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Could Estrogen Protect Women From COVID-19?

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Abstract

The apparent gender differences in favor of women in the risk of contracting and dying from coronavirus disease 2019 (COVID-19), and the fact that such trends have also been observed in recent epidemics including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), have prompted the obvious question: Are the reasons life-style or biological? True, women generally make healthier lifestyle choices as compared to men. Women do not smoke or drink as much as men, and they have a lower burden of those diseases (heart disease, diabetes or chronic lung conditions) that are known to be significant factors in the higher death rates among men with COVID-19. But there is compelling evidence for a role for biological factors. Genes are likely to play an important role. The X chromosome, of which women possess two, contains the largest number of immune-related genes of the whole human genome, theoretically giving women double the advantage over men in mounting an efficient and rapid immune response. A fundamental difference between women and men is their hormonal milieu, and it is not unreasonable to suppose that the dominant female hormone estrogen could influence the response to infection. In this paper we evaluate the evidence and mechanisms by which estrogen could provide protection to women from a variety of viruses, perhaps including the coronavirus that causes COVID-19.

Keywords: COVID-19; Coronavirus; Infection; Estrogen; Protection

Introduction

It is generally accepted that the coronavirus that causes the

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new coronavirus disease 2019 (COVID-19) pandemic infects and kills more men than women [1]. A sex and gender dichotomy on the impact of epidemic-prone infections has long been recognized by the World Health Organization (WHO) [2]. Studies investigating the role of gender in morbidity and mortality in patients with COVID-19 have shown that men with COVID-19 are more at risk for worse outcomes and death, independent of age [1, 3]. Men tend to develop more serious infections than women and the number of men is about 2.4 times that of women in the deceased patients [3]. Sex-disaggregated data from 26 countries indicate that the overall case fatality ratio in men is higher than that in women [4].

Virus infections in particular appear to have a stronger predilection for men, and this was observed in recent epidemics including the severe acute respiratory syndrome (SARS) [5] and Middle East respiratory syndrome (MERS) [6]. The reasons for these female-male differences are not fully understood, but they are likely to involve interplay between life-style factors and biological influences. From a life-style perspective, women tend to drink and smoke less than men, and consequently often have a lower burden of those diseases, such as cardiovascular disease, diabetes and chronic lung disease, which appear to increase mortality risk in COVID-19 patients [7]. However, biological influences could be even more important. The immune response is likely to play a critical role, and it is generally known that there are gender differences in immune responsiveness [8]. Within the whole human genome, the X chromosome contains the largest number of immune-related genes [9], and women possess two of these chromosomes versus one in men. In 2008, a list of 79 X-linked genes with a possible role in sex-based differences in immune responses was presented [10]. Some of these include *ELK1*, *EPAG*, *GTD*, *NEMO* and *CYBB* in addition to immune response related proteins, receptors and transcriptional and translational control receptors.

It is teleologically sound to suppose that any difference in immune responsiveness between men and women is likely to be mediated through hormonal mechanisms, with the dominant female hormone estrogen playing a central role. If estrogen modulates the immune system, an obvious human model is a comparison of such responses between men and premenopausal women (estrogen replete) and postmenopausal women (naturally estrogen deficient), in particular studying the impact of estrogen supplementation in the latter. A wealth of data from such research is now available.

While estrogen is often thought of in terms of its key role in normal sexual development and reproduction, and while there is evidence of its immunomodulatory activities de-

Estrogen

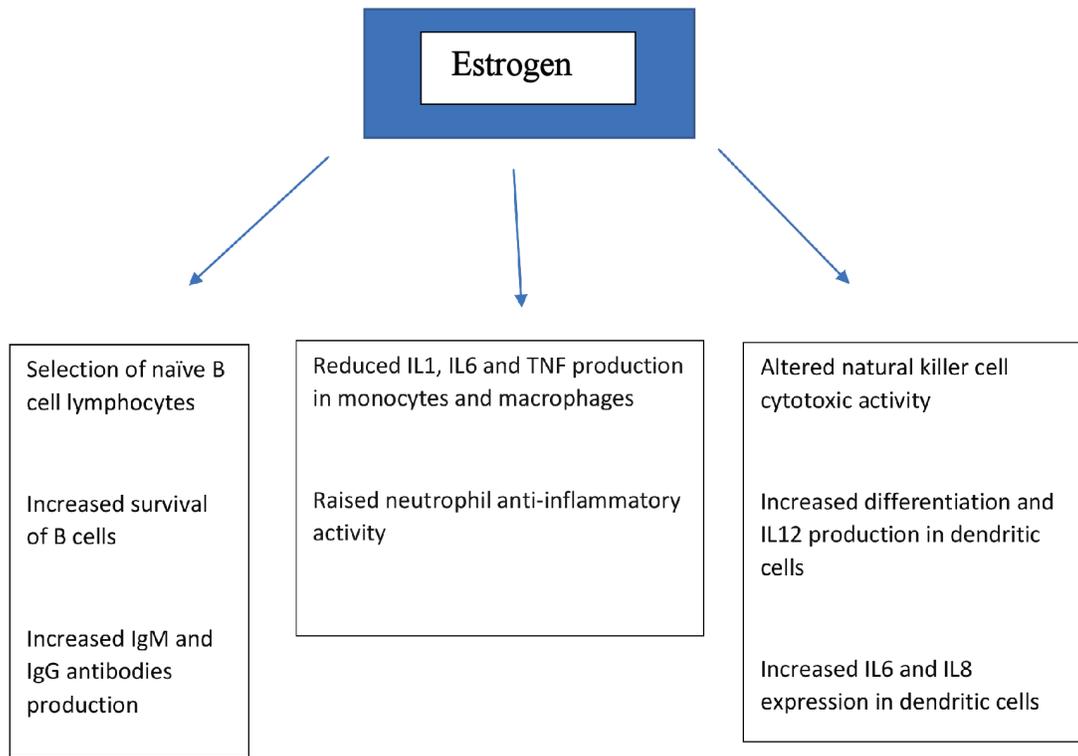| Selection of naïve B cell lymphocytes	Reduced IL1, IL6 and TNF production in monocytes and macrophages	Altered natural killer cell cytotoxic activity
Increased survival of B cells	Raised neutrophil anti-inflammatory activity	Increased differentiation and IL12 production in dendritic cells
Increased IgM and IgG antibodies production		Increased IL6 and IL8 expression in dendritic cells

Figure 1. Effects of estrogen on various components of immune system. IL: interleukin; TNF: tumor necrosis factor; Ig: immunoglobulin.

scribed above, the presence of estrogen receptors in a variety of other body tissues and organ systems (including endometrium, breast, ovarian stromal and granulosa cells, hypothalamus, hippocampus, kidney, bone, heart, blood vessels, lungs, intestinal mucosa, prostate and endothelial cells) suggests that estrogen plays an important role in a wide range of physiological processes including immune response [11], by a variety of mechanisms that include direct action on relevant cell types/tissue. Figure 1 shows the effect of estrogen on various components of the immune system. There are data from *in vitro* studies, animal experimentation and human research that support this contention. We searched the Medline/PubMed medical literature database using terms coronavirus, COVID-19, estrogen, estrogen, viral, infections and immunity to search for scientific manuscripts related to our topic, and here we explore some of the evidence and discuss the underlying mechanisms as they might relate to COVID-19 infection.

Antiviral Actions of Estrogen: Effect on Innate Immunity and Non-Immune Systems

Several lines of research have shown that estrogen exhibits antiviral properties. Smith et al [12] (2000) compared the influence of estrogen versus progesterone on the vaginal transmission of simian immunodeficiency virus. Six ovariectomized macaques were treated with estrogen, another six with progesterone, and a further six were untreated, followed by intra-

vaginal inoculation with the virus. All six untreated (control) macaques and five (83%) progesterone-treated animals became infected following intravaginal inoculation, while none of the six estrogen-treated macaques was infected. The block to infection occurred at the vaginal epithelium and/or lumen, since estrogen-treated macaques became infected following subepithelial inoculation of virus. Johansen et al [13] (2013) used molecular probes to identify drugs with antiviral activity against the Zaire Ebola virus. They identified a set of selective estrogen receptor modulators (SERMs), including clomiphene and toremifene, which acted as potent inhibitors of viral infection. Antiviral activity was confirmed for both of these SERMs in an *in vivo* mouse infection model. This antiviral activity occurred even in the absence of detectable estrogen receptor expression, and both SERMs inhibited virus entry after internalisation, suggesting that clomiphene and toremifene are not working through classical pathways associated with the estrogen receptor. Instead, the response appeared to be an off-target effect where the compounds interfered with a step late in viral entry and triggering of fusion. Yet another study in 2017 analyzed the structures of SERMs and their incidental biological activity (cholesterol accumulation) as a plausible mechanism of their inhibitory effects on Ebola virus infection [14]. They demonstrated that the same dosages of SERMs which induced cholesterol accumulation also inhibited Ebola infection. SERMs reduced the cellular sphingosine and subsequently caused endolysosomal calcium accumulation, which in turn led to blocking the Ebola entry.

Robinson et al [15] tested the hypothesis that estrogen protects against influenza A virus infection by altering the recruitment and activity of innate immune cells and T cells. Chemokine concentrations were measured, and innate and adaptive immune cells were enumerated from the lungs of estrogen and placebo-treated ovariectomized female C57BL/6 mice following infection. Females treated with estrogen experienced less morbidity but had similar lung virus titres to placebo-treated females. Females treated with estrogen had lower induction of CCL2 but higher CCL3 and CXCL1 responses in their lungs than placebo-treated females. Pulmonary recruitment of neutrophils, natural killer (NK) cells, macrophages, and dendritic cells was increased following infection, but only neutrophil numbers were greater in estrogen-treated than placebo-treated females. Neutrophil depletion in estrogen-treated females increased morbidity, reduced pulmonary production of chemo-attractants for neutrophils, and reduced interferon production by virus-specific CD8 T cells. This study showed that neutrophils appear to mediate both inflammation and tissue repair during influenza virus infection and are regulated by estrogen to improve the outcome of infection in females [15].

In an *in vitro* study, Peretz et al [16] (2016) found that estrogenic compounds reduced influenza A virus replication in primary human nasal epithelial cells derived from females but not males [16]. These estrogenic compounds reduced peak viral titre, which did not involve changes in the secretion of chemokines or interferons, but rather was associated with reduced metabolic processes. The effects of estrogens were specific for cultures from female but not male donors. The antiviral effects were most likely mediated by signalling through estrogen receptors.

Antiviral effects of estrogens have also been reported previously against viruses such as human immunodeficiency virus (HIV), hepatitis C (HCV), Ebola and human cytomegalovirus (HCMV) [16]. Studies have shown that estradiol can prevent HIV transcription by blocking HIV promoter activity in peripheral blood mononuclear cells and preventing HIV entry in CD4$^+$ T cell and macrophages [17, 18]. Estradiol also prevents the production of infectious HCV particles [19]. SERMs, including clomiphene and raloxifene, inhibit HCV infection at multiple stages of the virus life cycle and act as adjuvants to improve antiviral HCV therapies in postmenopausal women [16, 20, 21].

Tamoxifen (TAM) is an estrogen-receptor antagonist, and there was a study aimed to investigate the effect of TAM on infection with vesicular stomatitis virus (VSV) [22]. Vero cells (mice) were treated with different concentrations of TAM for 24 h and then infected with VSV. Additionally, mice were pretreated with 4 mg TAM, 1 day and 3 days before infection with VSV. Treatment of Vero cells with TAM suppressed the viral replication of VSV *in vitro* and *in vivo*. The inhibitory effect of TAM on VSV replication correlated with an enhanced interferon response and stimulation of macrophages [22].

Estrogen treatment in ovariectomized animals reduces expression of vascular monocyte chemoattractant protein-1 (MCP-1) and leukocyte infiltration into injured tissues, such as arteries and lung [23, 24]. Estrogen is also able to modulate the activation of different macrophage immune phenotypes [25].

Estrogen Modulates the Adaptive Immune System

The human immune system demonstrates significant sex differences. Women tend to have a more responsive immune system compared to men so that the outcome and survival rates from infections or sepsis are often better in females than in males [26].

We investigated the effect of therapeutic doses of 17β-estradiol on cellular immune responses in postmenopausal women [27]. Fifteen healthy women who had undergone a natural menopause were treated with estrogen in the form of 100 mg patches applied twice weekly for 3 out of every 4 weeks over a 3-month period, followed by combined estrogen and progestogen formulations as long-term therapy.

Blood samples were taken on two occasions prior to treatment and at weeks 1, 3, 4, 7, 9, 12 and 24 after commencing therapy. Lymphocyte subsets (CD2, CD4, CD8, CD19, human leukocyte antigen-DR (HLA-DR) and NK) were studied in each blood sample using a monoclonal antibody kit and a two-color fluorescence flow cytometer. One-way mixed lymphocyte reactions (MLRs) were performed using the same stimulator throughout. Delayed hypersensitivity skin tests (DHTs) were carried out twice before treatment and at weeks 3, 4, 12 and 24. Lymphocyte subsets did not change significantly with treatment, but both the MLRs and the DHTs were significantly depressed, maximally so by the third week of treatment showing that therapeutic doses of estrogen modulate certain immune responses. All these observations certainly confirm an interaction between the endocrine and immune systems. The hormonal milieu undoubtedly influences the immune system and sex steroids in particular appear to be potent modulators of the immune response [27].

In a 2016 study the effect of estrogen therapy on lymphocyte subset frequency and the immune response to influenza vaccine in three groups (younger women with regular menstrual cycles, not on hormonal contraception; postmenopausal women not on hormone replacement therapy; and postmenopausal hysterectomized women on estrogen-only replacement) were studied. The results suggested that humoral immunity is increased by estrogen therapy [28]. A summary of observational studies evaluating effects of estrogen on immune response is presented in Table 1 [12-17, 19-25, 27, 28].

Numerous additional studies, beyond the scope of this article, have confirmed male-female differences in the immune response, that generally the female immune response is superior, and estrogen driven, but also that this superior response could explain the higher prevalence of auto-immune disease in women when the immune system behaves in an aberrant fashion [29-33]. An age relationship between estrogen levels and monocyte/macrophage number and function has also been identified, with studies suggesting impaired macrophage function in postmenopausal women [34].

Estrogen Protects Cardiovascular Health

Cardiovascular complications are known to be a factor in a

Table 1. Summary of Observational Studies Evaluating Effects of Estrogen on Immune Response

Year	Authors	Study conclusion
1992	Manyonda et al [27]	Therapeutic doses of estrogen modulated immune responses in postmenopausal women.
2000	Smith et al [12]	Estrogen prevented vaginal transmission of simian immunodeficiency virus in macaques.
2004	Miller et al [23]	Estrogen attenuated the early vascular injury response by negatively modulating proinflammatory mediator expression and chemotactic activity of injured vessels for neutrophils in rats.
2007	Hsieh et al [24]	Estrogen showed protective effect on lung injury mediated via downregulation of lung MIF and TLR4-induced cytokine/chemokine production.
2010	Hayashida et al [19]	Estrogen inhibits the production of hepatitis C infectious particles in human cells.
2012	Furusyo et al [20]	Selective estrogen receptor modulator improved the efficacy of treatment of postmenopausal women with chronic hepatitis C.
2013	Murakami et al [21]	Selective estrogen receptor modulators seemed to target multiple steps of hepatitis C viral life cycle: attachment, entry, replication, and post replication events in viral culture system.
2013	Johansen et al [13]	Selective estrogen receptor modulators (SERMs) acted as potent inhibitors of viral infection in mice.
2013	Rodriguez-Garcia et al [17]	Estrogen reduces susceptibility of human $CD4^+$ T cells and macrophages to HIV infection.
2014	Robinson et al [15]	Estrogen regulates neutrophils mediated inflammation and tissue repair during influenza virus infection in mice.
2015	Villa et al [25]	Estrogen accelerates the resolution of inflammation in macrophagic cells in mice cell lines.
2016	Peretz et al [16]	Estrogenic compounds reduced influenza A virus replication in primary human nasal epithelial cells derived from females.
2016	Engelmann et al [28]	Estrogen affected immune homeostasis and humoral immune responses in postmenopausal women.
2017	Fan et al [14]	Selective estrogen receptor modulators (SERMs) selectively inhibited Ebola virus entry and infection in human cell lines.
2019	Lamin et al [22]	Tamoxifen protects from vesicular stomatitis virus infection in mice.

HIV: human immunodeficiency virus.

large proportion of deaths from COVID-19. Estrogen plays a crucial role in the regulation of female cardiovascular health [35], offering protection through repair of endothelial injury and collateral vessel formation in ischemic tissue [36]. The direct actions of estrogen include the increased synthesis and release of endothelia nitric oxide (NO) and prostacyclin vasodilators and anti-platelet agents and reduced production of proinflammatory mediators in situations of vascular injury [36, 37]. While young women generally have much lower rates of cardio-metabolic disease than their age-matched male counterparts, this protection is lost during the menopausal transition [35, 38, 39], when the risks of cardiovascular disease approach and often equal those experienced by men, thus emphasizing the potential role of estrogen. The beneficial effects of hormone replacement therapy (HRT) in preventing atherosclerotic cardiovascular disease are most pronounced when the therapy is initiated before the development of advanced atherosclerosis [36]. A randomized controlled trial showed that the initiation of HRT in women early after menopause significantly reduces the risk of mortality, myocardial infarction or heart failure without resulting in an increased risk of breast cancer or stroke [40]. Starting HRT less than 10 years since a woman's menopause has been shown to lead to a significant reduction in death and a reduction in cardiovascular disease [41], and this has been confirmed in a variety of studies [35, 41]. For women with premature ovarian insufficiency, HRT has ben-

eficial effects on plasma lipids, insulin resistance and also on endothelial function, and is recommended at least until the average age of natural menopause (51 years) [39]. Estrogen and its receptors therefore play a key role in health and disease in women. The protection against cardiovascular disease in women of reproductive age is related to estrogen since estrogen levels and receptor expression differ significantly between sexes.

Concluding Remarks

The gender differences in favor of women in both infection and mortality risk from COVID-19 infection are likely to be multifactorial. While life-style factors could be important, the dominant female hormone estrogen is likely a key player, acting via a variety of mechanisms that include immune-modulation and non-immune mechanisms. The ample evidence from *in vitro*, animal and human experimentation has been presented above. Perhaps the old adage "man flu" is, after all, far from being just a pejorative term against men to show the superiority of the fairer sex, but actually has a scientific basis [42]. However, our knowledge is far from complete, and further human studies are needed to enable translation into therapeutic approaches. The postmenopausal woman, where estrogen levels have naturally fallen, but can be readily replaced (with HRT), is an ideal human model for further research.

Conflict of Interest

There are no conflicts of interest to declare. None of the authors have any conflicts of interest to declare.

Author Contributions

RP and VST wrote the first draft of manuscript. JO and IM revised the draft. All authors approved the final draft of the manuscript.

Data Availability

The authors declare that data supporting the findings of this study are available within the article (review article only).

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