Received: 30 April 2021 | Revised: 17 August 2021 | Accepted: 18 August 2021

DOI: 10.1002/rmv.2290

REVIEW

WILEY

Crosstalk between estrogen, dendritic cells, and SARS-CoV-2 infection

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Funding information

Agência Nacional de Inovação; European Regional Development Fund, Grant/Award Number: POCI-01-02B7-FEDER-069745; Programa Operacional Temático Factores de Competitividade

Abstract

The novel coronavirus disease 2019 (Covid-19) first appeared in Wuhan and has so far killed more than four million people worldwide. Men are more affected than women by Covid-19, but the cellular and molecular mechanisms behind these differences are largely unknown. One plausible explanation is that differences in sex hormones could partially account for this distinct prevalence in both sexes. Accordingly, several papers have reported a protective role of 17β-estradiol during Covid-19, which might help explain why women appear less likely to die from Covid-19 than men. 17β-estradiol is the predominant and most biologically active endogenous estrogen, which signals through estrogen receptor α, estrogen receptor β, and G protein-coupled estrogen receptor 1. These receptors are expressed in mature cells from the innate and the adaptive immune system, particularly on dendritic cells (DCs), suggesting that estrogens could modulate their effector functions. DCs are the most specialized and proficient antigen-presenting cells, acting at the interface of innate and adaptive immunity with a powerful capacity to prime antigen-specific naive CD8+ T cells. DCs are richly abundant in the lung where they respond to viral infection. A relative increase of mature DCs in bronchoalveolar lavage fluids from Covid-19 patients has already been reported. Here we will describe how SARS-CoV-2 acts on DCs, the role of estrogen on DC immunobiology, summarise the impact of sex hormones on the immune response against Covid-19, and explore clinical trials regarding Covid-19

KEYWORDS

dendritic cells, estrogen, SARS-CoV-2

Abbreviations: ACE2, angiotensin-converting enzyme 2; AP-1, activator protein 1; APCs, antigen presenting cells; AR, androgen receptor; ATF-2, activation transcription factor 2; catl, gonadotropin-realizing hormone; cDCs, conventional DCs; Cat, cathepsin; CLRs, C-type lectin receptors; Covid-19, 2019 novel coronavirus disease; CoVs, coronaviruses; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DNA, deoxyribonucleic acid; E2, 17β-estradiol; ERs, estrogen receptors; EREs, estrogen-response elements; Flt3L, Fms-like tyrosine kinase 3 ligand; FSH, follicle-stimulating hormone; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPER, G-protein-coupled estrogen receptor; HSCs, hematopoietic stem cells; IFN, interferon; IL, interleukin; IP-10, interferon-gamma induced protein 10; IRAK, interleukin-1-receptor- associated kinases; IRF, interferon regulatory factor; ISGs, IFN-stimulated genes; JAK-STAT, janus-kinase/signal transducer and activator of transcription; LBD, ligand-binding domain; LH, luteinizing hormone; moDCs, monocyte-derived DCs; mRNA, messenger RNA; MDPs, macrophage-DC progenitors; MAPKs, mitogen-activated protein kinases; MCP-1, monocyte chemoattractant protein-1; MDMs, monocyte-derived macrophages; MERS-CoV, middle east respiratory syndrome coronavirus; MHC, major histocompatibility complex; MIP-1a, macrophage inflammatory protein-1 alpha; MyD88, myeloid differentiation primary response gene 88; NEMO, NFκB essential modulator; NF-kB, nuclear factor kappa-light- chain-enhancer of activated B cells; NOD-like, nucleotide-binding oligomerization-domain; pDCs, plasmacytoid DCs; PAMPs, pathogen-associated molecular patterns; PAR, protease-activated receptors; PAR-1, protease-activated receptors-1; PC1, prohormone convertase; PKR, protein kinase R; PRRs, pattern recognition receptors; RBD, receptor binding domain; RIG, retinoic acid-inducible gene-I; RNA, ribonucleic acid; ssRNA, single-stranded RNA; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SP1, specificity protein 1; T reg cells, T regulatory cells; Th, T helper; TLR, toll-like receptor; TMPRSS2, transmembrane serine protease 2; TNF- α, tumor necrosis factor-α; TRAF6, tumor necrosis factor receptor-associated factor 6.

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1 | INTRODUCTION

The novel coronavirus disease 2019 (Covid-19) was first recognized in late 2019 in Wuhan, China as caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has become the biggest pandemic of this century.[1] The morbidity and mortality of Covid-19 have not decreased despite our efforts to prevent and treat the disease, accumulating more than 186 million people infected and more than four million deaths until now.[2] Epidemiological data shows a higher mortality rate in men over women.[3] Several reports clearly demonstrate that female patients have a better prognosis than males, most probably due to differences in the levels of sex hormones, namely in 17β-estradiol.[4] Indeed, the hormonal fluctuations during physiological changes in premenopausal women warrant their stronger immune protection.[5] Estrogen exerts its effects through the estrogen receptors (ERs) ERα, Erβ, and G-protein-coupled estrogen receptor (GPER) 1, which are widely expressed in most tissues, including in mature cells from the innate immune system, such as dendritic cells (DCs), and the adaptive immune system,[6-8] suggesting that estrogens could have a relevant role in the response mediated by the immune system against several pathogenic agents, including SARS-CoV-2.[9] DCs play a key role in generating a robust immune response, as they are the most powerful antigen-presenting cells (APCs) with the ability to stimulate the activation of T and B lymphocytes, providing a crucial link between innate and adaptative immunity. Typically, DCs are found in an immature state at areas of the body that are close to the outside environment, which includes skin, and mucosa of respiratory and gastrointestinal tracts. Upon exposure to an infectious agent, DCs capture and process it, displaying the resulting antigens on major histocompatibility complex (MHC)-I or MHC-II molecules. Simultaneously, DCs start to mature and migrate towards the draining lymph nodes where they present the processed antigens to naïve T cells, initiating a specific immune response.[10] Nevertheless, if the innate and adaptive immune responses are dysregulated and pro-inflammatory cytokine production becomes uncontrolled, this cytokine storm[11] leads to infection exacerbation, extrapulmonary respiratory failure, or death. Thus, estrogens can play a key role in modulating the immune response during SARS-CoV-2 infection, keeping the balance between pro-inflammatory and anti-inflammatory responses.

This review surveys the state of the knowledge regarding estrogen-dependent regulation of DCs biology and its impact upon SARS-CoV-2 progression. Finally, we will display some clinical trials whose purpose is to understand whether the drugs or drug associations which interference with the signaling pathways triggered by sex hormones are beneficial for Covid-19 patients.

2 | SARS-CoV-2

Covid-19, which can drive in highly infectious pneumonia, was first reported in Wuhan, Hubei Province, China, in December 2019[12] and was latter recognized as a pandemic by the World Health Organization. Covid-19 is triggered by SARS-CoV-2, a designation assigned by the Coronavirus study group of the International Committee on Taxonomy of Viruses.[13]

Coronaviruses (CoVs) belong to the *Coronaviridae* family and *Orthocoronavirinae* subfamily. This subfamily is genotypically and serologically divided into four genera, the α, β, ɣ, and δ coronaviruses. SARS-CoV-2 is a β-coronavirus, enveloped, with a nonsegmented positive-sense single-stranded ribonucleic acid (RNA) genome. CoV genome is the largest among all the RNA viruses and codes for at least four major proteins: spike (S), envelope (E), membrane (M), nucleocapsid (N), and other accessory proteins that help the replicative process and facilitate entry into host cells (Figure 1a).[14]

CoVs originally transmitted from animals to humans are transferred from one individual to another through the kind of aerosolization caused by coughing or sneezing to reach the respiratory tract.[15] Bats and rodents represent the common reservoir hosts for α and β-CoVs, compared to birds for the ɣ and δ genera. However, this type of virus is able to jump from its physiological reservoirs to other animals, representing an example of virus evolution with high genetic variability and great potential for recombination. These features are expected to change the biological characteristics of the virus and are important factors predisposing to novel pandemics.[16]

Crucial steps of the invasion of the host cell by SARS-CoV-2 are the identification of target cells, S protein cleavage, and entry into the host cell. In brief, S protein contains two major functional domains, the *N*-terminal region (named S1) and a *C*-terminal region (named S2). S1 includes a receptor-binding domain (RBD) that recognizes and binds to its target cell surface receptor, namely, angiotensin-converting enzyme 2 (ACE2). S2 is crucial to the fusion between the virus envelope and the membrane of the target cell, allowing virus genetic material entry. After binding to ACE2, an initial cleavage of S protein at the S1/S2 site is required. This S2′cleavage site can be recognized and cut by the host transmembrane serine protease 2 (TMPRSS2), as well as by prohormone convertase 1 (PC1), trypsin-like proteases and cathepsins.[17] The proteolysis produces a mature S2 fusion protein, which allows virus entry into host cells.[18] This pre-activation of the S protein can also be made by furin, which is ubiquitously expressed (except in muscle cells, where furin is expressed in a low level) and localized at the cell surface and in intracellular compartments, being able to process both cytosolic and extracellular substrates.[19] The abundant intracellular furin suggests that infected cells might release pre-activated viruses, that can be fused without an ACE2 interaction, increasing virus transmissibility.[18] Type II alveolar cells express ACE2, allowing the infection by SARS-CoV-2. After entry into the host cell, viral positive-sense RNA links to cellular ribosomes and uses cell machinery to produce new viral proteins and genomes, and, ultimately, progeny virions.[20] Subsequently, the host cell releases inflammatory mediators that activate alveolar macrophages to release cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α). The excess cytokines reach the hypothalamus and direct it to increase the body temperature (fever).[21] Additionally, this cytokine burst destroys the endothelial layer inside the capillary around the alveolus, promoting vessel dilation and capillary permeability. As a result,

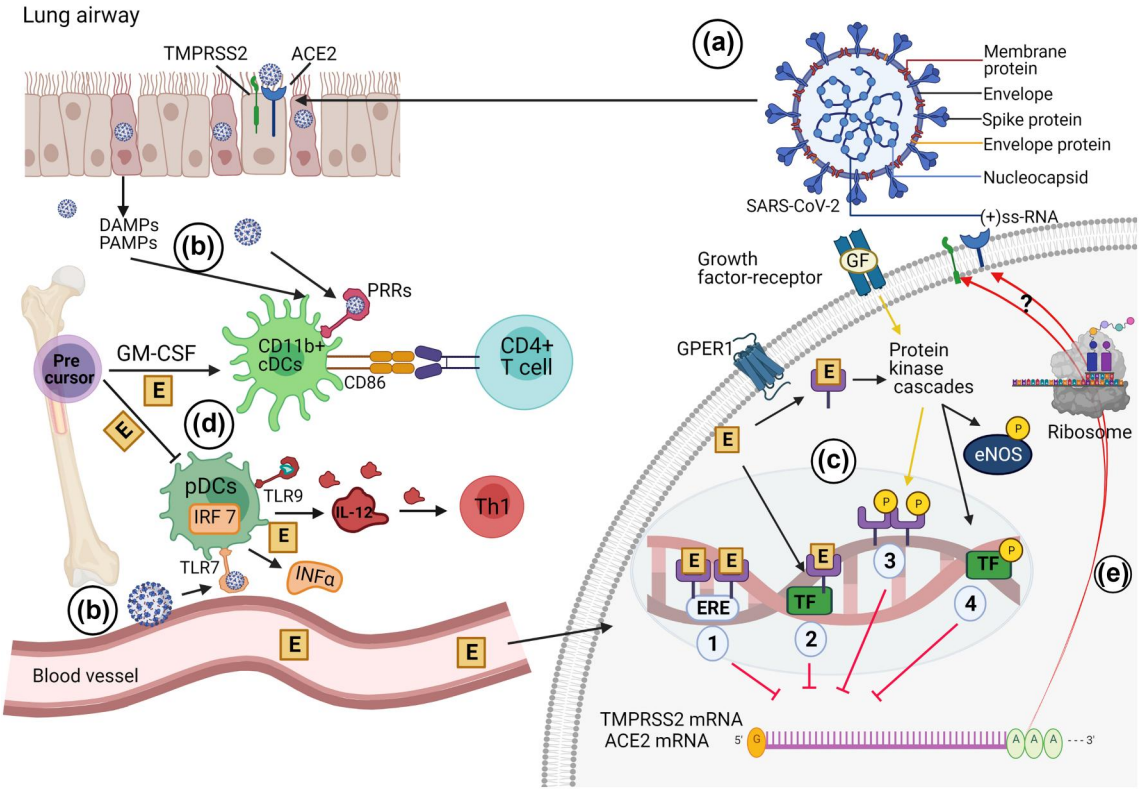

FIGURE 1 (a) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) structure. SARS-CoV-2 is a virus enveloped with a nonsegmented positive-sense single-stranded ribonucleic acid ((+)ss-RNA) genome. Their genome codes for four major proteins: spike protein, envelope protein, membrane protein, nucleocapsid, and accessory proteins.[11] (b). How SARS-CoV-2 acts on dendritic cells. When SARS-CoV-2 infects the respiratory tract, the host innate immune system detects viral infection by using pattern recognition receptors, including toll-like receptor (TLR), to recognize damage-associated molecular patterns and pathogen-associated molecular patterns, like-proteins, lipoproteins, and nucleic acids of viral origin.[30] The plasmacytoid dendritic cells (pDCs) could recognize ss-RNA viruses via TLR7.[31] Subsequently the interferon regulatory factor (IRF)-7 is activated to induce the production of pro-inflammatory cytokines, such as interferon (INF)-α.[32] (c). Estrogen receptors (ERs). Following interaction with its ligand (E), the ER can modulate cellular function through nuclear genomic (1,2,3) or non-genomic (4) mechanisms. (1) Nuclear E-ER binds directly to estrogen receptor element in target gene promoters. (2) Nuclear E-ER is tethered through protein-protein interactions to a transcription factor complex (TF) that contacts the target gene promoter. (3) Growth factors activate protein-kinase cascades, leading to phosphorylation (P) and activation of nuclear ER at the target gene. (4) Membrane E-ER complexes activate protein-kinase cascades, leading to altered functions of proteins in the cytoplasm (e.g., activation of eNOS) or the regulation of gene expression through phosphorylation (P) and activation of a TF.[55] (d). How estrogen acts on dendritic cells. The culture of bone marrow cells in the presence of granulocyte-macrophage colony-stimulating factor, has shown the crucial role of estrogen (E) in the culture medium in promoting the development of $CD103^+$ cDCs and $CD11b^+$ cDCs.[59] The $CD11b^+$ cDC subset displayed higher levels of cell surface CD86, exhibiting superior ability to induce the proliferation of naive $CD4^+$ T cells.[62] However, estrogen decreases the absolute number of pDCs, led to a more mature phenotype development and an enhanced capacity to produce interleukin (IL)-12 in response to TLR9 stimulation.[65] Subsequently, the high levels of IL-12 induce the differentiation of Th1 cells.[66] The higher levels of estrogen also enhance the TLR7-dependent production of IFN-α by pDCs, increasing the immune response against the virus.[63,64] (e). The effect of 17β-estradiol levels on angiotensin-converting enzyme (ACE)2 and transmembrane serine protease (TMPRSS)2 expression. Pre-treatment of the VERO E6 cell line with 17β-estradiol showed that estrogen significantly downregulated TMPRSS2 messenger RNA (mRNA) expression.[6] Similarly, normal human bronchial epithelial cells pre-treated with 17β-estradiol expressed lower levels of ACE2 mRNA.[73] In spite of the downregulation of TMPRSS2 mRNA and ACE2 mRNA, it might not translate into a reduction of protein expression at the cell surface (red arrow). Created with BioRender.com

the alveolar edema reduces the production of surfactants, leading to alveolar collapse and impaired gas exchange mechanism. Consequently, the contraction of bronchial smooth muscle induces or enhances cough sensitivity. On the other hand, the inflammatory mediators, such as prostaglandin and bradykinin, also promote the cough reflex by sensitizing cough receptors.[22]

If the lung inflammation is severe, it can stimulate a systemic inflammatory response, increasing vessel permeability. As a result, the plasma fluid leaks into tissue space, decreasing blood volume, which may eventually reduce the perfusion of the organs and lead to multiple organ failure.[23]

3 | DENDRITIC CELLS

DCs play a key role in generating a robust immune response, as they are the most powerful APCs with strong migration ability. Mature DCs can stimulate the activation of T and B lymphocytes, providing a

crucial link between innate and adaptative immunity. They are able to recognize, capture, process, present antigens, and produce cytokines in the presence of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs).[24] PAMPs are recognized by pattern recognition receptors (PRRs), for instance, Toll-like receptors (TLRs), nucleotide-binding oligomerization-domain (NOD-like) receptors, C-type lectin receptors (CLRs), protein kinase R (PKR), and retinoic acid-inducible gene-I (RIG-I)-like helicase.[10]

DCs originate from $CD34^+$ hematopoietic stem cells (HSCs) in the bone marrow and the generation of most DC subsets is controlled by cytokine Fms-like tyrosine kinase 3 ligand (Flt3L), whereas during physiological stress granulocyte-macrophage colony-stimulating factor (GM-CSF) mobilizes and stimulates the production of monocyte-derived DCs (moDCs). Human DCs can be divided into plasmacytoid DCs (pDCs) and conventional/myeloid DCs (cDCs). pDCs are able to produce type I interferons (IFN) upon viral infection, however, in their immature state, they may be involved in immune suppression. cDCs can be also subdivided according to their location: (i) lymphoid organ-resident DCs, (ii) peripheral tissue-resident DCs, and (iii) circulating DCs. Immature DCs are located at body surfaces with potential antigen entry, such as the skin and mucosal at the respiratory, genitourinary, and gastrointestinal systems.[10] During maturation, DCs migrate to lymphoid tissues where they can activate naive B and T lymphocytes, the latter through antigen presentation by peptide-MHC complexes on the surface of DCs. DCs may also interact with cells of the innate immune system, such as macrophages, natural killer cells, and mast cells, thus modulating the global immune response.[24] When the maturation process of DCs is blocked, it directly affects the initiation of the adaptative immune response and, consequently, pathogen clearance.

DCs, along with the alveolar macrophages, constitute the first line of sentinel cells in the innate immune response against respiratory viral infection. In steady state, lung DCs of mice can be subdivided into $CD11c^{hi}$ $CD103^+$ cDCs (human cDC1 subset) that belong to the CD8a type cDCs, $CD11c^{hi}$ $CD11b^+$ cDCs (human cDC2 subset), and $CD11c^{dim}$ pDCs (human pDCs subset). During inflammation, moDCs are recruited to the lung and some of $CD11c^{hi}$ $CD11b^+$ cDCs can acquire a $CD103^+$ $CD11b^+$ phenotype. $CD103^+$ $CD11b^+$ cDCs migrate from the intraepithelial base to the draining mediastinal lymph nodes to primarily induce the $CD8^+$ T cell immune response against respiratory viruses.[25]

4 | HOW SARS CoV-2 ACTS ON DENDRITIC CELLS

Due to the recentness of the SARS-CoV-2, many reports have turned to accumulated evidence on previous coronaviruses. Taking this into account, the data herein described crosses the information available from the SARS-CoV and Middle East Respiratory syndrome coronavirus (MERS-CoV) to fill the knowledge gap on the new SARS-CoV-2.[26] When SARS-CoV-2 reaches the respiratory tract, as stated previously, it enters into cells expressing ACE2.[17] As DCs in intra-alveolar septa of the lung express ACE2, it is plausible to speculate that these immune sentinels can be infected by SARS-CoV-2.[27] In addition to the recognition receptors, DCs also express attachment receptors, such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), which augments infection by SARS-CoV of already permissive cells, that is cells that express ACE2.[28] In fact, Chan et al. reported that patients with reduced DC-SIGN expression had a lower risk of having severe SARS-CoV infection.[29] The host innate immune system detects viral infection by using PRRs, including TLR, to recognize PAMPs like-proteins, lipoproteins, and nucleic acids of viral origin.[30] For example, Lee et al. demonstrated that pDCs could recognize single-stranded RNA viruses via TLR7 upon transport of cytosolic viral replication particles into lysosomes through autophagy (Figure 1b).[31] Additionally, upon infection of APCs by SARS-CoV-2, including pDCs and macrophages, several transcription factors, such as interferon regulatory factor (IRF), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mitogen-activated protein kinases (MAPKs), are activated to produce pro-inflammatory cytokines.[32] Typically, during a viral infection, TLR7 recognizes single-stranded RNA through the myeloid differentiation primary response gene 88 (MyD88) pathway. MyD88 forms a complex with interleukin-1-receptor-associated kinases (IRAK)-1 and IRAK-4 and tumor necrosis factor receptor-associated-factor 6 (TRAF6), which can activate transcription factor IRF7. The activated IRF7 is then translocated to the nucleus and promotes the synthesis of type I IFN. Type I IFN subsequently activates the downstream Janus kinase/signal transducer and activator of transcription (JAK-STAT) signal pathway, promoting the expression of IFN-stimulated genes (ISGs). ISGs restrict viral replication and induce apoptosis to protect the host cells from virus spread.[33]

However, CoVs have been developing strategies to escape the host immune response, namely those involving DCs.[34,35] Cong et al. observed that immature moDCs were permissive for MERS-CoV, whereas mature moDCs were not, without upregulation of pro-inflammatory cytokines and chemokines.[36] Taking into account that the maturation state of DCs is required to activate T cells,[37] infection of immature moDCs may impair the adaptive immunity against the virus. In contrast to MERS-CoV, the infection of moDCs by SARS-CoV is abortive,[38] which may contribute to enhanced viremia and pro-inflammatory response verified in severe cases of SARS-CoV.[39,40]

DCs infected by both SARS-CoV and MERS-CoV are unable to stimulate the expression of anti-viral cytokines (IFN α and IFN β), inducing comparable levels of TNF α and IL-6. MERS-CoV induces higher expression of IL-12, IFNγ, interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1a), and IL-8 than SARS-CoV.[41,42] Law et al. demonstrated that SARS-CoV-infected DCs display a lack of IL-12 production that compromises the differentiation of $CD4^+$ T cells into T helper (Th)1 cells thus impacting cell-mediated immunity.[41]

Yang et al.[43] reported that moDCs and monocyte-derived macrophages (MDMs) support SARS-CoV-2 protein production but did not efficiently support virus replication and generation of infectious virus progenies.[43] Furthermore, SARS-CoV-2 did not activate any IFN gene upregulation in both infected moDCs and MDMs. Although SARS-CoV-2 triggers pro-inflammatory cytokines expression from infected MDMs, it did not activate the expression of these genes in infected moDCs with the exception of IP-10. Despite successful virus entry and protein production, the infection mitigates the extent of signal transducer and activator of transcription 1 (STAT1) phosphorylation in moDCs, whereas it did not have a modulatory effect in MDMs. Thereby, SARS-CoV-2 destabilises IFN signaling in moDCs through antagonizing STAT1 phosphorylation, suggesting potential manipulation of the IFN signaling pathways by the virus, which might delay viral clearance thus contributing to Covid-19 pathogenesis.[43]

In a case report of a non-severe Covid-19 patient, blood $CD14^+$ $CD16^+$ monocytes showed lower levels on days 7, 8, and 9 when compared to those of healthy donors, which may reflect the migration of these cells into the site of infection.[44] According to Ancuta et al. $CD14^+CD16^+$ cells differentiated in vitro from peripheral blood monocytes display DCs characteristics,[45] corroborating the critical role of DCs in the immune response against SARS-CoV-2.

A recent study evaluated the proportion and functionality of cDC and pDC subsets derived from acute and convalescent Covid-19 patients and healthy donors.[46] The convalescent patients showed an increased frequency of $CD11c^+$ cDCs in total DCs. Furthermore, the cDC:pDC ratio in acute patients was higher (especially in severe cases) in comparison with those in the healthy donors and convalescent patients, suggesting a link between the reduction in pDCs and poor outcomes.[46] Both acute and convalescent patients presented a significant decrease in expression of the co-stimulatory molecule CD86, which is also related to cDCs maturation.[46]

These findings suggest that SARS-CoV-2 negatively impacts DCs numbers and functions, which may explain the worst Covid-19 outcomes.[43] Therefore, the knowledge of the mechanisms through which SARS-CoV-2 mitigates DCs function is of paramount importance to identify new molecular targets for disease treatment. Accordingly, the Aivita Biomedical Company, focused on the design of personalized vaccines, and has recently started an adaptive Phase I-II trial (NCT04386252) of a vaccine consisting of autologous DCs loaded ex vivo with SARS-CoV-2 S protein, with or without GM-CSF, to prevent Covid-19 in adults. Nevertheless, there is no more information about the results of this trial.[47]

5 | ESTROGEN RECEPTORS

As previously mentioned, there are two classical nuclear ERs: ERα and ERβ, and one transmembrane ER, GPER1, which are encoded by *ESR1* on chromosome 6, *ESR2* on chromosome 14, and *GPER* on chromosome 7, respectively.[6,48] ERα is mainly expressed in immune cells, reproductive tissues, breast, bone, kidney, liver, and white adipose tissue, while ERβ is found in the immune cells, lung, central nervous system, cardiovascular system, ovary, prostate, male reproductive organs, colon, and kidney.[48] GPER1 is also expressed in the immune, central nervous, reproductive, renal and cardiovascular systems.[49] ER activity is regulated by endogenous estrogens and they are subdivided into four types: estrone (E1), 17β-estradiol (E2), estriol (E3), and estetrol (E4). The E2 is the predominant and most biologically active estrogen.[50] ERα and ERβ share five structural and functional domains: two transcriptional activation function domains, a deoxyribonucleic acid (DNA)-binding domain, a hinge domain, and a ligand-binding domain (LBD).[51] Following interaction with its ligand, ERα and ERβ can modulate cellular function through nuclear genomic or non-genomic mechanisms. The genomic mechanism involves the direct or indirect binding of the estrogen receptor to transcriptional control regions of targeted genes, while the non-genomic mechanism, initiated by receptors localized to caveolae in the plasma membrane, signal through kinase pathways.[52] Of note, the palmitoylation of ERα cysteine 447 is critical for directing ERα to the plasma membrane through physical interactions with caveolin-1.[53] Occupation of the LBD results in a conformational change in the receptor, driving it to an activated state. Subsequently the receptor allows or prevents interaction with coactivators if the ligand is an agonist or an antagonist, respectively.[54] The transcriptional responses may follow a classical pathway where ligand-activated ERs can interact with estrogen-response elements (EREs) found in the promoters of target genes. ERs may also follow a tethered signaling pathway, interacting with other transcription factor complexes and binding to non-ERE sequences (Figure 1c).[50] The transcription factor complexes include activator protein 1 (AP-1), STATs, activation transcription factor 2 (ATF-2), NF-κB, specificity protein 1 (SP1), which are bound to their cognate DNA binding sites.[55] The genes regulated by ERα are distinct from those regulated by ERβ in response to estrogens, which act as agonists in all tissues, even though they can produce opposite effects. Tee et al. reported that only 38 of the 228 (17%) genes are regulated by both ERα and ERβ with E2. Furthermore, they verified that the regulation of some gene expression by E2 was dose dependent.[56] The recruitment of different coregulatory proteins (such as co-activators, chromatin modulators, and basal transcription factors) to EREs also impacts gene transcription.[50,56] Different types of EREs in target promoters, the differential utilization of coregulators, and the relative expression of ERα and ERβ in different cell types justify the complexity of ER-mediated gene transcription.[56]

GPER1 is a 7-transmembrane G protein-coupled receptor which mediates both rapid genomic and nongenomic transcriptional responses estrogen-dependent, such as activation of adenylyl cyclase and transactivation of epidermal growth factor receptor.[49,57] Interestingly, this receptor showed to have an impact on the expression of IL-6, once their blockage leads to IL-6 expression decrease.[8]

6 | HOW ESTROGEN ACTS ON DENDRITIC CELLS

Estrogens exert their effects through ERs which are expressed in mature cells from the innate and the adaptive immune system,[7] namely DCs, suggesting that estrogens could modulate their effector functions.[9]

moDC express ER transcripts, however, B cells had the highest levels of *ESR1* messenger RNA (mRNA). In addition, B cells and pDCs expressed the highest levels of *ESR2* mRNA when compared with any other immune cell type.[58]

The culture of bone marrow cells or highly purified progenitors, such as macrophage-DC progenitors (MDPs), in the presence of GM-CSF, has shown the crucial role of E2 in the culture medium in promoting the development of $CD11c^{hi}$ $CD103^{+}$ cDCs and $CD11c^{hi}$ $CD11b^{+}$ cDCs.[59] Indeed, ERα-signaling controls the level of IRF4 in GM-CSF-stimulated MDPs, and thus promotes efficient development of the IRF4- dependent $CD11c^{hi}$ $CD11b^{+}$ cDCs subset.[60] This cell subset, present in the gut, lung and skin is essential in driving $CD4^{+}$ T cell-mediated responses and effector T cell development.[61] Vladislava Paharkova-Vatchkova et al. demonstrated that $CD11b^{+}$ DC from E2-supplemented medium displayed higher levels of cell surface CD86, exhibiting superior ability to induce the proliferation of naive $CD4^{+}$ T cells.[62] The high levels of estradiol also influence T regulatory (Treg) cell populations.[63]

The presence of Flt3L in the culture medium generates cDCs and pDCs subsets from bone marrow progenitors. However, E2 downregulates the development of these Flt3L-driven $CD11c^{+}$ DCs through ERα-signaling in progenitors, compromising the absolute number of pDCs, whereas cDCs were slightly changed.[9,64] The decreased pDCs absolute number led to a more mature phenotype development and an enhanced capacity to produce IL-12 in response to TLR9 stimulation.[65] Subsequently, the high levels of IL-12 induce the production of IFNγ and the differentiation of Th1 cells, leading to a greater antiviral immune response.[66] Of relevance, estrogen also regulates the TLR7-mediated antiviral response of pDCs[65] and human pDCs constitutively express high levels of IRF-5 and IRF-7, with basal levels of IRF-5 higher in women in comparison with men.[67] Interestingly, IRF-5 controls the INF-α release upon TLR7 stimulation.[68] Thus, the higher levels of estrogen enhance the TLR7-dependent production of IFN-α by pDCs, increasing the immune response against the virus (Figure 1d).

This new knowledge enhanced our comprehension on the ER-dependent signaling mechanisms by which estrogen modulates the development and function of DCs. These studies also provided new insights into the mechanism of sex bias in the E2/Erα signaling. Thereby, E2 induces a quick response by DCs to a viral infection with an increased ability to stimulate T cells and fast viral clearance. These results may account for sex-based differences during Covid-19 disease where women can develop a faster and strong immune response decreasing their susceptibility.

7 | COULD ESTROGEN LEVELS EXPLAIN THE EPIDEMIOLOGICAL DIFFERENCE ON COVID-19 BETWEEN SEX?

There are differences between sexes concerning their responses towards SARS-CoV-2 infection, with male patients showing doubled probabilities of requiring intensive care and higher mortality than female patients.[69] Furthermore, younger patients are more protected against adverse outcomes.[3]

The sex hormones may explain the sexual dimorphism in SARS-CoV-2 symptom severity and mortality. Indeed, in addition to the role of estrogen on DCs, as previously explained, it is well known that E2 modulates immune cell responses and increases anti-inflammatory effects by delaying neutrophil apoptosis, enhancing neutrophil annexin-1 expression without increasing their activation, and reducing monocyte and macrophage inflammatory cytokine release.[70-72]

Interestingly, Robertha et al. measured the levels of ACE2 and TMPRSS2 after pre-treatment of the VERO E6 cell line with 17β-estradiol and showed that estrogen significantly downregulated TMPRSS2 mRNA expression.[6] In addition, Kimberly et al. demonstrated that normal human bronchial epithelial cells pre-treated with 17β-estradiol expressed lower levels of ACE2 mRNA (Figure 1e).[73] However, the reduction of TMPRSS2 and ACE2 mRNA expression might not translate into a reduction of protein levels at the cell surface.[73] Two of the crucial steps of SARS-CoV-2 infection are the identification of target cells and S protein cleavage. If ACE2 is less expressed, it will decrease the number of target cells recognized by the virus. Besides, the decrease of TMPRSS2 expression compromises the cleavage of the S2 protein and the virus entry into host cells.[18] Gennadi V. Glinsky[74] reported that estradiol affects the expression of 203 out of 332 (61%) human genes encoding protein targets of SARS-CoV-2 (namely, *ACE2* and *Furin*), thus potentially interfering with functions of 26 of 27 (96%) SARS-CoV-2 viral proteins.[74] Both downregulated and upregulated genes induce expression changes that would alter the stoichiometry of viral/human protein interactions.[74] Thus, high levels of 17β-estradiol might influence the expression of the host receptors and proteases, compromising SARS-CoV-2 entry into target cells, which might explain the increased male and elderly population susceptibility to Covid-19.[73]

TLR7 is a receptor expressed on DCs that responds to single-stranded viral RNA.[75] Berghöfer et al.[76] demonstrated that pDCs in the peripheral blood of women produced more type I IFNs in response to TLR7 ligands than pDCs from men.[76] The authors observed that pDCs from postmenopausal women exhibited a reduced TLR7-mediated response by comparison with premenopausal women, which was partially preserved by hormone replacement therapy with E2,[76] suggesting that estrogens have a key role in regulating TLR-mediated response.[67,68]

Progesterone can also modulate immune responses by binding to progesterone receptors located in immune cells, including natural killer cells, T lymphocytes, macrophages, and DCs, as well in

non-immune cells, such as epithelial and endothelial cells in the respiratory tracts, where it modulates cellular signaling and activity against infections. Signaling through progesterone receptors stimulates the epidermal growth factor amphiregulin, thus promoting proliferation and respiratory epithelial cell repair. The fast recovery of the lung tissue verified in female patients decreases their susceptibility to opportunist infections, which are an important cause of mortality.[77] Furthermore, progesterone promotes the skewing of Th cell responses from Th1 toward Th2 and the production of anti-inflammatory cytokines, such as IL-4 and IL-10. Besides, progesterone inhibits the production of proinflammatory cytokines by DCs, such as IL-1β and IL-12.[78,79]

Androgens, such as dihydrotestosterone and testosterone, may also have a protective role in younger men. This effect results from the interaction with the androgen receptor (AR).[80] It was reported that testosterone modulates the immune response, downregulating the expression and function of inflammatory cytokines, including, IL-6, IL-1β, and TNF-α.[81] Furthermore, testosterone enhances Treg and suppresses Th17 differentiation, thus attenuating pro-inflammatory immune response.[82] Testosterone also reduces both neutrophil and eosinophil recruitment, impairing Th2 activation, B cell proliferation and consequently humoral response.[83,84] It is important to highlight that aromatase uses the androgenic substrates to convert them into their respective estrogen, which might trigger an anti-inflammatory effect.[85] Nonetheless, low levels of testosterone, typically verified with aging, may revert these immunological features predisposing patients to systemic inflammation and worse clinical outcomes. On the other hand, androgens seem to enhance TMPRSS2 gene expression, triggering a greater viral entry of SARS-CoV-2 into target cells.[86]

Thereby, aging may impair immune response against SARS-CoV-2 infection, particularly in men. Physiological levels of testosterone lead to a dampened immune response, allowing systemic SARS-CoV-2 spreading with the injurious clinical outcome on one side, but protecting them against cytokine storm on the other side.[81-86] In addition, progesterone seems to keep the balance between pro and anti-inflammatory immune responses, enhancing the estrogen antiviral response against SARS-CoV-2.[77] Thus, not only estrogen but sex hormones all together might justify the differences between genders (Figure 2) and age rates.

8 | X-CHROMOSOME IMMUNE-RELATED GENES AND THE IMPLICATIONS FOR COVID-19

Discrepancies between sex can be justified by other mechanisms, such as the imbalance expression of genes on the Y- and X-chromosomes, since immune-related genes linked to chromosome X appear to be more activated in female immune cells. Females carry polymorphic X-chromosomes from both parents, providing an advantage to be potentially heterozygous, whereas males carry only the maternal X-chromosome, who are definitely hemizygous.[87] During early female embryonic development, one of the X-chromosome is inactivated randomly for gene expression, resulting in cellular

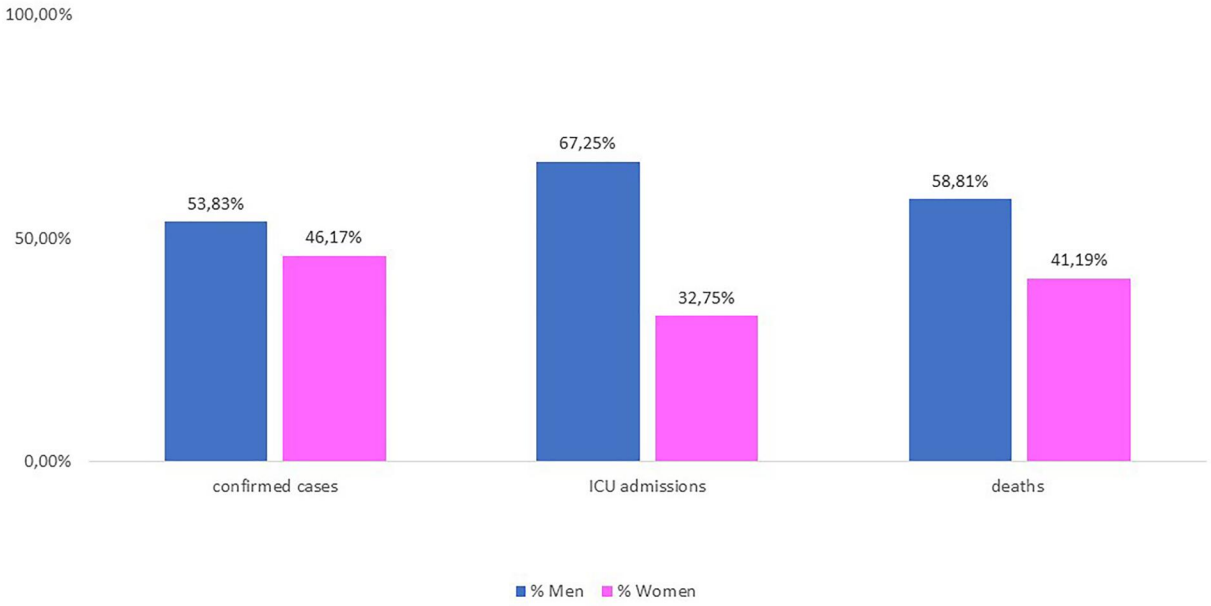

FIGURE 2 Sex differences during novel coronavirus disease 2019 (Covid-19). Sex differences from confirmed cases, intensive care unit admissions, and deaths during Covid-19. There are differences between sexes with male patients showing higher morbidity and mortality than female patients. The data was obtained from Global Health 5050 on 17th August 2021[3]

mosaicism for the expression of X-related proteins, which may contribute to sex-related dimorphism, favouring females with greater adaptability to counteract the progression of the SARS-CoV-2 infection.[88] The X-chromosome has the *ACE2 gene*[89] as well as genes related to the immune system, including *TLR7*, *TLR8*, *IRAK1*, NFκB essential modulator (*NEMO*), among others.[90] To understand the impact of polymorphism in the *TLR7*, *IRAK1*, and *NEMO* genes on Covid-19 we will describe the signaling cascade initiated by TLR7 after the recognition of single-stranded RNA (ssRNA) viruses. TLR7, which is located in the endosomes, recognizes ssRNA trough the MyD88. Then, it is formed a complex with IRAK-1, IRAK-4 and TRFA6. If TRFA6 activates IRF7, this IRF will be translocated to the nucleus to promote the synthesis of the type I IFN.[33] On the other way, TRFA6 could activate the complex formed by NFκB-inhibitory kinases (IKKα and β) and NEMO. The phosphorylation of the NFκB-inhibitory proteins by IKKα and IKKβ activates the NFκB and increases the pro-inflammatory response.[33,91]

Fallerini et al. reported that missense deleterious variants in the X-linked recessive *TLR7* gene may increase susceptibility to Covid-19 in 2.2% of severely affected young males patients.[92] The polymorphism in the human *IRAK1* haplotype seems to persistently increase its kinase activity, which translates into an augmented pro-inflammatory immune response.[33,93] Taking into account that ACE2 is directly correlated with the SARS-CoV infection,[94] Hussain et al. explored the binding affinity between ACE2 variants and SARS-CoV-2 S protein. The team highlighted only two probable alleles (rs73635825 (S19P) and rs143936283 (E329G)) of *ACE2* that may impact the susceptibility and/or resistance against SARS-CoV-2.[95] Nevertheless, human *ACE2* gene is localized on Xp22, in an area where genes are reported to escape from X-inactivation.[96] Souyris et al. also demonstrated that *TLR7* escapes from X-inactivation in B lymphocytes and myeloid cells.[97]

Thereby, the cell mosaicism and the inactivation of the X-chromosome may be the explanation for the immune hyper-responsiveness verified in females.[98]

9 | PREGNANCY

Women may be more protected than men during physiological changes, including pregnancy or the menstrual cycle when the oscillation of reproductive steroids warrants stronger immune protection. Studies have noted that some SARS-CoV-2 positive pregnant women with mild Covid-19 symptoms or asymptomatic on admission to obstetrical unit, intensified symptom severity immediately postpartum in coincidence with the drastic hormonal decrease following childbirth.[5] In the early stages of pregnancy, peripartum, and postpartum a proinflammatory response is reported, despite the rest of pregnancy revealing an anti-inflammatory response. The proinflammatory state is characterized by Th1 and Th17 cells, resulting in the overproduction of pro-inflammatory mediators, including TNF-α, IL-6, IL-1β. The anti-inflammatory state is Th2 and Treg cells dominated with the overproduction of anti-inflammatory cytokines, such as IL-4, IL-10, and TGF-β. The anti-inflammatory response is amplified by placental hormones (estriol, estradiol, progesterone, human chorionic gonadotropin, prostaglandins), type 2 macrophages, and leukemic inhibitory factor.[99] IL-10 can promote anti-inflammatory and antifibrotic actions in the lungs and other tissues by suppressing the Th-1 immunity.[100] Further, IL-10 also enhances B lymphocytes survival and antibody production, which is crucial to balance the immune suppression and activation.[101] In contrast, protease-activated receptors (PAR)-1 and IL-6 are responsible for the deterioration of Covid-19 patients due to the overproduction of proinflammatory cytokines and the activation of the coagulation pathway.[102] This will lead to exacerbated inflammatory response and procoagulant-anticoagulant imbalance, which in turn results in extensive tissue damage, diffused micro thrombosis, and multiorgan failure. In a normal pregnancy, however, PAR-1 is undetectable after the first trimester.[103] Taken together, the high secretion of placental hormones, increased levels of IL-10, and the domination of Th-2 response may contribute to the relatively benign course of Covid-19 in normal pregnancy. However, the risk of having severe Covid-19 is higher in the first trimester of pregnancy and in the postpartum period. This risk is similar to the non-pregnant population.[79]

10 | ACTIVE CLINICAL TRIALS

As we conveyed in this document, there is a connection between the different sex hormone levels and the severity of Covid-19. Concretely, both estrogens and progesterone seem to display a protective effect, and testosterone seems to lead to a dampened immune response, allowing systemic SARS-CoV-2 spreading.[104] With these principles in mind, it is possible to speculate that interfering with the signaling pathways triggered by sex hormones may control the severity of Covid-19. In fact, some clinical trials are assessing this hypothesis currently. In Figure 3 we present active clinical trials aiming to understand whether the drugs or drug associations are beneficial for Covid-19 patients.

Some of the strategies are the sex hormones previously described, whereas other incorporate molecules that are not even hormones. However, those molecules modulate the cell response due to the presence of sex hormones by different mechanisms. Next, we will deeply discuss their mechanisms.

Degarelix, Enzalutamide, and Bicalutamide are drugs that are currently used to treat prostate cancer.[105,106] However, their mechanisms of action are slightly different from one another. Degarelix is a selective, competitive, and reversible antagonist of the gonadotropin-realizing hormone (GnRH) receptors located on hypophysis. As a result, the gonadotropins (which are luteinizing hormone—LH, and follicle-stimulating hormone—FSH) release is decreased, leading consequently to the reduction of testosterone releasing levels by testicles.[107] On the other hand, both Enzalutamide and Bicalutamide reduce the efficiency of the AR migration towards the nucleus, which results in the impairment of the AR-mediated

Study ID	Drug / Combination of drugs	Population	Phase	Primary outcomes	State	Refs
NCT04359329	Estradiol	Male ≥ 18 years of age or female ≥ 55 years of age	2	Hospitalization, ICU transferation, intubation, and death at 30 days	Recruiting	137
NCT04539626	Estrogen	Male ≥ 18 years of age and female ≥ 55 years of age with non-severe COVID19	Not applicable	Hospitalization, oxygen therapy use, intubation, and death at 7, 14, and 21 days	Recruiting	138
NCT04853069	Oestrogen	Adult males > 18 years of age, Post-menopausal women	2	Disease progression for mild and moderate and severe cases at 28 days	Not yet recruiting	139
NCT04801836	Estetrol	Postmenopausal women who have not used hormone replacement therapy; Men ≥18 years	2	% of recovering at 28 days	Active, not recruiting	140
NCT04365127	Progesterone	Male ≥ 18 years	1	Change in clinical status at 7 days	Completed, without posted results	141
NCT04397718	Degarelix	Male veterans hospitalized due to COVID-19	2	Hospitalization, requirement of mechanical ventilation, or death at day 15 after randomization	Recruiting	142
NCT04475601	Enzalutamide	Women and men ≥50 years	2	The time to need mechanical ventilation or discharge from the hospital	Recruiting	143,144
NCT04509999	Bicalutamide	Male ≥ 18 and ≤ 70 years	3	Improvement of COVID-19 symptoms at 28 days	Recruiting	145
NCT04853927	Proxalutamide	Male and females age ≥18 years	3	Death (28 days)	Recruiting	146
NCT04446429	Proxalutamide	Male age ≥18 years	Not applicable	COVID-19 Hospitalization at 30 days	Completed, with posted results	147
NCT04853134	Proxalutamide	Female age ≥18 years	3	COVID-19 Hospitalization at 30 days	Active, not recruiting	148
NCT04652765	Camostat	Male and females age ≥60 years	1	Hospitalization at day 28	Recruiting	149
NCT04345887	Spironolactone	Male and females age ≥18 years	4	Hospitalization, oxygen, and death	Not yet recruiting	150
NCT04865029	Estradiol and Progesterone	Women and men ≥18 years	2	Limitations on activities through day 28	Not yet recruiting	151
NCT04389580	Isotretinoin + Tamoxifen	Patients ≥ 18 and ≤ 70 years with severe respiratory failure requiring admission to ICU	2	Lung injury at 7 days	Not yet recruiting	152
NCT04568096	All trans-retinoic acid+tamoxifen	Adult SARI patients≥18 and ≤ 80 years	2	lung injury score	Not yet recruiting	153
NCT04424134	Spironolactone + bromhexine	Women and men ≥18 years	3	Change from the baseline in clinical assessment score COVID-19	Recruiting	154

FIGURE 3 Active studies of drugs that interfere with cell signaling triggered by sex hormones.[37-154] Adapted from[105]

signaling pathway.[106] Currently, the potential of Proxalutamide to manage prostate cancer has been studied. This molecule antagonizes the receptor of androgens and reduces the transcriptional levels of the same receptors and, therefore, their expression.[108] According to what we stated previously, and supported by other works, androgens promote the transcription of TMPRSS2,[109] which favours SARS-CoV-2 infection.[110] So, inhibiting the pathways related to androgens will decrease TMPRSS2 expression, which can mitigate the viral entrance

on cells and, so, its replication.[111,112] Moreover, Proxalutamide is also able to lower the expression of ACE2, which can act synergistically with the activity of lowering the expression of TMPRSS2.[113]

Spironolactone is a mineralocorticoid/aldosterone antagonist[114] that is commonly used in cases of resistant hypertension.[115] However, due to its antiandrogenic activity, it could be used by transgender females, as well. As a matter of fact, spironolactone seems to reduce the synthesis of testosterone, antagonizes ARs, and agonizes ER.[116] Taking into account both, the anti-hypertensive (because of the inhibition of the rennin-angiotensin-aldosterone system) and the anti-androgen effects of spironolactone (impacting on the TMPRSS2 expression), it was proposed that spironolactone would be a helpful tool on managing Covid-19 positive patients.[117]

In order to convey as many targets as possible to stop Covid-19, it is possible to use combinations of molecules and some are currently being studied. The first combination that we will spell out is the association of isotretinoin with tamoxifen. Isotretinoin is a drug that is currently used to treat acne because of its antiandrogenic effect.[118] Indeed, isotretinoin diminishes the serum levels of dihydrotestosterone,[119,120] which is an androgen that promotes the transcription of TMPRSS2.[109,121] The effect of androgens on regulating the expression of TMPRSS2 was previously reported on lungs.[122] For this reason, it is tempting to speculate that suppressing the activity of androgens (including testosterone and dihydrotestosterone) will lead to the reduction of the expression of TMPRSS2, which results in less viral infection,[111] and this suppression may be induced by isotretinoin. Beyond its antiandrogenic activity, Sinha et al. have found out recently that isotretinoin is a strong downregulator of ACE2 receptors, being the strongest one out of over 20000 molecules tested in vitro.[123] Taken together, isotretinoin may have a protective role due to suppression of TMPRSS2 and ACE2 expression.

Tamoxifen exerts effects beyond the ones related to estrogens. Reportedly, tamoxifen inhibits the acidification of lysosomes and endosomes and decreases the rate of vesicular transport. Therefore, there are a lot of implications for many cellular functions.[124] Concretely, lysosomes and endosomes contain cathepsins. Cathepsins (Cat) are acidic proteases that display many functions on cells according to their location in the cell. Apparently, CatL is involved in the entrance process of SARS-CoV-2: firstly, SARS-CoV-2 binds to the ACE2 receptor and it is endocytosed. Here, TMPRSS2 and CatL perform an initial S protein proteolysis on the cell surface. Secondly, in the endosomes, CatL cleaves the S1 subunits, and the bound between SARS-CoV-2 and ACE2 is disrupted. Lastly, the viral membrane fuses with the endosome membrane, and the viral ssRNA is released into the cytosol.[125] Plus, cathepsin L may act synergistically with TMPRSS2,[126] but also as the mean of S protein priming when TMPRSS2 is absent from the cells' surface.[110]

The next drug conjugation that is being assessed is the concomitant use of tamoxifen and all trans-retinoic acid. Also known as retinoic acid, all trans-retinoic acid displays many important functions,[127] for instance, the inhibition of the responses induced by the bradykinin B_1 receptor, displaying the immunomodulatory and anti-inflammatory effects of retinoic acid on Covid-19.[128] The rationale behind targeting those receptors was originated by the hypothesis that bradykinin B_1 receptor on endothelial cells of the lungs is upregulated in Covid-19 patients. This may be explained by the decrease of ACE2 receptors resultant of SARS-CoV-2 entrance in cells.[129] Accordingly, after the SARS-CoV-2 entrance into the cells via ACE2, this receptor won't be available to inactivate the potent ligand of the B_1 receptor des-Arg^9 bradykinin. As this ligand is not inactivated, the B1 receptor will remain in its active form, which results in vascular leakage and angioedema.[130] So, inhibiting bradykinin B_1 receptors may be an option to reverse the Covid-19-induced angioedema in the lungs.[131] Besides, all trans-retinoic acid displays an inhibitory effect on IL-6-driven induction of proinflammatory T_H17 and a promontory effect on the anti-inflammatory T_{reg} cell differentiation.[132]

The last drug conjugation that we will explore has a similar purpose to the previous one. Indeed, the conjugation of spironolactone and bromhexine may be very beneficial. Despite its use as a mucolytic agent,[133] bromhexine showed to inhibit the TMPRSS2 in situations of infection by influenza A, MERS-CoV,[134] and, more recently, SARS-CoV-2.[135] Besides, bromhexine, after its metabolism, is converted into ambroxol, which is also active. Interestingly, ambroxol displayed anti-SARS-CoV-2 activity by blocking ACE2.[136]

As all these putative therapeutically options have the lungs as main target and, as previously stated, DCs are highly present in this organ. Therefore, it seems reasonable to hypothesise that all those molecules can also impact DCs function. The resulting effect on DCs can condition the immunological response by, for instance, interfering with antigen recognition, or even, with antigen presentation to T cells.[25,27]

11 | CONCLUSION

Sex differences in Covid-19 have consequences in the hormonal and genetic signaling pathways, with male patients showing higher probabilities of requiring intensive care and mortality than female patients. The literature suggests that premenopausal women have a fast recovery of the lung tissue and can mount a strong and quick innate immune response, thereby decreasing their susceptibility to Covid-19 disease. The explanation for this discrepancy can be attributable, at least partially, to sex hormones, for instance, 17β-estradiol, progesterone and testosterone. Indeed, high levels of 17β-estradiol downregulate TMPRSS2 mRNA and ACE2 mRNA expression, jeopardizing SARS-CoV-2 recognition and entry into host cells. Concerning males, physiological levels of testosterone lead to a dampened immune response, allowing systemic SARS-CoV-2 spreading with the injurious clinical outcome on one side, but protecting them against cytokine storm on the other side. Thus, sharp variations in testosterone levels may justify the higher vulnerability among males during aging. Additionally, the imbalanced expression of the immune-related genes on the Y- and X-chromosome, the cell mosaicism, and the random inactivation of

the X-chromosome unique in females may be the explanation for the immune hyperresponsiveness and for the worst prognosis in males.

The evidence that pregnancy did not add risk to deterioration associated with Covid-19 and the higher mortality observed in postmenopausal women Covid-19 positive corroborate the hypothesis that estrogens can trigger a protective effect in Covid-19. However, it is important to fully understand the complex interaction of sex hormones in different environments, knowing that the effects of E2 as a pro- or anti-inflammatory stimulus must be adjusted to the viral infection phase.

Lastly, there are active clinical trials aiming to find out therapeutic strategies disrupting the signaling pathways triggered by sex hormones and able to control the severity of Covid-19. The results of these studies are eagerly awaited, and our hope is that such efforts will soon be successful and drive the development of safe and effective treatments for Covid-19.

ACKNOWLEDGMENTS

This research work has been carried out in the framework of project "TECNICOV - Desenvolvimento de testes rápidos para a monitorização de anticorpos em soro e saliva," co-financed by the European Regional Development Fund (POCI-01-02B7-FEDER-069745) through the Operational Programme for Competitiveness Factors (COMPETE) and by national funds through the National Innovation Agency.

CONFLICT OF INTEREST STATEMENT

No conflict of interest declared. Tecnimed Group Company had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the study.

AUTHOR CONTRIBUTIONS

Maria Teresa Cruz, Anália do Carmo, and Daniela Mateus conceptualized the manuscript. Daniela Mateus and Ana Isabel Sebastião wrote the first draft of the manuscript. Ana Miguel Mato. and Mylène A. Carrascal revised and edited the final version of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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How to cite this article: Mateus D, Sebastião AI, Carrascal MA, Carmo Ad, Matos AM, Cruz MT. Crosstalk between estrogen, dendritic cells, and SARS-CoV-2 infection. *Rev Med Virol*. 2022;32(3):e2290. https://doi.org/10.1002/rmv.2290