combination of E2 and P4 may represent a safe and viable therapeutic option that needs to be tested in clinical trials to mitigate severe COVID-19 outcomes.

Abbreviations: COVID-19, coronavirus disease–2019; E2, 17β-estradiol; ER, estrogen receptor; HCC, hepatocellular carcinoma; IL-1β, interleukin-1β; IL-6, interleukin-6; ISARIC, International Severe Acute Respiratory and Emerging Infections Consortium; MERS-CoV, Middle East respiratory syndrome coronavirus; MHT, menopausal hormone therapy; P4, progesterone; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SERM, selective estrogen receptor modulator; TNF-α, tumor necrosis factor α; Tregs, regulatory T cells.

Coronavirus Disease–2019 Mortality is Lower in Women Compared to Men

Since the beginning of the 21st century, 2 previous deadly zoonotic betacoronavirus outbreaks have crossed the species barriers to infect humans and exhibited the same apparent female protection from severe outcomes. The first SARS-CoV outbreak emerged in 2002 in Guangdong province, China, and among 1755 hospitalized patients in Hong Kong the case fatality rates was 13% in women compared to 22% in men [1]. During the ongoing Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak that began in 2012 in Saudi Arabia, among 425 reported cases, disease occurrence was lower among women (38% of cases) and the case fatality rate was 23% for women compared to 52% for men [2]. Today in China, Europe, and the United States, COVID-19 severity and mortality is consistently lower in women than in men [3-8]. Taking the most representative series to date, in the cohort of 1099 COVID-19 hospitalized patients in Wuhan, China, only 42% of the patients were women [4]. Among severe cases (ie, admitted to an intensive care unit, requiring mechanical ventilation, or fatal), women accounted for 32% of patients [4]. Similarly, women represented only 18% of all COVID-19 admissions in intensive care units in the Lombardy region of Italy [9]. In New York City, among 5700 hospitalized patients, women represented 33% of cases and 39% of deaths [7]. The International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) in a prospective observational cohort study of more than 17 000 patients in the United Kingdom reported that among hospitalized patients, women accounted for only 40%, with a 20% lower mortality than in men [10]. Although advancing age is associated with greater risk of mortality in both sexes, female protection remains evident [11]. An analysis of COVID-19 data from Italy, Spain, Germany, Switzerland, Belgium, and Norway reveals that among all age groups older than 20 years, fatality rates are greater for males than females [12]. In contrast, male-female differences in the rate of confirmed SARS-CoV-2 infections are age dependent in all countries, being greater among females age 10 to 50 years and greater among males younger than 10 years and older than 50 years [12]. We interpret these data to suggest that biological sex differences contribute to female-biased protection against death, but sex-associated risk of exposure may affect rates of infection differently for males and females at differential ages. A question then arises as to what biological factors are protective in women compared to men, and how can we harness these modifiable factors to mitigate COVID-19 morbidity and mortality?

Role of the Proinflammatory Cytokine Storm in Coronavirus Disease–2019 Outcomes

Severe COVID-19 outcomes are associated with delayed and exaggerated innate immune responses, including hypercytokinemia and inflammatory cell infiltration in the lungs. Our current understanding of the disease, which is rapidly evolving as we write this review, is that patients with COVID-19 do not die from damage caused by virus replication, they die from the consequences of a so-called "cytokine storm" [13-16]. In an attempt to protect the body from SARS-CoV-2, immune cells infiltrate the lungs, causing hyperactivation of monocytes and macrophages, and elevated production of proinflammatory cytokines (eg, interleukin-6 [IL-6], interleukin-1β [IL-1β], tumor necrosis factor α [TNF-α]) and chemokines (eg, monocyte chemoattractant protein-1 (MCP-1/CCL2)) [15]. The cytokine storm is also associated with lymphopenia, and a study in 21 patients from Wuhan reported a decrease in CD4+ and CD8+ T cells, as well as suppressed interferon γ production by CD4+ T cells, which was associated with COVID-19 severity [15]. The local outpouring of chemokines and cytokines attracts more inflammatory cells, such as neutrophils and monocytes, into lung tissue, resulting in lung injury. Ironically, the cytokine storm is a result of the immune system responding to infection in an effort to protect the host, but results in acute respiratory distress syndrome and multiorgan failure [13, 14]. Increased production and elevated local and systemic IL-6 is hypothesized to be central to the development of the cytokine storm [17, 18]. Accordingly, therapeutic strategies targeting the inflammatory response such as IL-6 blockade [19] or the transplantation of mesenchymal stem cells to restore immune tolerance [20] are showing promising preliminary results in mitigating the cytokine storm. Here we discuss a paradigm in which therapy with the steroid hormones E2 and P4 could mitigate this virally induced innate immune inflammatory response.

Females Generally Exhibit Greater Immune Responses to Viruses

Females generally develop heightened immune responses compared to males. In 1967, Butterworth et al reported that women produce higher levels of circulating immunoglobulins IgG and IgM than men [21], which was subsequently confirmed by multiple studies. Accordingly, following vaccination against influenza, yellow fever, rubella, measles, mumps, hepatitis, herpes simplex 2, rabies, smallpox, and dengue

viruses, protective antibody responses are twice as high in women than in men (22). Women also have higher frequencies of CD4+ T helper cells than men (23). The biological reasons why females develop a more robust immune response than males against pathogens, including viruses, likely explain the observed female protection from COVID-19 fatal outcomes. First, females enjoy the genetic benefit of 2 X chromosomes and being a mosaic of X-linked genes (ie, randomly expressing alleles inherited from their mother or father), including more than 60 immune-response genes (24). By contrast, males have only one X chromosome inherited from their mother. Several studies show that genetic diseases associated with deleterious X-linked alleles are more frequently observed in males (25). Generally, there should be no dosage effect associated with position of 2 X chromosomes in females. Incomplete inactivation of immunoregulatory genes on the X chromosome in females, however, can cause a gene dosage imbalance between the sexes (26, 27), which is implicated in female-biased autoimmune diseases (28) and vaccine efficacy (29). The Y chromosome also has immunoregulatory functions (30) that are linked to influenza outcomes, at least in mice (31).

Sex steroids are potent immune-modulators and the different concentrations of estrogens, P4, and androgens between women and men, in addition to the genetics described previously, are likely to influence COVID-19 immune responses and inflammatory outcomes. This is especially important because acute and severe illnesses, such as COVID-19, may alter the function of the hypothalamic-pituitary gonadal axis and decrease the endogenous production of estrogens and P4. Hormones are also amenable to therapeutic intervention. Later, we discuss immunomodulation provided by high physiological serum concentrations of estrogens and P4 as it relates to SARS-CoV-2 infection. This background knowledge is paramount to appreciate the potential benefits that E2 and P4 treatment could provide in the context of SARS-CoV-2–mediated hyperinflammation and acute respiratory distress syndrome.

Estrogens, Progesterone, and Immune Function

Estrogen receptors (ERs) are expressed in all immune cells, serving as transcriptional regulators of cellular function. In human peripheral blood mononuclear cells, CD4+ T lymphocytes express higher levels of ERα messenger RNA than ERβ, whereas B cells express higher levels of ERβ than ERα messenger RNA (32). Peripheral blood CD8+ T cells and monocytes express low but comparable levels of both ERs (32). Therapy with E2, leading to serum concentrations equivalent to ovulation or pregnancy, possess beneficial immunomodulatory and anti-inflammatory actions in mice and humans (reviewed in [24, 33]). In most experimental human or rodent models, the anti-inflammatory actions of E2 on innate immunity includes the suppression of the production of proinflammatory cytokines, for example, IL-6, IL-1β, and TNF-α, by monocytes and macrophages (a major factor in the COVID-19 cytokine storm) and a strong inhibition of CCL2, thus preventing innate immune cells migration into inflamed areas, particularly neutrophils and monocytes. E2 stimulates CD4+ T-helper cell production of anti-inflammatory cytokines, for example, interleukin 4 (IL-4), interleukin 10 (IL-10), and interferon γ. Generally, high E2 concentrations favor helper T-cell type 2 (Th2)-type anti-inflammatory responses. E2 decreases interleukin 17 production by proinflammatory Th17 helper cells. E2 enhances the expansion of regulatory T cells (Tregs) thus promoting immune tolerance. E2 also stimulates antibody production by B cells (Figure 1).

There is strong evidence in metabolic bone disease and virus-induced liver disease that estrogens inhibit disease pathogenesis through suppression of IL-6 production. For example, estrogens inhibit osteoclast development and resorptive function in bone by inhibiting IL-6 gene transcription and production (34). Additionally, the incidence of chronic hepatitis B–induced hepatocellular carcinoma (HCC) in humans shows a strong male predominance. IL-6 is believed to be a key component in inflammation-associated tumorigenesis of HCC (35). In a retrospective study of postmenopausal women with chronic hepatitis C, progression to liver fibrosis was decreased in women who took menopausal estrogen therapy, compared to women who did not (36). In a rat model of chemically induced HCC, males produced more IL-6 from liver Kupffer cells and were more prone to HCC than females (37). Estrogens protected males from HCC via inhibition of IL-6 production by Kupffer cells.

In a mouse model of acute lung inflammation by instillation of bacterial lipopolysaccharide, males and ovariectomized females exhibited increased lung infiltration of polymorphonuclear cells with elevated production of IL-6, IL-1β, and ICAM-1 (intercellular adhesion molecule-1), which was reduced by E2 treatment of males and ovariectomized females (38). In preclinical models of influenza infection, estrogens exhibit powerful immunomodulatory actions leading to a more appropriate innate immune response in the lungs, which is associated with decreased proinflammatory cytokines
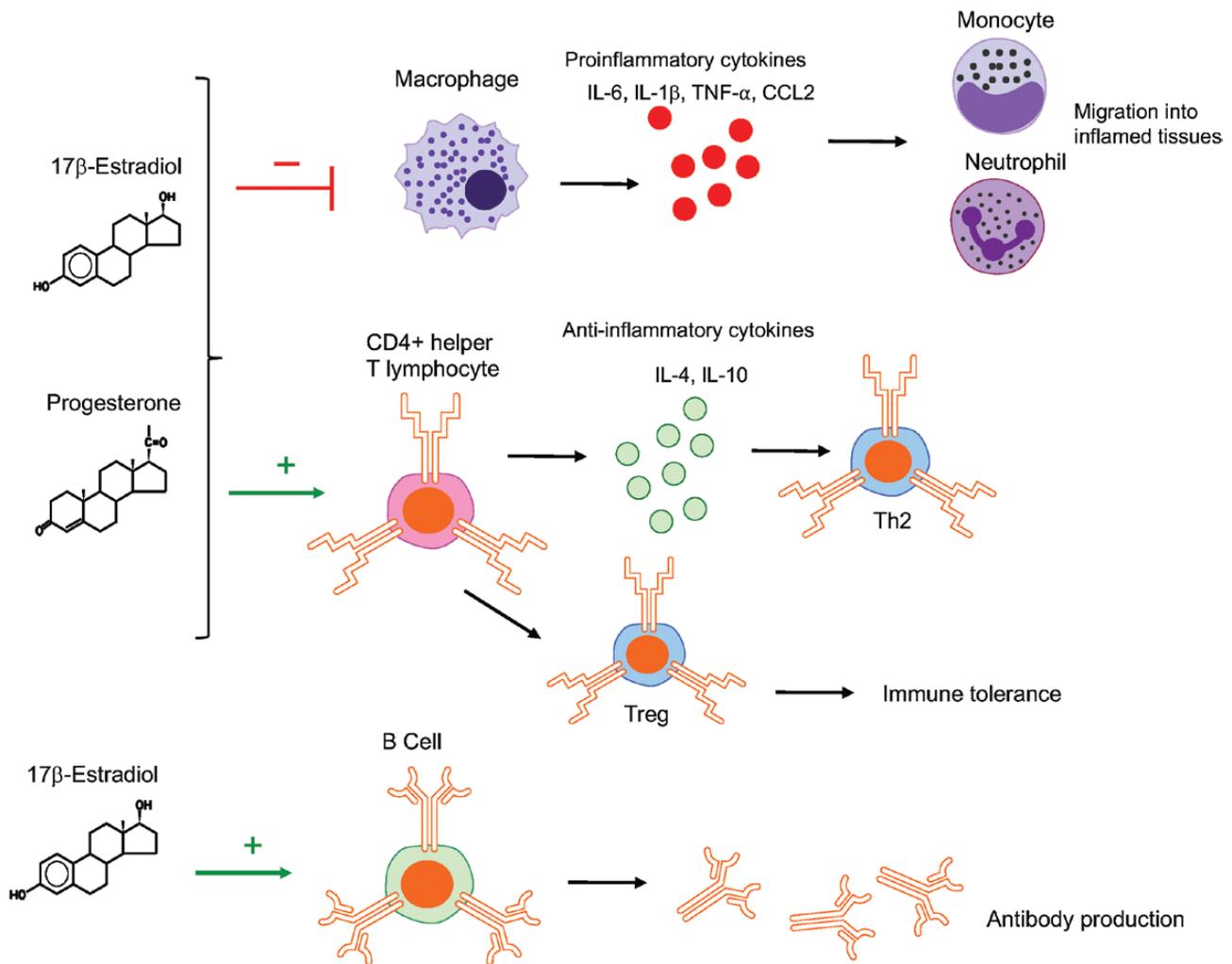
Figure 1. Anti-inflammatory and immunomodulatory actions of estradiol and progesterone. High physiological concentrations of 17β-estradiol (E2) suppress the production of proinflammatory cytokines, for example, interleukin 6 (IL-6), interleukin 1β (IL-1β), and tumor necrosis factor α (TNF-α), and chemokine CCL2 by macrophages, thus preventing neutrophils and monocytes migration into inflamed areas. Progesterone (P4) also inhibits proinflammatory cytokines IL-1β and interleukin 12 production by macrophages and dendritic cells. High concentrations of E2 or P4 stimulate CD4+ T-helper cell production of anti-inflammatory cytokines, for example, interleukin 4 and interleukin 10, and favor Th2-type anti-inflammatory responses. E2 and P4 also enhance the expansion of regulatory T cells (Treg), thus promoting immune tolerance. Finally, E2 stimulates antibody production by B cells.

and chemokine responses before the clinical disease develops (39-41). In primary human nasal epithelial cell cultures, estrogenic compounds, including E2, signaling through ERβ significantly reduce influenza virus replication (42). Further, SARS-CoV-2 and SARS-CoV both produce deadly pneumonias with the same apparent female protection. In a mouse model of SARS-CoV infection, female mice developed lower virus titers, lower infiltration with inflammatory monocyte, macrophages, and neutrophils producing fewer inflammatory cytokines (IL-6, IL-1β, and TNF-α) and chemokines (CCL2), resulting in milder pulmonary damage and a lower female mortality (20%) compared to males (80%) (43), a sex distribution similar to that observed in patients with SARS. Importantly, the endogenous production of E2 in female mice was instrumental in this protection. Castration of males had no effect on the disease,

while surgical removal of the ovaries or treatment with the ER antagonist fulvestrant in female mice infected with SARS-CoV resulted in the same pulmonary damages and mortality rate as in males. Further, treatment of ovariectomized mice with the Food and Drug Administration–approved selective estrogen receptor modulator (SERM) tamoxifen—a mixed ER agonist and antagonist prescribed for the treatment of breast cancer—restored the female protection. This study indicates that in a murine model of SARS-CoV infection, ovarian hormones and especially estrogens protected females from lethal pneumonia, and tamoxifen mimicked the female-biased protection. A screening of multiple Food and Drug Administration–approved compounds for anticoronavirus activity identified tamoxifen and toremifene (another SERM) among the top 10 most effective and safe drugs at inhibiting MERS-CoV and

SARS-CoV infections in vitro (44). Toremifene also inhibits Ebola virus infection in vitro and in vivo in mice (45). The mechanism of toremifene action seems related to the multiple cationic amphiphile structure of the molecule that impairs the late step of virus entry or fusion. Taken together, these findings suggest that E2 and related SERMs have 2 potential protective mechanisms of action against SARS-CoV–mediated pneumonias in mice: 1) an estrogen-dependent decrease in the deadly innate immune response and cytokine storm in the lungs, thus preventing respiratory failure, and 2) specific to SERMs, an off-target direct inhibition of SARS-CoV replication and cytopathic effects.

P4 is another important immunomodulatory and anti-inflammatory hormone that is produced at high levels by the placenta during pregnancy. Progesterone receptors are expressed in most immune cells, including epithelial cells, macrophages, dendritic cells, lymphocytes, mast cells, and eosinophils (24). However, P4 can also signal via glucocorticoid and mineralocorticoid receptors. P4 inhibits proinflammatory cytokines IL-1β and interleukin 12 production by human and rodent macrophages and dendritic cells. Progesterone favors the skewing of CD4+ T-helper cell responses from Th1-type toward Th2-type and the production of anti-inflammatory IL-4 and IL-10 cytokines (24, 46, 47). Treatment of cord blood cells with P4 increases the percentage of FOXP3+ Treg cells (thus promoting immune tolerance), while decreasing the frequencies of proinflammatory Th17 cells (Fig. 1). Administration of P4 at concentrations mimicking the luteal phase to progesterone-depleted adult female mice conferred protection from lethal influenza A virus pneumonia (48). In these mice, P4 treatment decreased the inflammatory environment of the lungs, improved pulmonary function, and promoted cell proliferation and pulmonary repair, which resulted in an earlier recovery, without effects on viral load. Interestingly, in this case, P4 treatment promoted faster recovery by increasing transforming growth factor β, IL-6, interleukin 22, and the numbers of regulatory Th17 cells expressing CD39. Importantly, progesterone promoted pulmonary tissue repair by upregulating the epidermal growth factor amphiregulin in the lungs (48). Although influenza A virus infection is different and produces an immune reaction different from that induced by SARS-CoV-2 (eg, the beneficial effect of IL-6), this study provides important insight into the immunomodulatory and healing effects of P4. Further, P4 also seems to exhibit antiviral activity in VeroE6 cells infected with SARS-CoV-2 (49).

Pregnancy and Coronavirus Disease–2019

During pregnancy, the innate and adaptive immune responses shift from an inflammatory to an anti-inflammatory phenotype to avoid fetal rejection and favor passive transfer of maternal antibodies to the fetus (reviewed in [50]). These effects, which are relevant to COVID-19 protection, are largely mediated by E2 and P4. During pregnancy, increased levels of E2 suppress many cytotoxic and innate immune inflammatory responses but stimulate antibody production by B cells (33, 51). In fact, one of the most important immunological features of pregnancy is the increase in B-cell responses with enhanced antibody production due to dual stimulation by estrogens and P4, the production of which is maximal in the third trimester (33, 51). P4 also stimulates the synthesis of progesterone-induced binding factor (PIBF) by lymphocytes, which promotes the differentiation of CD4+ T cells into Th2 cells secreting anti-inflammatory cytokines, including IL-4, interleukin 5, and IL-10 (52). This explains why during pregnancy B-cell/antibody-driven diseases, like systemic lupus erythematosus, exacerbate; whereas T-cell–driven diseases with cytotoxic and innate immune responses, like rheumatoid arthritis or multiple sclerosis, improve (50, 53).

Pregnant women are not protected from SARS-CoV-2 infection but seem to be relatively protected from the severe outcomes of SARS-CoV-2. Currently, studies evaluating COVID-19 outcomes during pregnancy have not yet separated outcomes occurring during pregnancy (ie, when E2 and P4 concentrations are high) from those in the immediate postpartum period (ie, when E2 and P4 concentrations are undetectable). In a Chinese retrospective series of 82 women (28 pregnant women, 54 reproductive-aged nonpregnant women) hospitalized in Wuhan with confirmed COVID-19, pregnant women exhibited comparable severity of disease, virus clearance time, and length of hospital stay compared with reproductive-aged nonpregnant women (54). The authors concluded that pregnant women infected with SARS-CoV-2 have comparable clinical course and outcomes compared with control women. However, in this study, the nonpregnant women received more antiviral, corticosteroid, and immunoglobulin therapies than pregnant women and therefore the groups were not comparable in terms of treatments and related outcomes. A larger retrospective review of 118 pregnant women admitted for COVID-19 pneumonia in China reported only 9 cases (8%) of severe pneumonia with hypoxemia. Notably, in 6 of these

women, including 1 requiring mechanical ventilation, the exacerbation of pneumonia occurred during the postpartum period, after serum concentrations of E2 and P4 had already dropped (55). Therefore, the actual number of severe cases in this study was 3 (2.5% of the pregnant population), which is less than the severity of COVID-19 in Chinese women in a similar age range (around 6%) (2). In fact in the only published series of 9 pregnant women with fatal COVID-19, a detailed analysis of the cases reveals that 7 of these women deteriorated and died in the hours or days following delivery (56). Therefore, larger studies addressing COVID-19 mortality during pregnancy compared to early postpartum as a primary end point are needed to determine whether the hormonal environment of the third trimester is protective.

Immunomodulation by Hormone-Based Therapies in Women

Treatment of postmenopausal women with menopausal hormone therapy (MHT) and use of oral contraceptives by women during reproductive age are accompanied by concomitant physiological changes associated with increased concentrations of estrogens and progestins. Thus, the effects of these 2 hormones cannot be separated. Most studies assessing the effect of MHT using E2 alone or in combination with progestins showed that MHT inhibits the production of proinflammatory cytokines (eg, TNF-α, IL-1β, and IL-6) by peripheral blood mononuclear cells ex vivo or in vivo in the serum of MHT-treated women (57-60). In addition, transdermal E2 blunted the proinflammatory cytokine responses to an inflammatory challenge (59). The anti-inflammatory effect of E2 therapy in menopausal women with regard to low-grade systemic inflammation seemed to be observed mostly following transdermal rather than oral E2 administration and was not reproduced by conjugated equine estrogens. Bazedoxifene belongs to a new generation of SERMs used in combination with estrogens in oral menopausal hormone therapy. In obese female mice, treatment with bazedoxifene decreases IL-6 and multiple markers of systemic inflammation (61). However, this effect was not observed in a pilot randomized trial of 8-week treatment with oral estrogens and bazedoxifene in obese postmenopausal women (62). Likely, the absence of beneficial effect of orally administered estrogens on systemic inflammation is related to the first-pass liver metabolism following oral estrogen administration, which increases C-reactive protein production and markers of inflammation (63).

Transdermal and oral estrogen therapy with or without progestin increases CD19+ B-cell numbers and activity (64). Accordingly, the stimulating effects of menopausal therapy with estrogens and progestins on B cells promotes the progression of systemic lupus erythematosus in postmenopausal women (33).

Classical ERα, ERβ, and P4 receptors are present both in extranuclear and nuclear pools in most cells (65). To what extent each receptor cellular pool has collaborative or unique effects on immune function has not been determined and will be of interest for the design of future studies assessing the effect of sex-steroid receptor ligands in modulating immune functions.

Repurposing Estrogens and Progesterone to Mitigate Coronavirus Disease–2019 Mortality?

High physiological concentrations of E2 and P4 possibly synergize to mitigate innate immune cells production of proinflammatory cytokines, promote T cells' anti-inflammatory responses and immune tolerance, and stimulate antibody production by B cells (Fig. 1). In individuals with confirmed COVID-19, acute hormone therapy with E2 and P4 could mitigate the cytokine storm while increasing antibody production. Pandemics such as SARS-CoV-2 provide little time for drug development. Repurposing existing and approved drugs that have already been tested in humans—and for which detailed information is available on their pharmacology, formulation, dose, and potential toxicity—provides an expedited and safe approach for off-label use of potentially life-saving therapeutics. As discussed earlier, acute E2 and P4 treatment would be expected to blunt innate immune inflammatory responses and at the same time stimulate B-cell responses and antibody production (33, 51) without noticeable side effects. A critical advantage of estrogen, SERMs, and progestin compounds is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from decades of clinical and basic studies. Hormone therapy is used by millions of women for contraception and prevention of menopausal symptoms. It is widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately. As this review is being written, 2 clinical trials are testing E2 (ClinicalTrials.gov identifier NCT04359329) or P4 (ClinicalTrials.gov identifier NCT04365127) individually in COVID-19 patients. It is worth considering the potential benefit of hormone therapy alone or in combination therapy with antiviral drugs or IL-6 blockade as an immune modulation in

single-center off-label clinical trials. In an outbreak like this, and while we are waiting for a safe and efficient vaccine to be developed, the systematic investigation of clinically approved drugs is a priority to determine which compounds may mitigate the disease and to invest resources to begin full-scale production.

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Additional Information

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