









## REVIEW

# Immunological aspects and gender bias during respiratory viral infections including novel Coronavirus disease-19 (COVID-19): A scoping review

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## Abstract

The human immune system is not adequately equipped to eliminate new microbes and could result in serious damage on first exposure. This is primarily attributed to the exaggerated immune response (inflammatory disease), which may prove detrimental to the host, as evidenced by SARS-CoV-2 infection. From the experiences of Novel Coronavirus Disease-19 to date, male patients are likely to suffer from high-intensity inflammation and disease severity than the female population. Hormones are considered the significant pillars of sex differences responsible for the discrepancy in immune response exhibited by males and females. Females appear to be better equipped to counter invading respiratory viral pathogens, including the novel SARS-CoV-2, than males. It can be hypothesized that females are more shielded

**Abbreviations:** ACE2, angiotensin-converting enzyme-2; Bcl-2, B cell lymphoma-2; CD22, cluster of differentiation 22; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CLR, C-type lectin receptors; COVID-19, novel coronavirus disease-19; DAMP, damage-associated molecular patterns; DC, dendritic cells; IL, interleukin; ISG, IFN-stimulated genes; NK, natural killer cells; NLR, nucleotide-binding oligomerization domain (NOD)-like receptors; PRR, pattern recognition receptors; RAS, rennin-angiotensin system; RBD, receptor-binding domain; RLR, retinoic acid-inducible gene-like receptor; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; Shp-1, SH2 domain-containing protein-tyrosine phosphatase; TGF- $\beta$ , tissue growth factor- $\beta$ ; TH1, T helper 1 cells; TLR, toll-like receptors; TMPRSS, type II transmembrane serine protease 2; TNF- $\alpha$ , tumor necrosis factor-alpha; Vcam-1, vascular cell adhesion 1.

from disease severity, probably owing to the diverse action/influence of estrogen and other sex hormones on both cellular (thymus-derived T lymphocytes) and humoral immunity (antibodies).

**KEYWORDS**

COVID-19, estrogen, gender biasness, immune system, inflammation, SARS-CoV-2, sex hormones

## 1 | INTRODUCTION

Respiratory viral diseases are commonly caused by adenovirus, influenza virus, rhinovirus, parainfluenza virus, respiratory syncytial virus (RSV), coronavirus, which replicate and grow in the respiratory tract.<sup>1</sup> These viruses enter via the mucosal lining of respiratory, alimentary, and genitourinary tracts. They may also enter the human body through the outer surface of the eye and exposed skin, where they replicate by interfering with the synthesis of interferons (IFNs) and macrophages in the host system. After the dissemination in the body, the virus develops mechanisms, such as constant variations of surface glycoproteins, to escape from the host's defense mechanism, that is, circulating antibodies and various immune cells. Most respiratory viruses, including influenza and coronaviruses, have several hosts and are typically transmitted from animals to humans, and subsequently human to humans.<sup>2–4</sup> During repeated jumps/spillovers from one host to the other (animal–human, animal–animal, human–animal, and human–human), these viruses undergo significant antigenic variations, which invariably help them to counter the immune responses of the host and establish the infection. The available data on the SARS-CoV-2 pandemic indicates higher incidence, morbidity, and mortality among the male population than women suggesting a peculiar predisposition.<sup>5–8</sup> The differential mortality rate between women and men associated with COVID-19 might suggest a protective role for the sex steroids.<sup>9</sup> SARS-CoV-2 utilizes transmembrane protease serine 2 (TMPRSS2) to facilitate its entry into the host cell. TMPRSS2 contributes to the process of viral entry by facilitating the Spike glycoprotein into the S1 (receptor-binding) and S2 (membrane fusion) domains.<sup>10</sup> Preliminary investigation using single-cell RNA sequences analysis identified that the expression of TMPRSS2 was significantly higher in males as compared to females.<sup>11</sup> Although the findings were significant, our understanding of the relationship between TMPRSS2 expression and lung immunity remains poorly understood. However, it has opened up new opportunities to utilize sex steroid-based therapies<sup>11</sup> for improving the disease outcome in patients with COVID-19.

Androgen receptors can simplify the process of viral entry as they are transcription promoters for TMPRSS2. Therefore, the androgen receptor gene variants can affect androgen sensitivity, and diseases such as

prostate cancer and androgenetic alopecia can contribute to COVID-19 outcomes and hospitalization.<sup>6</sup> In addition to the significant differences between women and men in immune response, nonimmune components, like autophagy, mitochondrial functionality, and cholesterol biosynthesis have marked changes that contribute to the unique response trajectories<sup>12</sup> against the COVID-19. Gene expression studies<sup>13</sup> conducted using individual expression profiles obtained from upper airway and blood have already identified profound discrepancies in the responses to SARS-CoV-2 infection. The COVID-19-infected male patients have an increased risk of cardiovascular complications, leading to increased mortality. High cardiovascular vulnerability exhibited by male patients can be attributed to the male-biased regulation of multiple pathomechanisms<sup>14</sup> observed in COVID-19. The expression of angiotensin-converting enzyme-2 (ACE2) in various tissues, including the heart is male-biased as it is an X-linked gene.<sup>15</sup> Therefore, the circulating and tissue levels of ACE2 might impact the virulence of SARS-CoV-2 in the heart. The plasma concentrations of ACE2 were observed to be high in male patients with heart disease,<sup>16</sup> contributing to a higher fatality rate in men due to COVID-19. This review investigates the gender influence and underlying immunological mechanisms in the outcome of respiratory viral infections, including SARS-CoV-2.

### 1.1 | Methodology of literature selection

A systematic literature review was performed to collect data by exploring authentic academic databases, including ScienceDirect, Scopus, PubMed, and Google Scholar, as well as, public and government health organization websites, such as the World Health Organization (WHO), European Centre for Disease Prevention and Control of the European Union (ECDC), National Institutes of Health of United States (NIH), Guidelines International Network, and Chinese guidelines on Novel Coronavirus<sup>9</sup> resources. For critical covering and compilation of most recent and relevant literature contents, the key terms searched include respiratory viral infections, coronavirus, immunological aspects, factors affecting COVID-19 mortality, androgen and progesterone, ageing, inflammation, innate and adaptive immune responses, gender disparity, and its influence on the immune response, and estrogen action on T and B cells. After literature assortment, the obtained data were carefully examined, and only closely matched studies were considered for critical discussion while excluding the irrelevant or generalized studies.

## 2 | RESPIRATORY VIRAL INFECTIONS

Respiratory diseases are the leading causes of morbidity and death in children and older people.<sup>17</sup> Respiratory viral infections affect the airways (breathing passage) and lungs. Most virus-associated respiratory infections are self-limited and affect the upper airway, causing milder symptoms such as sore throat and running nose.<sup>18</sup> Nevertheless, these can involve the lower airways in the elderly and children resulting in wheezing, breath shortness, and pneumonia.<sup>1,19</sup> The viruses are inhaled through the droplets and aerosols that float in the air when a person sneezes, coughs, and talks. The virus enters, invades with the help of specific receptors, and multiplies within the respiratory epithelial cells. Although the human body is equipped with innate immunity as the first line of defense in mucus lining and secretory antibodies, the virus may successfully evade inactivation from the nasopharynx to the alveolar membrane by triggering activation of innate immune-related responses.<sup>20–22</sup> The host-cell and virus interaction occur in three stages: The phase where immune cells sense the virus, initiate innate antiviral responses, and stimulate the adaptive immune response. Later, T and B immune cells clear the infection by executing infected/damaged cells and enforcing antibody-assisted immune responses, respectively. In the final phase, innate immune cells fix damaged tissues and produce mediators that implement the immune response's homeostasis.<sup>23</sup> Type I and III INFs are secreted by immune and tissue-specific cells, which elicit an immune response that is purely antiviral. Lung dendritic cells (DCs) presenting viral antigens drain into lymphatics, where they encounter naïve T cells. These cells activate and re-enter the lungs to react with resident myeloid cells, causing the release of either pro- or anti-inflammatory cytokines to act against infected cells.<sup>24</sup>

Among the most common respiratory infection-causing viruses, the Influenza virus is responsible for frequent seasonal viral infections. Influenza virus has four main types, wherein the Type A (H1N1, H2N2, H3N2, H5N1, H5N5, H5N8, H7N7, H7N9, H7N3, H9N2, H10N8), and B (Yamagata and Victoria) cause frequent human and animal/bird infections, and Type C, and D are mostly confined to animals (pigs and cattle). Influenza viruses adapt themselves in different hosts, and undergo genetic variations, and are responsible for epidemics and pandemics. Moreover, RSV belonging to the Paramyxovirus group causes annual outbreaks of respiratory infections, especially during the winter season. The onset of pulmonary symptoms peaks during wet months in the geographical regions with high annual precipitation and cooler months in hot and dry areas. The RSV causes both upper and lower respiratory tract infections in adults, with the virus entering through contact with mucous membranes and by droplet aerosols. Increasing severity of RSV infection is noted in persons with comorbid conditions, adults with severe combined immunodeficiency, and lung or bone marrow transplantation patients.<sup>25</sup> Other viruses that are responsible for respiratory infections include the Rhinovirus, Coronavirus, human

Adenovirus, Enterovirus, Parainfluenza virus (Types 1–4), and human Metapneumovirus.<sup>26,27</sup>

## 3 | VARIABLE CLINICAL OUTCOMES IN RELATION TO GENDER

A previous study had assessed the variations in the plasma viral load, anti-SARS-CoV-2 antibodies, and cytokines among males and females diagnosed with moderate COVID-19. The results of the study revealed that there was no significant difference in the viral load among both sexes. Nevertheless, it was observed that in males, higher activities of plasma cytokines and weakened T cell responses correlated with the worst disease outcomes. In contrast, female patients revealed increased activities of innate cytokines that positively correlated with unfavorable disease outcomes and efficient T cell activities during favorable outcomes.<sup>28</sup> Increased ACE-2 expression on the cells of various organs attributed to the sex hormones, and X-chromosome mediated overexpression of androgens and ACE-2 receptors could predispose males to severe complications<sup>29</sup> of COVID-19. Significance of age and sex influences on the COVID-19 mortality rates, a study from Italy by Gallo et al.<sup>30</sup> proposed data be interpreted based on standardized mortality trend ratio, that considers both age and sex factors and its relation to the disease outcome. Although the X-chromosome codes for the pattern recognition receptors (PRRs) and toll-like receptors (TLRs) that effectively function to identify and destroy the pathogens, an exaggerated and uncontrolled inflammatory response that potentially harms host cells may be attributed to the increased severity of COVID-19 among males.<sup>31</sup> In another study from China by Zheng et al.,<sup>32</sup> it was noted that increased age and male sex were risk factors for respiratory oxygen supplementation and other intensive care support among more than 1700 patients observed.<sup>32</sup>

Age-related decreased expression of sex steroid hormones may facilitate increased proinflammatory responses during COVID-19. Estrogen and testosterone may act as anti-inflammatory mediators and minimize disease severity. Therefore, the therapeutic efficacy of hormone replacement therapy among COVID-19 patient management must be explored.<sup>33</sup> A higher risk of death from COVID-19 was associated with the male sex. Other risk factors of death in both the sexes included were older age, presence of co-morbidities like cardiovascular disease, cerebrovascular disease, diabetes, chronic kidney disease, hypertension, obesity, smoking, chronic obstructive pulmonary disease, and co-infection with human immunodeficiency virus (HIV) and tuberculosis.<sup>34,35</sup> An assessment of the effect of age and sex on COVID-19 mortality in the European region was recently reported. The results of the study revealed that irrespective of age, the male gender was noted to be at increased risk of death due to COVID-19 than the female counterparts of the same age.<sup>36</sup> COVID-19 is reported to cause venous thromboembolic events that can result in adverse outcomes. The exaggerated coagulopathic state observed in patients can lead to the formation of fibrin clots.

There will be an elevation in the D-dimer level when these clots start to dissolve. It has been already established that an elevated D-dimer level is associated with the disease severity<sup>37</sup> in COVID-19. In a meta-analysis involving 33 970 patients (60.1% men), men were found to experience venous thromboembolic events more than women (1.5-times more) in the case of hospitalized patients<sup>38</sup> with COVID-19. Myocardial injury is another important COVID-19-related adverse event and it is sex-dependent.<sup>39</sup>

#### 4 | INNATE IMMUNE MECHANISM DURING SARS-COV-2 INFECTION

The cells of innate immunity, mainly white blood cells (WBCs), such as basophils, eosinophils, monocytes, mast cells, DCs, natural killer (NK) cells, macrophages, neutrophils, and Langerhans cells, are activated at the site of viral entry. The PRRs present in host cells play a crucial role in initiating an immunological reaction during infection. They are evolutionarily conserved molecules that help in the detection of invading pathogens. They identify molecules, namely damage-associated molecular patterns (DAMPs), released from pathogen-infected, damaged, and dead cells alerting the immunity system of imminent danger to host cells. PRRs are categorized into four major classes, including TLRs, C-type lectin receptors (CLRs), a retinoic acid-inducible gene I (RIG-1)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors. TLRs are single membrane spanned receptors seen on the surface of macrophages, DCs, NK cells, and T and B lymphocytes. TLRs are subdivided into ten types (TLR1 to TLR10). TLRs identify dangerous molecules, such as viral nucleic acids, and proteins present in infected cells activating the innate immunity and DCs, which cause the release of proinflammatory cytokines. If the innate immunity cannot eliminate infection, the adaptive immune responses are stimulated via antigen-presenting cells. The adaptive immune response eliminates infection by activating both cells mediated immunity (CMI) and humoral immunity (HI).<sup>40</sup> RLRs function as intracellular pattern recognition molecules that recognize the virus and facilitate the host immune response. They stimulate the release of inflammatory cytokines, including IFNs, and control the response by phosphorylation or ubiquitination.

NLRs are intracellular sensor molecules that work alongside pathogen-associated-inflammatory response. This causes the release of cytokines, chemokines, cell adhesion molecules, and immunoreceptors during the early response to an infection, activates genes, and enables the adaptive immune response.

The infected host cells trigger the secretion of virus-specific IFNs  $\alpha$  and  $\gamma$  (IFN- $\alpha$  and IFN- $\gamma$ ), various pro-inflammatory cytokines that include interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Interferons induce the adaptive immune response against the virus via stimulating many genes in immune cells. The activated immune cells either destroy infected cells or cause the deactivation of virus particles with antibodies.<sup>41</sup> Stimulation of the IFN- $\alpha$  and IFN- $\gamma$  is critical to restrict the virus proliferation within

the host cells at the initial phase of the infection. IFN- $\gamma$  and IFN- $\alpha$  are incredibly effective in limiting the SARS-CoV-2 spread and can orchestrate innate and adaptive immunity. Viruses, including SARS-CoV, and MERS-CoV employ various mechanisms to attack the immune response governed by type I IFNs. Recently, reports have confirmed the expression of that IFNs during SARS-CoV-2 infection.<sup>42</sup> Furthermore, Signal transducer and activator of transcription 1 (STAT1) translocation following IFN-stimulated genes (ISGs) stimulation have also been determined in the SARS-CoV-infected lungs. In addition to these observations, plasmacytoid DCs (pDCs) also secrete IFNs during SARS-CoV infection indicating the indispensable role of IFNs throughout viral infections. Although the genetic basis of limiting the effect of COVID-19 infection is not entirely understood, ISGs, lymphocyte antigen 6 complex locus E, and IFN-induced transmembrane family (IFITM) proteins may regulate virus-host cell interactions both in favor and against infection.<sup>43</sup>

#### 5 | GENDER VARIATION AND ITS INFLUENCE ON THE IMMUNE RESPONSE

The infectious disease outcome significantly varies between males and females. Females suffer less severe consequences from infection than males due to the strong cellular and humoral immunity. It is also perceived that males are vulnerable to infections and cancers, whereas females are more prone to autoimmune diseases. For example, males exhibit 40% more viral RNA than females in acute HIV infection and show a higher mortality rate in hepatitis infected cases. Moreover, numerous viral, bacterial, and fungal infections lead to gender-biased diseases. Hepatitis, syphilis, influenza, fungal meningitis, and Lyme diseases are commonly occurring in males, whereas tapeworm, onychomycosis, and bacteremia infections are often diagnosed in females.<sup>44</sup> These differences could be due to several factors including gonadal hormones, expression of receptors of gonadal hormones, microbiome, epigenetics, and the X chromosome associated genes. In comparison to males, females appear to have a more robust immunity system compared to their male, which makes them more efficient in clearing the infection. It also makes them more susceptible to autoimmune diseases by aggressively responding to self-antigens.<sup>45</sup>

Many recent reports have demonstrated a clear sex-specific bias for COVID-19 with males more likely to exhibit increased mortality rates compared with female patients with similar ages, ethnic, and social backgrounds.<sup>46,47</sup> The male patients are likely to show more serious manifestations accompanied by a higher fatality rate though it also varies among various countries.<sup>48,49</sup> The highest ratios of COVID-19-associated male/female mortalities have been reported in Greece, Romania, Netherlands, and the Dominican Republic.<sup>49,50</sup> This sex-biased illness severity and mortality might be ascribed to varying immune response, where females mount more robust cell-mediated, innate, and humoral immunity levels than male counterparts.<sup>51</sup> Though females have a more robust immune response following infection with pathogenic microorganisms, the intensified immune response can also trigger immunopathology. A study of 198 COVID-19 patients showed women

had better T cell activation compared to men. In men, age was negatively correlated with poor T cell response, which was associated with adverse disease outcomes. This was not seen in women who fared worse if levels of innate immune cytokines were higher.<sup>28</sup> It is crucial to examine the reasons for gender variation in disease manifestations and outcomes in SARS-CoV-2.

An increase in proinflammatory cytokines level was recorded in the case of MERS and SARS-CoV. SARS-CoV positive individuals showed an increased level of cytokines, including IL-6, IL-12, IL-1B, putative internal head protein 10 (IP10), IFN $\gamma$ , and monocyte chemoattractant protein 1 (MCP1), whereas IL-17, IL-15, TNF- $\alpha$ , and IFN $\gamma$  levels raised in MERS-infected persons. The explicit mechanism of COVID-19 immunopathology remains to be elucidated, and the utmost information is based on MERS and SARS-CoV. Some studies have revealed an increased concentration of TNF- $\alpha$ , IL-2, IL-6, IL-7, IL-10, IFN $\gamma$ , MCP1, granulocyte stimulating factor, and macrophage inflammatory protein 1- $\alpha$  in critical ill COVID-19 patients.<sup>52,53</sup> Among these modulators, IL-10 and IL-6 displayed a positive association with milder COVID-19 cases, whereas a strong correlation was observed between IL-6 and severely infected groups.<sup>53</sup> Therefore, inhibiting the activity of IL-6 has been suggested to decrease the severity<sup>52</sup> of COVID-19, and clinical investigations are underway to scrutinize the therapeutic effectiveness of antibodies against IL-6 or IL-6 receptors.

High mobility group box 1 protein (HMGB1) is another important contributing factor and immunomodulator for sex-biased risk of COVID-19, severity, and mortality. The release of endogenous DAMPs by stressed or infected cells can trigger the immune response by interaction with PRRs.<sup>54,55</sup> A large number of DAMPs, like IL-33, decorin, biglycan, defensin, RNA, DNA, versican, F-actin, fibrinogen, and HMGB1, have been recognized.<sup>54</sup> As the chronic inflammatory disorders led to an increase in HMGB1 level, the severity extent in COVID-19-infected persons with associated inflammatory comorbidities might be linked with HMGB1.<sup>55</sup> Sex-biased differential immune response results in different mechanisms to clear out the injured cells and viral load. Apoptosis is typical in stressed/infected pulmonary endothelial cells in females, whereas males are recognized to undergo necrotic cell death. Physiological conditions such as pregnancy make the women tolerant to the allogenic paternal antigen of the fetus. The fetal cells have been shown to enter the maternal blood and persist for a long time. Thus, women can precisely control the excessive immune activation to foreign antigens, as the virus-associated organ damages during the COVID-19 infection is due to excessive immune activation and inflammation of the individuals' own immune system. The cytokine release syndrome in COVID-19 patients is due to excessive immune activation, leading to tissue and organ damage. It is also known that the bats are able to live with the Coronaviruses by controlling the unwanted inflammatory response in the body and weakened INF activation.<sup>56</sup>

## 5.1 | Effect of the X chromosome on immunity

The X chromosome defines a few immunity-related functions of the cell. It regulates the genes coding for immunity, which are necessary

to synthesize microRNAs (miRNAs).<sup>57</sup> As females carry two X chromosomes and forestall excessive X chromosomal responses, it is inactivated by transcriptional silencing during developmental stages, resulting in cellular mosaicism.<sup>57</sup> Two X chromosomes give women an advantage in mounting more efficient and rapid immune response which may also be related to female hormones. Behavioral differences between women and men also play some role in differential susceptibility and outcomes. Women are less likely to smoke or drink and have a lower burden of chronic diseases such as hypertension; chronic lung diseases hence may have lower mortality due to COVID-19.

## 6 | VITAMIN D AND SEX DIFFERENCES

Among the factors that affect COVID-19 mortality, the vitamin D status of the patient has received a special position due to the role played in modulating the immune system as well as on the renin-angiotensin system.<sup>58</sup> A sex-specific association was previously suggested between the vitamin D status and mortality rate of COVID-19 in the older population.<sup>58</sup> The deficiency of vitamin D is believed to predispose susceptible individuals to infections, including COVID-19. In a recent study conducted among the patients from Switzerland, older males showed a higher susceptibility to vitamin D deficiency than their female counterparts.<sup>59</sup> Vitamin D deficiency can further increase X-chromosome-linked the renin-angiotensin system (RAS) activity, making the male population susceptible to ACE2 receptor dysregulation. This association is believed to improve the COVID-19-associated mortality in males mainly because of the increased susceptibility to COVID-19 induced cytokine storm. Vitamin D has a key role in limiting the rapid increase of circulating pro-inflammatory cytokines, thereby controlling the hyperinflammatory stage/cytokine storm-related to COVID-19.<sup>59</sup> Furthermore, the role played by Vitamin D3 in controlling the differentiation of T regulatory cells is estrogen-dependent. Estrogen was found to enhance the gene expression of vitamin D receptors in CD4+ T cells and at the same time reduce the expression of the cytochrome P450 component of 25-hydroxyvitamin D(3)-24-hydroxylase enzyme, CYP24A1, that inactivates Vitamin D3. The immunomodulatory potential of Vitamin D3 is sex-related, therefore leading to a role in the outcome and lethality of COVID-19. Therefore, vitamin D supplementation can be considered an important strategy to reduce COVID-19 infection.

### 6.1 | Estrogen

The fourth type of estrogen, estetrol, is found only during pregnancy. The molecular structure of estrogen and its various forms is shown in Figure 1. The estrogen activities influence females' health, as noted by the development of fibroids, predisposed to breast cancer when it increases, and susceptible to osteoporosis in case of decreased activities.<sup>60</sup> The role of estrogen during microbial infections has been



recently established.<sup>61</sup> For example, Channappanavar et al.<sup>62</sup> infected both males and females mice with SARS-CoV to investigate the role of sex hormones on the survival rate of animals. Results showed a more severe form of the disease in ovariectomized female mice than that to control. Furthermore, the death number was not increased in castrated male mice due to SARS-CoV infection compared to the control group, indicating a pivotal role of estrogen in the disease onset. Moreover, interference of 17 $\beta$ -estradiol with hepatitis C life cycle suggests a direct inhibitory action of steroid on the virus, rather than only the modulation of host intracellular homeostatic processes. As the sterols can potentially modify virus infection capacity, pretreatment with estrogen is likely to enhance protective actions in acute lung injury due to the anti-inflammatory effects.<sup>63</sup> A previous study suggested that estrogen could lower the activities of CMI, HI, and innate immune cells, like neutrophils and NK cells.

Estrogen was found to inhibit proinflammatory cytokines, like IL-4, IL-10, IL-12, and TGF- $\beta$ , regulate immune cell apoptosis, and enhance the activities of anti-inflammatory cytokines, including NK cells, IL-1, and IL-6. IL-17, IL-23, and TNF- $\alpha$ . The effect of sex hormones on the vulnerability to Zika and herpes simplex virus-2 (HSV-2) infection has previously been explored.<sup>64</sup> Additionally, the immunomodulatory activities of 17 $\beta$ -estradiol and its effect on innate immune responses against bacterial infection were reported. A previous study noted that in females, the replication of influenza A virus within nasal epithelial cells was inhibited compared to that in males. This was supported by the fact that respiratory epithelial cells have receptors for binding estrogen, that is, G-protein-coupled estrogen receptor. These receptors were noted to regulate cell function, signaling pathways, and the inflammatory response.

## 6.2 | Estrogen receptor (ER)

There are two subcategories of ER, ER $\alpha$ , and ER $\beta$ . These are studied for their potential role in immunomodulation. Estrogens receptors present on the immune cells regulate cellular functions of the adaptive and innate immune system and play a noteworthy

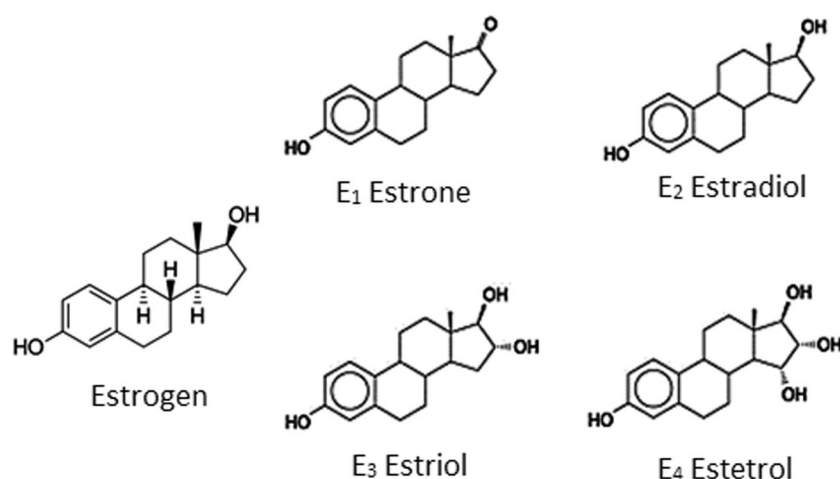
contribution in immune cell development.<sup>65</sup> ER-mediated regulation of immune cells and signaling may have a key role in the gender-based differences in innate and adaptive immune pathways. Most immune cells, including plasma cells, monocytes, macrophages, DCs, T lymphocytes, mast cells, and NK cells, express ER $\alpha$  and ER $\beta$ , which are self-regulated.

## 6.3 | Role of ER receptors

ERs mediate the immunomodulatory effector function through different mechanisms including acting as a ligand-dependent transcription factor for regulating gene expression and promoting epigenetic changes, participating in membrane-initiated steroid signaling to generating rapid responses and dose-dependent effect on innate immune cell function and development.<sup>65</sup> The immune-related functions of ERs are mediated more by the ER- $\alpha$  subset than by ER- $\beta$ . ER- $\alpha$  participates in activating, proliferation, and differentiation of T cells. It contributes to T cell-mediated inflammation and regulates T cell functions. ER  $\alpha$  enhances the proinflammatory responses of TLR and the cytotoxicity of NK cells and ameliorates the activities of TNF- $\alpha$ , IL-1, and -6. ERs can modulate invariant natural killer T cells (iNKT), a type of T cell that causes the death of infected and tumor cells. Experimental studies on mice have shown increased cytokine production in females only in estrogen presence but not in ovariectomized mice and mice supplied with artificial iNKT cell ligands. Thus, estrogen induces proinflammatory cytokines and NK cells that clear abnormal cells and pathogens more efficiently.<sup>66</sup>

## 6.4 | The action of estrogen on T and B cells

T cell-mediated immune responses are necessary to prevent auto-immune disorders and cancers. The estrogen hormone modulates the T cells differentiation, including cytotoxic, helper, and memory cells. It regulates the transcription of the IFN- $\gamma$  gene, which is essential for the release of cytokines, type II IFNs, and other immune cells



**FIGURE 1** The molecular structure of estrogen and its derivatives

participating in adaptive and innate immune responses.<sup>63,67,68</sup> Estrogen upregulates the Th-1 transcription factor T-bet, a key transcriptional factor of type 1 T helper cells (Th1) required to clear pathogens and maintain immunity. It suppresses the microRNA-dependent expression of IFN- $\gamma$ . MicroRNAs are noncoding RNAs that regulate RNA silencing and gene expression. The regulation of microRNA transcription during viral infection correlates with the interferon signaling pathway. Estrogen suppresses host sterol synthesis, especially the mevalonate-isoprenoid branch of cholesterol biosynthesis, required by the virus to survive in the host. It facilitates the expansion of Treg cells, and self-tolerance to antigens and prevents the development of the autoimmune disease.<sup>69,70</sup>

## 6.5 | Estrogen action on B cells

### 6.5.1 | Cluster of differentiation 22 (CD22) gene

Estrogen affects cell differentiation, function, and activity by regulating the CD22 gene belonging to the sialic acid-binding immunoglobulin type lectin (SIGLEC) family. This gene is expressed predominantly on mature B cells and helps to regulate the over-activity of immune cells and prevents autoimmunity.

### 6.5.2 | Shp-1 gene

The gene codes for the Src homology 2 (SH2) domain-containing protein-tyrosine phosphatase, which plays an important role in lymphocyte activation, differentiation, and proliferation. It also regulates B cell receptor (BCR)-mediated signal transduction, which is related to B cell activation. The binding of antigen to the BCR initiates signal transduction, which involves a change in the receptor oligomerization (interaction of more than one polypeptide chain) and other assessment regarding the antigen type and its presentation to the helper T cells.

### 6.5.3 | B cell lymphoma-2 (Bcl-2) gene

Bcl-2 is a gene belonging to the family of Bcl-2 proteins associated with the regulation of apoptosis either by inducing or inhibiting cell death.

### 6.5.4 | Vascular cell adhesion 1 (Vcam-1) gene

This codes for Vcam protein 1 and is also called CD-108. It is an adhesion molecule mediating the adhesion of lymphocytes, eosinophils, monocytes, and basophils to the vascular endothelium. Experimental studies have provided evidence regarding whether estrogen protects isolated primary B cells from B cell receptor-mediated apoptosis and its ability to activate B cells to autoreactivity, making women more prone to autoimmune diseases.<sup>71,72</sup>

## 6.6 | Role of estrogen in ACE2

ACE2 acts as a coreceptor for the SARS-CoV-2 entry into the host cell. ACE is an important enzyme of RAS.<sup>73</sup> Estrogen activity on the ACE receptor may profoundly contribute to the gender-based differences between males and females in the SARS-COV-2 infection and outcome of COVID-19.

## 6.7 | RAS

RAS is a cascade of hormone systems that maintains blood pressure (BP), fluid and electrolyte balance. A drop in renal blood volume or decreased BP stimulates the release of Renin from renal cells (juxtaglomerular cells), which acts on angiotensinogen secreted by hepatic cells. Renin cleaves angiotensinogen into angiotensin I (Ang. I), and further metabolism of these products is executed by ACE.<sup>73</sup>

## 6.8 | ACE

It is secreted by the epithelium of the lungs and kidneys. It may be ACE1 mostly or along with ACE2. ACE1 acts on Ang. I and converts it into angiotensin II (Ang. II), which acts as a vasoconstrictor, raising BP. ACE2 antagonizes the activity of Ang. II by converting Ang. II into angiotensin 1-7, which helps in BP homeostasis and regulates the transport of neutral amino acids (alanine, valine, leucine, etc.) across the membrane by acting as a vasodilator. However, respiratory viruses such as Coronavirus use ACE2 on the membrane as a coreceptor for their entry into the host cell, as they have more affinity to bind to these receptors.<sup>74</sup> Experimental evidence in rats found that ACE2 activity or its levels are lower in females than in male rats.<sup>75</sup> The decreased levels of ACE2 in females are due to estrogen (E2) activity, whose levels frequently change from birth to puberty, menopause, and pregnancy.

## 6.9 | Androgen and progesterone

Progesterone is a hormone produced by the corpus luteum during the menstrual cycle and placenta during pregnancy. It exerts its effects via progesterone receptors, which are seen on NK cells, T cells, macrophages, and DCs. Experimental studies revealed that progesterone relaxes bronchial smooth muscles during lung inflammation, acting as vasodilators, and in allergic inflammation of the lung, progesterone promotes the release of TH1 cells and cytokines, like IL-1 $\beta$ , -4, -5, -6, -10, -22, and TNF- $\alpha$ .<sup>76</sup> Treatment of lung inflammation with progesterone reduced inflammation and restored tissue homeostasis<sup>77</sup> (Figure 2). Progesterone increases Tregs' production that is anti-inflammatory and increases Th17 and CD8+, which protects from an adverse immune response that is fatal for the host.<sup>46</sup> Vitamin D regulates the immune response and modulates IL-6 via progesterone-induced blocking factor. Administration of

exogenous progesterone led to rapid recovery by elevating IL-6 and -22, TGF- $\beta$ , Th17 expressing CD39, and cell multiplication. It also reduced protein leakage in the airway and upregulated AREG in the lungs which improves pulmonary functions. Progesterone-based compounds alter cellular activity and signaling to influence infections outcome in the respiratory tract.

### 6.10 | Action of testosterone associated with immunity

Testosterone is the principal androgen present in males for male traits and reproductive activity. It exerts effects on binding with receptors, forming a complex that then moves into the nucleus, binds with transcriptional regulators on DNA, and controls its activity. Testosterone modulates the functions of lung immune cells, such as decreasing the expression of innate lymphoid cells 2 in males involved in allergic reactions attenuating airway inflammation.<sup>78</sup> Experimental evidence suggests that testosterone binds to T cell receptors and inhibits their differentiation and proliferation. It was found to have no role in B cell differentiation or proliferation. Testosterone inhibits T cell differentiation by activating the protein tyrosine phosphatase nonreceptor type 1 gene. This gene reduces tyrosine kinase 2 phosphorylation that stimulates lymphocytes and T cell differentiation. Thus, testosterone will increase Th1 responses and activate CD8 cells simultaneously. Additionally, it decreases NK cell and TNF- $\alpha$  activities and increases anti-inflammatory cytokines such as IL-10. Th1 mediates cell-mediated immune reactions towards intracellular viruses and bacteria via the secretion of IFNs and IL-2 and IL-10. IL-2 enables cell-mediated immunity through T cells and prevents autoimmune diseases. IL-10 facilitates host defense against pathogens invasion by shielding host tissue damage and maintaining tissue homeostasis.<sup>79,80</sup>

### 6.11 | Role of estrogen in DNA methylation, epigenetic changes, and histone modifications

DNA methylation implicates adding a methyl group at the 5-position of cytosine nucleotides adjacent to guanine, forming CpG islands. It is

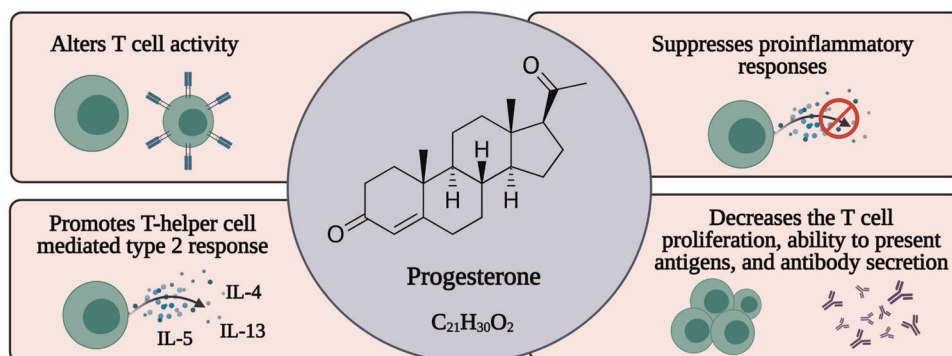
catalyzed by methyltransferase enzymes, and this methylation process of DNA causes a change in histone modification. Histone modification includes acetylation and methylation of histone protein of chromatin catalyzed by histone acetyltransferase and histone methyltransferase. These processes add acetyl or methyl groups to lysine residues (positive charge) of histone tails, changing their charge and subsequent gene activation. It can be demethylated or deacetylated by histone deacetylase or demethylase mask positive charge on histone inactivates gene function.<sup>81,82</sup> Estrogen receptors, especially ER $\alpha$ , directly interact with transcriptional factors involved in histone modification, such as acetylation, phosphorylation, and methylation. Thus, it has a role in these modifications in eliciting an immune response against viral infections. Estrogen receptors cause epigenetic changes in the genome. Epigenetic modifications include DNA methylation and histone modifications. These epigenetic changes are heritable wherein phenotypic changes are inherited by progeny. During such changes, the DNA sequence does not alter, but a difference in the reading frame of the DNA alters its function. Human breast epithelial cells undergo cancerous changes because of estrogen, mediated by ERs, their direct genotoxic effects, and the ability to cause DNA methylation.<sup>83</sup>

## 7 | IMMUNOLOGICAL RESPONSES IN GENDER FEMALE AND MALE

Sex hormones regulate the immune system via receptors present on immune cells, including proliferation, differentiation, and maturation. A review reports that 17 $\beta$ -estradiol (E2) and progesterone are strong immune-modulators, which may reverse the immune dysregulation, leading to the adverse clinical manifestation of cytokine storm<sup>84</sup> in COVID-19. Women exhibit a different immune response to vaccinations and gender-based biological factors are key determinants of susceptibility to infections and clinical outcomes.

### 7.1 | Innate immune responses

The immune response is the body's natural way of eliminating a pathogen and ensures cells' normal function. There are sex



**FIGURE 2** Actions of progesterone related to immunity



differences seen in all classes of vertebrates from insects to lizards and birds to mammals. In all these, there are lowered adaptive and innate immune responses in males compared to females.<sup>85</sup> The difference in the immunity related to innate immune cells among males and females is germline-encoded (a genetic constituent). There is a difference in identifying viruses by pattern recognition molecules by the immunity system of female and male genders. TLR activation, retinoic acid-inducible gene (RIG), and so forth, and release of type I IFNs and related responses were observed more often in females than in males.<sup>86</sup> There is increased cytokine stimulation and production by females than males, governed by gender-specific chromosomal expression. This differential expression of PRRs is important for gender-based responses by innate immune cells. The number and activities of innate immune cells vary between females and males. In males, the frequency of NK cells is higher, whereas the phagocytic activity of macrophages and antigen-presenting cell activity is higher among females.<sup>87</sup> The response of innate lymphoid cells and secretions of cytokines is also superior in females. Dysregulated activity of innate lymphoid cells leads to autoimmune diseases. Notably, females are more vulnerable to autoimmune diseases due to the lower number of these innate lymphoid cells.

## 7.2 | Adaptive immune responses

Adaptive immunity protects against invading pathogens and toxic metabolic byproducts while ensuring the safety of host cells (self). The thymus gland plays a pivotal role in adaptive immunity by producing mature T cells. These T cells constitute helper (cluster of differentiation 4 [CD4]) T cells, cytotoxic (cluster of differentiation 8 [CD8]) T cells, and memory cells. There is a high activity of helper T cells in females, whereas the increased activity of cytotoxic T cells was detected in males. The better fighting capacity against infection in females is due to increased CD4 T cells. In contrast, the ability to kill cancer cells and other invaders is greater among males due to the high activities of CD8 T cells. The greater antibody responses among females are attributed to increased B lymphocyte counts<sup>88</sup> (Figure 3).

## 8 | INFLUENCES OF SEX HORMONES ON LUNG FUNCTION

Gender-based differences in the prevalence of lung disorders, like asthma, cancer, pulmonary arterial hypertension, and obstructive pulmonary diseases have been observed. Several human and animal-based studies have detected substantial differences between males and females in respiratory physiology.<sup>89</sup> The sex hormone such as estrogen and its receptor can influence cellular processes such as proliferation and migration in the lung, which might affect the activity of vascular cells, pulmonary tissue, and immune cells. There is a varied functional difference shown by lung cells related to immunity, like macrophages, DCs,

eosinophils, NK cells, and neutrophils by the actions of sex hormones and it is discussed in detail below.

### 8.1 | Macrophages

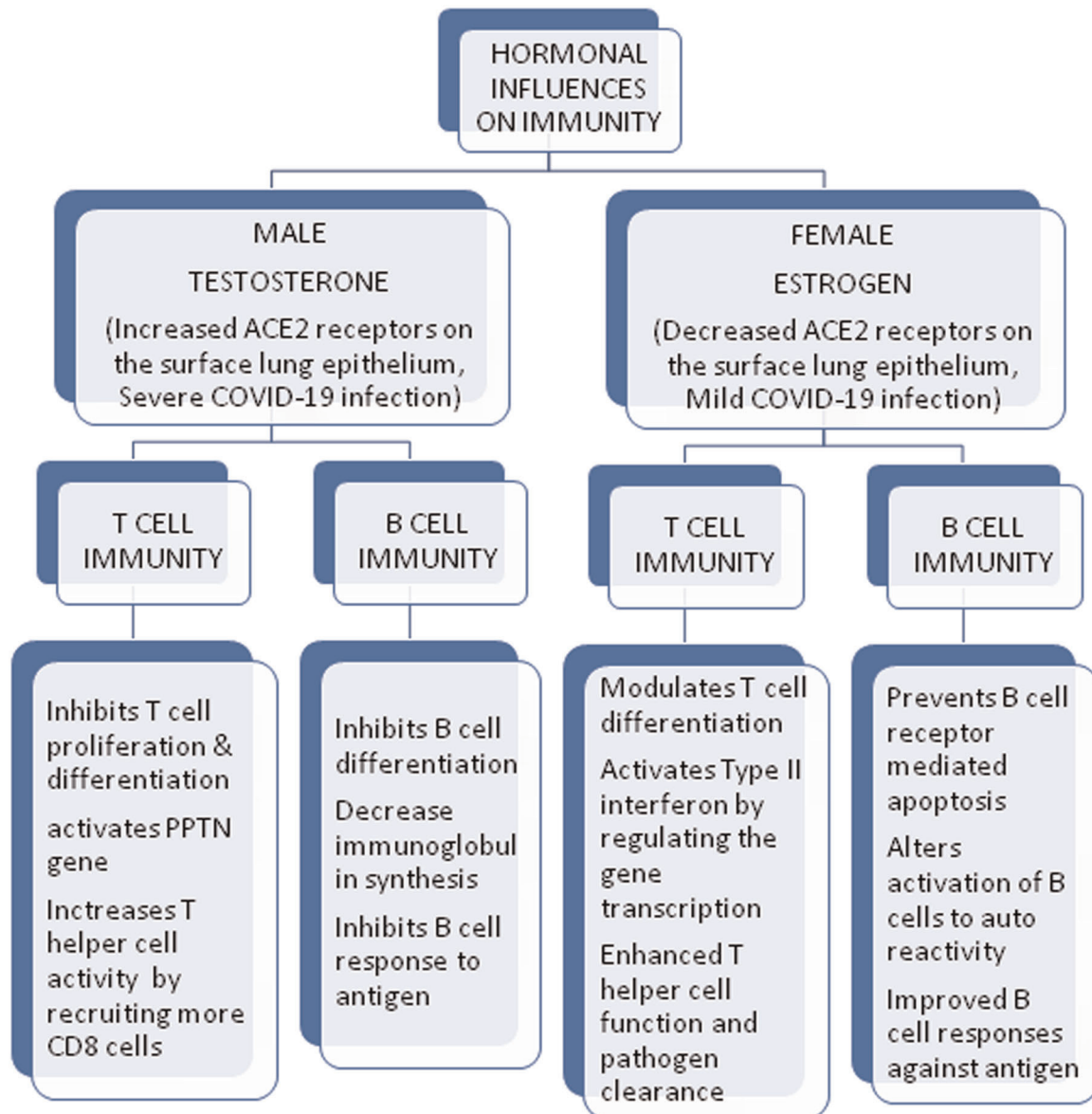
Macrophages of the lung, also called alveolar macrophages, are the first defense system seen in the lower airway tract. They are the mediators of inflammation and tissue remodeling. The female sex hormone estrogen shortens the proinflammatory phase and prolongs the inflammatory phase required for immunomodulation and remodeling.<sup>90</sup> Progesterone inhibits the release of NO from activated macrophages and inhibits tissue injury. The male sex hormone testosterone reduces the formation of the TNF- $\alpha$  in AM and decreases NO.<sup>91</sup>

### 8.2 | Dendritic cells

These are the cells that present antigen material to MHC (major histocompatibility cells) molecules and activate T cells. They also act as a connecting link between adaptive and innate immunity by giving instructions either to Th1 (antiviral) or Th2 (antiparasitic or allergic reaction) or Th17 (autoimmunity or antibacterial) type of response.<sup>92</sup> It elicits a Th1 immune response during viral infection and tries to clear the invading virus, but the respiratory virus can block the Th1 immune response. Mouse studies on DCs revealed that DCs are regulated by epigenetic modification, especially during the inflammatory response against a respiratory virus. The epigenetic modification of the transcriptional regulator H3K4me3 (activator of the release of type 1 IFNs) blocks the Th1 response and activates the Th2 response.<sup>93</sup>

### 8.3 | Eosinophils

They are immune system components involved in allergic airway disease and asthma. The development of asthma has environmental factors and most viral infections. Respiratory viruses trigger asthma during the infection process because these asthmatic individuals have low innate immunity (impaired type I & III IFNs).<sup>94</sup> Eosinophilic cells detect viral particles with the help of PAMPs and become activated. These activated eosinophils present these viral antigens to lymph nodes and activate T cells secreting cytokines, eliciting an inflammatory response.<sup>95</sup> The female hormone estrogen increases the activated eosinophils and inflammatory process compared to the male hormone testosterone.<sup>96</sup> Thus, females are more prone to eosinophilia than males.<sup>97</sup> It also decreases viral multiplication by the secretions of granular components that contain the enzyme NO synthase. NO (nitric oxide) acts against viral particles, inhibiting its replication process in the host.



**FIGURE 3** The hormonal differences between males and females on T and B cells

#### 8.4 | Neutrophils

They act as the front line of the immune system against any infection. During a respiratory viral infection, neutrophils identify viral particles via pattern-associated molecular recognition molecules. Neutrophils reach virus-infected sites and produce proinflammatory mediators (cytokines, chemokines, and IFNs) and toxic substances (peroxides, hydrolytic enzymes, and reactive oxygen species [ROS]) that induce pathological changes in the invaded virus.<sup>98</sup> These inflammatory substances that are released damage the virus's DNA, inhibit virus replication, and propagation or induce trigger apoptosis of virally infected cells, thus halting viral spread.<sup>99</sup> Neutrophils also induce innate immunity of the host, increasing antiviral host defense. Neutrophils in the process of host defense against a virus can also cause collateral damage to the host.<sup>100</sup> However, experimental

evidence suggests that the invaded virus can block the generation of ROS by neutrophils by impeding the chemokines released from virus-infected cells.<sup>101</sup> Females are prone to neutrophilic lung inflammation, as the estrogen hormone recruits more neutrophils at the infection site than males.<sup>102</sup>

#### 8.5 | Natural killer cells

They are components of the immune system that eliminate virus- or virus-infected cells or tumor cells. They are cytotoxic in nature and release interferons at a preliminary stage, sensing the presence of infection.<sup>103</sup> NK cells kill virally infected cells or viruses either by producing cytokines or by secreting cytolytic granules or death receptor-mediated cytotoxicity.<sup>104</sup> Activated NK cells secrete

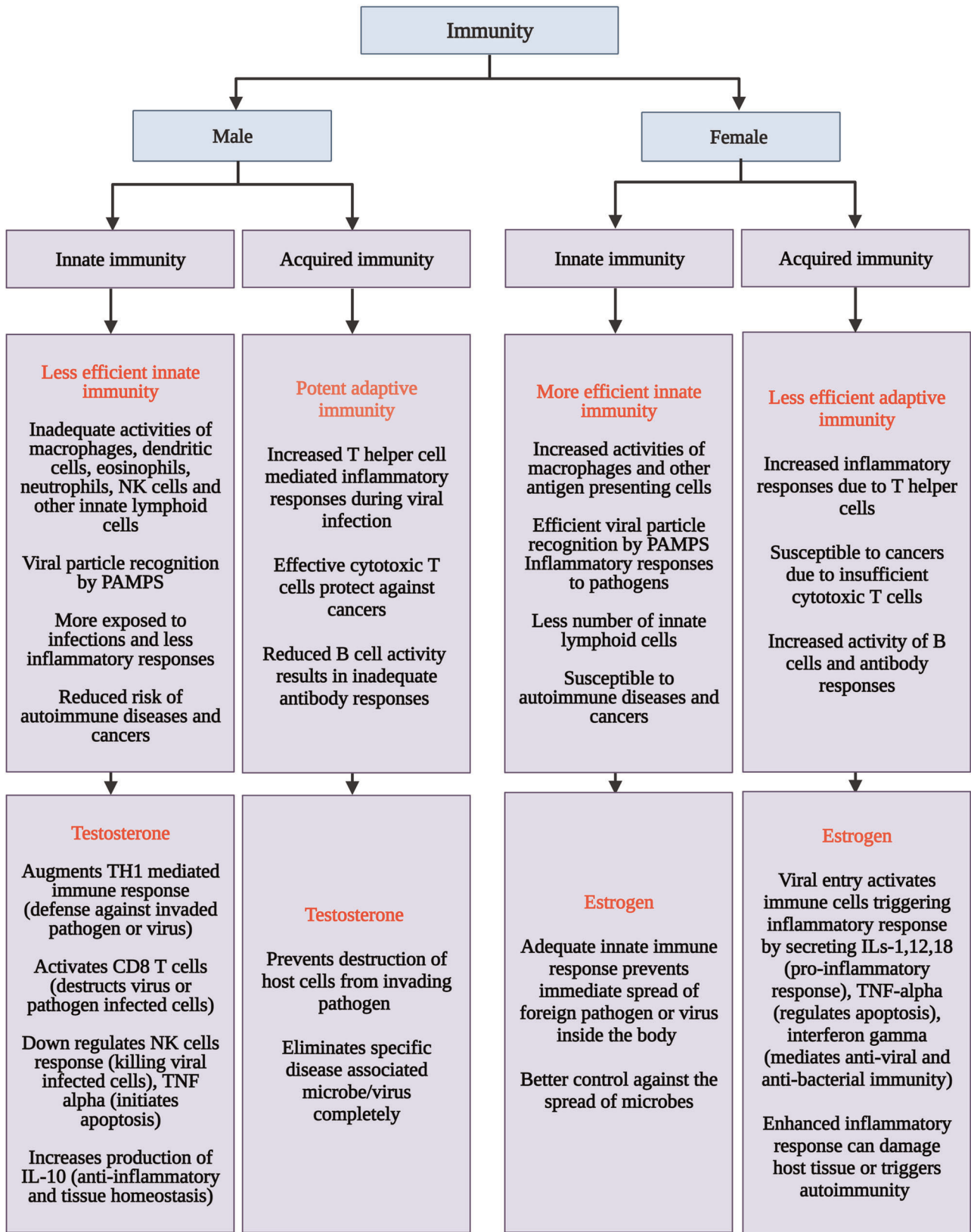


FIGURE 4 Flow chart of the differential activity of immunity, and hormonal influences in males and females

interferon-gamma, making the virus less sustainable in the host and protecting the cells from viral infection acting at distant sites.<sup>105</sup> However, viruses have multiple mechanisms to inhibit the NK cells functioning by downregulating cytotoxic function or by triggering apoptosis, thereby decreasing the number of NK cell activities.<sup>106</sup> Recent reports have shown NK cell-based therapies to protect against SARS-CoV-2 infection.<sup>107</sup> Due to the increased activities of NK cells in males compared to females, there is an elevated cytotoxic response in males<sup>108</sup> (Figure 4).

## 9 | CONCLUSION AND FUTURE PROSPECTS

Several reports have shown high mortality among men than women due to the ongoing pandemic COVID-19, and men are more likely to be affected by the COVID-19. Despite the men engaging in high-risk behaviors, such as smoking and ignoring social distancing, it appears that there is a significant gender-based difference between women and men in the immune system, susceptibility to COVID-19 infection, pathobiology of the disease, and disease outcome. Factors, like sex hormones, sex hormone receptor expression, ACE-2 receptor expression also contribute to the gender-based differences at the cellular and molecular level. Sex hormones such as estrogen, testosterone, progesterone, and so forth, play a key role in immune responses during infections. On the basis of the available literature, females appear to be better equipped to counter invading respiratory viral pathogens, including the novel SARS-CoV-2, than males.

Estrogen seems to regulate innate and adaptive immunity mechanisms, thereby influencing the activities of both cellular and humoral immune responses. ER expressed on immune cells also affects the effector functionalities of immune cells and their development. ACE2 receptors and their expression on various human cells are regulated by estrogen, which might contribute to the gender-based differences between males and females on vulnerability to COVID-19 infection and the disease outcome. Other sex hormones, like testosterone and progesterone, also significantly influence immune systems during infections. The differential mortality and morbidity rates due to COVID-19 in females and males may be because the sex hormones affect the immune responses during SARS-CoV-2 infection. Further studies in this regard are warranted to improve understanding regarding the influences of sex hormones and gender bias on the immunological responses during microbial infections and their impact on vaccine development.

### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

### AUTHOR CONTRIBUTIONS

Sabitha Vadakedath proposed the idea and provided an initial draft. Venkataramana Kandi provided the title, performed review of the

literature, and revised the manuscript. Ranjan K. Mohapatra participated in preparing the draft. Sabitha Vadakedath, Venkataramana Kandi, Ranjan K. Mohapatra, Venkata B. K. Pinnelli, Richa R. Yegurla, Praveen R. Shahapur, and Vikram Godishala performed the revision of the manuscript. Senthilkumar Natesan, Kranti S. Vora, Khan Sharun, Ruchi Tiwari, Muhammad Bilal, and Kuldeep Dhama updated and edited the review. All the authors were involved in the writing and proofreading of the manuscript. All authors have read and approved the final manuscript.

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