Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review Article

The pivotal link between ACE2 deficiency and SARS-CoV-2 infection: One year later

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1. Introduction

The pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) generated much interest on the basic pathogenetic mechanisms through which SARS-CoV-2 damages diverse organ systems including the lungs, heart, brain, kidney, and vasculature. [1] In June 2020, the *European Journal of Internal Medicine* hosted a paper from our group which presented a unified pathophysiological hypothesis on some basic aspects of the disease [2].

We moved from the observation that the imbalance between angiotensin II (Ang II) and Angiotensin$_{1\text{-}7}$ (Ang$_{1,7}$) caused by the interaction between SARS-CoV-2 and the angiotensin converting enzyme 2 (ACE2) receptors may be expected to play an important pivotal role on the clinical picture and outcome of COVID-19 [2]. Then, we raised the hypothesis that the reduced catalytic efficiency of ACE2 resulting from viral occupation and down-regulation of these receptors could be particularly detrimental not in subjects with ACE2 up-regulation, as initially believed, but, rather, in subjects with baseline deficiency of ACE2 receptor activity [2,3].

The main aim of this review is to summarize the evidence accrued over the past 12 months in this field, spanning from basic researches to human clinical studies.

2. Interaction of SARS-CoV-2 with ACE2

The mechanisms of virus entry into cells is mediated by the efficient binding of the Spike protein (which comprises S1 and S2 subunits) to ACE2 [4,5]. The ACE2 is a homolog to ACE with 40% structural identity [6]. It is a trans-membrane type I glycoprotein (mono-carboxypeptidase) composed by 805 amino acids which uses a single extracellular catalytic domain to cleave an amino acid from angiotensin I (Ang I) to form angiotensin$_{1\text{-}9}$ (Ang$_{1\text{-}9}$) and to remove an amino acid from Ang II to form Ang$_{1\text{-}7}$ [7]. ACE2 receptors are expressed in almost all human tissues (heart, vessels, gut, adipose tissue, thyroid, lung, kidney, testis, and brain) [8].

Briefly, the viral entry process consists of three main steps [4,9]. In the first step, the N-terminal portion of the viral protein unit S1 binds to a pocket of the ACE2 receptor [4]. In the second step, the protein cleavage between the S1 and S2 units is operated by the receptor transmembrane protease serine 2 (TMPRSS2, structurally contiguous to ACE2 receptor) which facilitates viral ingress and down-regulates surface ACE2 expression [10]. In the last step, the viral S2 unit undergoes a

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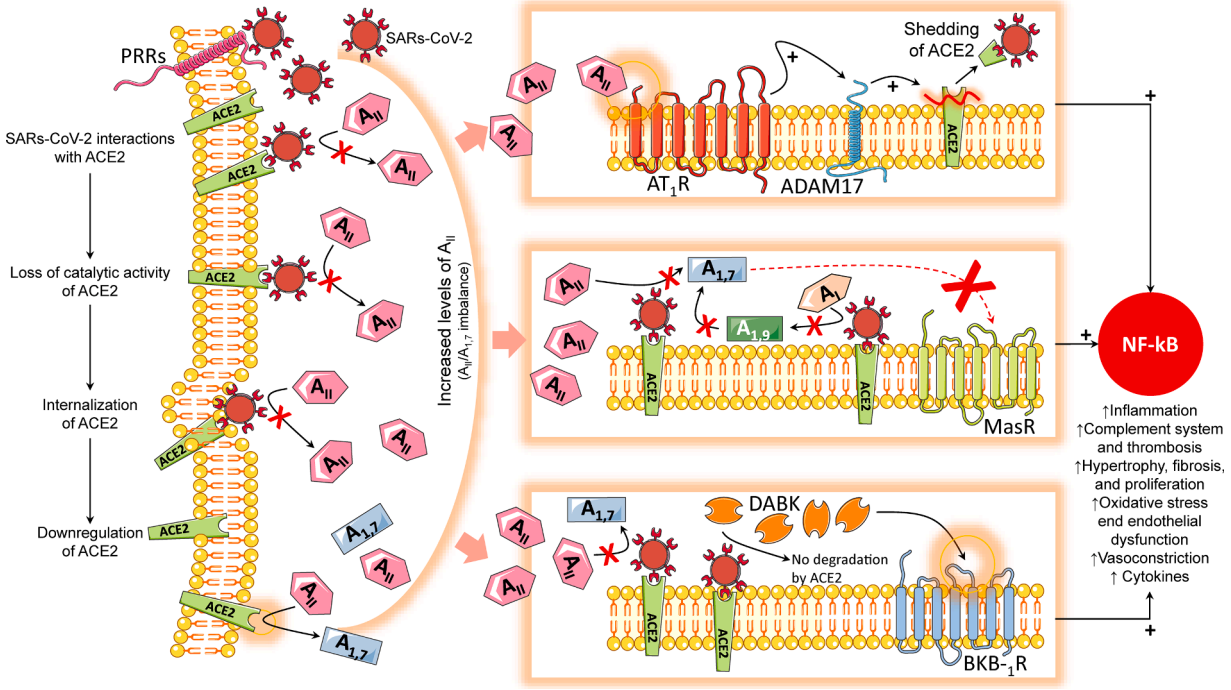

Fig. 1. Mechanisms implicated in the development of Angiotensin II storm during the SARS-CoV-2 infection. The deleterious effects of this phenomenon are also depicted. See text for details. Legend: A_I=angiotensin I; A_{II}=angiotensin II; $A_{1,7}$=angiotensin$_{1,7}$; $A_{1,9}$=angiotensin$_{1,9}$; ACE2=angiotensin coverting enzyme 2; ADAM17= disintegrin and metalloproteinase domain-containing protein 17; AT_1R=angiotensin II type 1 receptor; $BKB\text{-}_1R$=bradykinin B1 receptor; DABK= des-Arg$_9$ bradykinin; MasR=Mas receptor; NF-kB= nuclear factor kappa-light-chain-enhancer of activated B cells; PRRs=pattern recognition receptors; SARS-CoV-2= severe acute respiratory syndrome coronavirus-2.

conformational rearrangement after the cleavage of the viral protein by TMPRSS2, driving the fusion between the viral and cellular membrane and promoting the entry of the virus into cell, release of its content, replication, and infection of other cells [11].

3. Angiotensin II "storm"

As aforementioned, the failure of the counter-regulatory renin-angiotensin-aldosterone system (RAAS) axis, characterized by the decrease of ACE2 expression and generation of the protective Ang$_{1,7}$, appears to be strictly implicated in the development of severe forms of COVID-19 [2,3,12]. More specifically, ACE2 internalization, down-regulation and malfunction predominantly due to viral occupation, dysregulates the protective RAAS axis with increased generation and activity of Ang II and reduced formation of Ang$_{1,7}$ [2,3,12].

This has been corroborated by the findings of recent investigations supporting the evidence of the development of an "Ang II storm" [13] or "Ang II intoxication" [14] during the SARs-CoV-2 infection. The specific mechanisms of this condition are depicted in Figure 1.

The interplay between SARS-CoV-2 infection and RAAS is explained by the binding of S protein to ACE2 which triggers enzyme internalization via endocytosis [15] and down-regulation of its cell surface activity [10,16] with consequent increase in serum levels of Ang II (which remains almost intact because of the lack of conversion to Ang$_{1,7}$). Indeed, in the absence of ACE2 due to viral blockade and down-regulation, both Ang I and Ang II accumulate. Nonetheless, ACE is not engaged by the virus and the conversion of Ang I to Ang II continues unabated, leading to unopposed accumulation of Ang II. Elevated levels of Ang II develop an abnormal activation of the Ang II/Ang II type 1 receptor (AT_1R) component of the RAAS, producing end-organ damage through the activation of pro-inflammatory cascade (also enhanced by the activation of the complement system by pattern recognition receptors [PRRs]), pro-fibrotic cascade, pro-coagulant state, mitochondrial oxidative damage, reactive oxygen species production, and interleukin (IL) 6 (IL-6) up-regulation [14,15,17-19].

Furthermore, Ang II upregulation and accumulation activates disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) activity (perpetuating membrane shedding of ACE2 and RAAS over-activation), and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) pathway (as mediated by the AT_1R activation) [20,21].

These mechanisms lead to increased production of IL-6, tumor necrosis factor-α, IL-1β, IL-10, and IL-12 (citokynes storm) [22,23]. Finally, loss of activity of ACE2 reduce deactivation of des-Arg$_9$ bradykinin (DABK, which is a well-known pulmonary inflammatory factor) [24,25] promoting a pro-inflammatory synergistic effect with the derangement of the ACE2/Ang II/AT_1R and ACE2/DABK/bradykinin B_1 receptor (BKB-1R) axes [19,26,27].

ACE2 downregulation and malfunction (with subsequent imbalance in the RAAS and increase in Ang II levels) is implicated in several models of acute lung injury [28]. More specifically, downregulation of ACE2 is associated with alveolar wall thickening, bleeding, edema, and the recruiting of inflammatory cells [28].

In the specific setting of COVID-19, an investigation of epidemiological, clinical, laboratory, radiological characteristics, and potential biomarkers to predict disease severity in SARS-CoV-2 infected patients in Shenzen, demonstrated that Ang II levels in the plasma samples were significantly increased and linearly associated with viral load and lung damage in critically ill patients [29].

The findings in another study were similar and documented that plasma Ang II elevation was closely related to the SARS-CoV-2 infection [30]. Specifically, Wu and co-workers investigated the plasma Ang II levels in 82 non-hypertensive patients (42 mild cases, 25 severe cases, and 15 critically ill cases) infected by SARS-CoV-2 and 12 critically ill patients not infected by SARS-CoV-2 serving as control [30]. They documented that Ang II level was higher than that of normal range in the majority of COVID-19 cases (90.2%), especially the plasma Ang II positive rate in the critically ill COVID-19 patients (100%) [30].

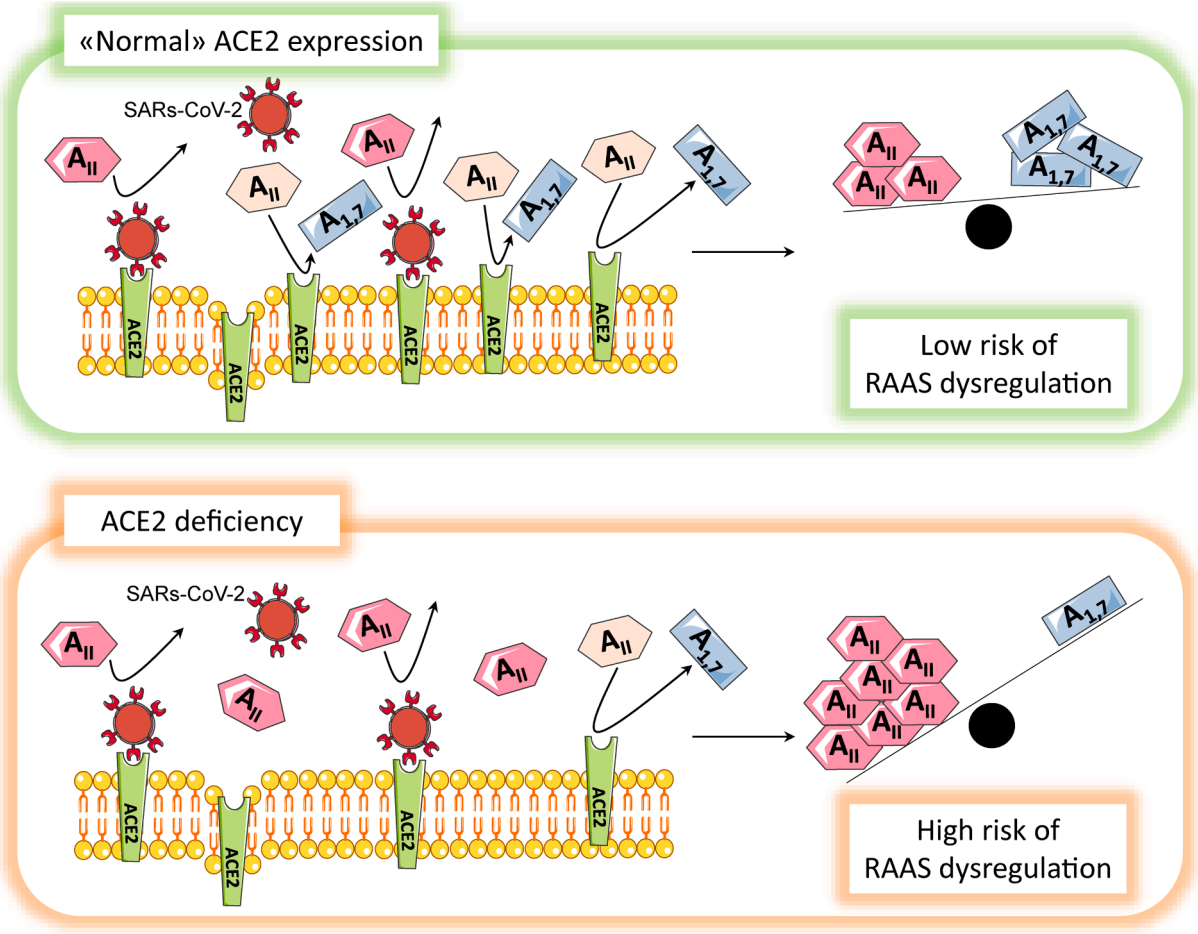

Fig. 2. Pre-existing ACE2 deficiency (as documented for elderly patients, diabetes mellitus, COPD, hypertension, and chronic disease) contributes to an unfavourable outcome in SARS-CoV-2 infection. See text for details. Legend: A_{II}=angiotensin II; $A_{1,7}$=angiotensin$_{1,7}$; ACE2=angiotensin coverting enzyme 2; RAAS=renin-angiotensin-aldosterone system; SARS-CoV-2= severe acute respiratory syndrome coronavirus-2.

Furthermore, they demonstrated a positive correlation between plasma Ang II levels and COVID-19 severity [30].

Conversely, a study by Rieder and co-workers [31] showed that mean serum concentrations of ACE2 and Ang II did not differ between SARS-CoV-2 positive patients and a control group of subjects presenting with similar symptoms in the emergency unit. Nonetheless, most of the patients included in this study had non-severe forms of COVID-19 and results reinforced the notion that RAAS dysregulation is predominantly to be expected only among critically ill COVID-19 patients [31].

4. Phenotypes of ACE2 deficiency

Observational studies and meta-analyses reported older age, male sex, and the presence of comorbidities (including hypertension, chronic obstructive pulmonary disease [COPD], diabetes mellitus, and history of cardiovascular events) as risk factors for increased disease severity in COVID-19 (2-1) [32–39]. Remarkably, all these conditions are associated with RAAS dysregulation and ACE2 deficiency [15,40,41] (Figure 2).

4.1. Sex

Different susceptibility to severe forms of COVID-19 between males and females may be explained by the different expression of ACE2. The human ACE2 gene is located on the X chromosome (Xp22.2 chromosomal region), in sites commonly escaping the inactivation (transcriptional silencing) of one X chromosome in mammalian XX cells [42]. Nonetheless, the silencing is not complete and some of the genes present on the silenced chromosome (including ACE2 gene) still undergo transcription and translation, "escaping" the inactivation [43]. As a consequence, XX cells over-express genes located in XCI sites, like ACE2 [44]. Furthermore, estrogen, the primary female sex hormone, regulates different components of the RAAS, including the up-regulation of the expression of ACE2 [45].

4.2. Age

Generally, respiratory viral infections are more frequent and severe in children than in adults. Nonetheless, the evidence that older age is an established risk factor for mortality and the marked disparities in disease prevalence and severity of COVID-19 between adult and paediatric populations [46] revealed a different scenario for SARS-CoV-2 infection.

Notably, ageing has been associated with decline in levels of ACE2 expression in experimental and human models [46–49]. Xie and co-workers [48] analysed the expression of ACE2 and the effect of ageing on its expression in lungs of rats. The analysis of the immunoreactive bands documented that ACE2 was predominantly expressed in alveolar epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vessels with similar content [48]. Furthermore, ACE2 expression was markedly reduced among older group [48].

Similarly, a study by Yoon and coworkers [47] evaluating the association between the change in the expression of ACE2 and arterial ageing in mice demonstrated that the expressions of ACE2 decreased with age [47].

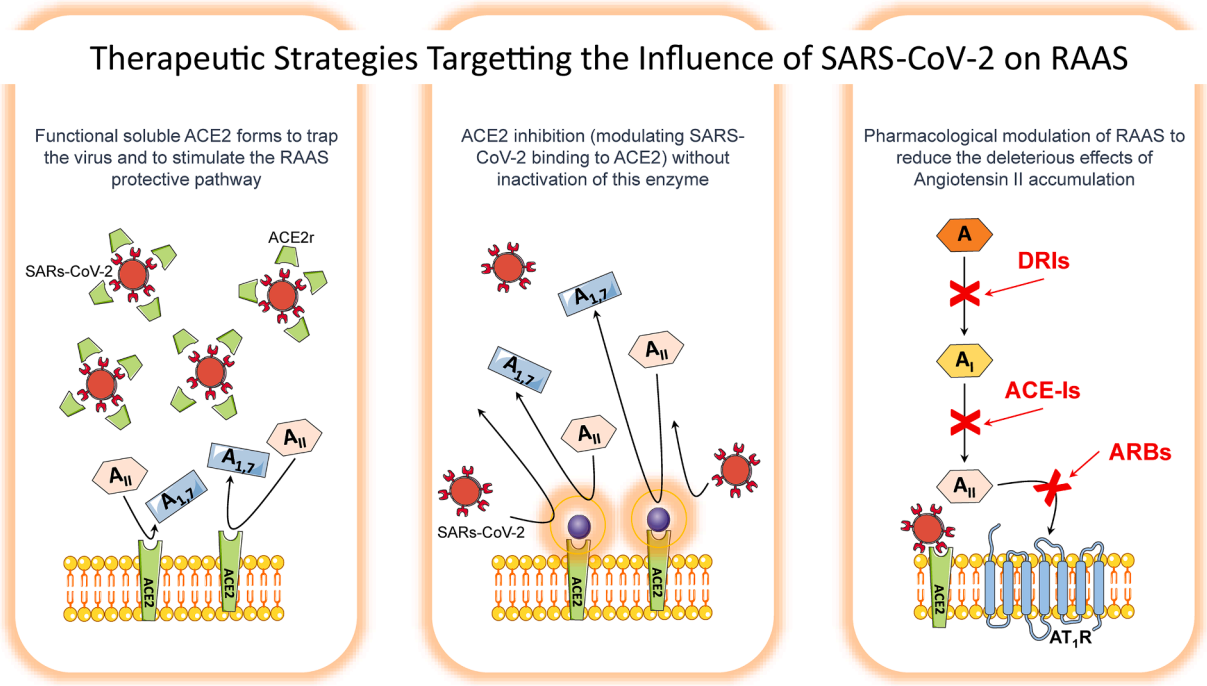

Fig. 3. Potential therapeutic approaches of restoring the ACE2/Angiotensin$_{1-7}$ pathway or to trap the virus and to stimulate the renin-angiotensin-aldosterone system protective pathway. See text for details. Legend: A_{II}=angiotensin II; $A_{1,7}$=angiotensin$_{1,7}$; ACE2=angiotensin coverting enzyme 2; ACE-Is=ACE inhibitors; ARBs=angiotensin receptor blockers; AT_1R=angiotensin II type 1 receptor; DRIs=direct renin inhibitors; RAAS=renin-angiotensin-aldosterone system; SARS-CoV-2=severe acute respiratory syndrome coronavirus-2.

A bioinformatic analysis of publicly available human genomics and transcriptomics gene expression data by Chen and coworkers [49] demonstrated that ACE2 expression decreases during ageing in many tissues. Specifically, the Authors documented a decrease in ACE2 expression with age in blood, adrenal gland, colon, nervous system, adipose tissues, and salivary gland [49].

4.3. Lung disease (with hypoxia)

One of the main clinical feature of lung disease, including COPD, is hypoxia [50]. In a normoxic condition, the dynamic balance between the expression of ACE and ACE2 regulates the RAAS system. However, under conditions of chronic hypoxia ACE is upregulated by factor 1 (HIF-1) in human pulmonary artery smooth muscle cells (hPASMCs), whereas ACE2 expression is markedly decreased [51]. Furthermore, a recent investigation of the binding affinity between receptor-binding domain (RBD) and S1 at different oxygen concentration levels documented that ACE2 is down-regulated under hypoxia [52].

4.4. Cardiovascular risk factors and comorbidities

RAAS over-activation in hypertension increases AT_1R stimulation by Ang II, promoting downregulation of ACE2 and upregulation of ACE expression [53]. Disruption of tissue ACE/ACE2 balance results in changes in blood pressure (BP), with increased ACE2 expression protecting against increased BP, and ACE2 deficiency leading to increased BP [54,55].

Similarly, diabetes mellitus is associated with a reduction in ACE2 expression and with Ang$_{1,7}$-generating system downregulation [56,57].

ACE2 deficiency is also documented in several experimental models of cardiac complications, including left ventricular systolic dysfunction and heart failure with pulmonary congestion, myocardial infarction, and coronary artery disease [58–62]. The absence of ACE2 also results in increased mortality and infarct size expansion, adverse ventricular remodelling and greater systolic dysfunction after myocardial infarction [61].

5. Therapeutic strategies

Some hypotheses have been made on the potential therapeutic approach of restoring the ACE2/Ang$_{1-7}$ pathway. The development of ACE2 inhibitors, approaches to enhance ACE2 (including soluble ACE2 and ACE2 activators), and pharmacological modulation of RAAS may be useful to build an armamentarium finalized to block the transition from infection to severe forms of COVID-19.

A reasonable treatment strategy deserving further investigation would be delivering functional soluble ACE2 forms to trap the virus and to stimulate the RAAS protective pathway (Figure 3, left panel). Indeed, high-affinity variants of soluble ACE2 bind to spike protein of SARS-CoV-2 and thereby neutralize infection as decoy receptors. These high-affinity variants outcompete native ACE2 present on cells by binding with the S protein of SARS-CoV-2, making native ACE2 on cell surfaces readily available for conversion of Ang II to Ang$_{1,7}$ [63].

Although clinical evidence on this aspect is scarce, the administration of the soluble human recombinant ACE2 was able to reverse the lung-injury process in preclinical models of other viral infections [64,65].

Among the different therapeutic strategies, the blockade of SARS-CoV-2 from binding to human cell receptors is the object of both in-silico and in-vivo experiments [66,67]. Keeping in mind that SARS-CoV-2 uses ACE2 as a Trojan horse to invade target cells, ACE2 inhibitors with insurmountable inhibition of ACE2, blocking or attenuating the binding of the viral Spike protein to the pocket of the ACE2 receptor, have the potential to prevent viral internalization into ACE2-expressing cells. However, pharmacological inhibition of ACE2 may exert enzymatic activities with or without inactivation of ACE2. Thus, the real challenge in the field of ACE inhibition is to modulate SARS-CoV-2 binding to ACE2 without blocking the crucial protective properties of this enzyme (Figure 3, middle panel) [12].

The increase in Ang II levels in COVID-19 also suggests that the use of

drugs balancing the RAAS may be used repurposing on SARS-CoV-2 infected patients [12,68,69] (Figure 3, right panel). Indeed, cardiac ACE2 expression is markedly enhanced in response to RAAS blockade by ACE-inhibitors (ACE-Is) [70], angiotensin receptor blockers (ARBs) [71–73], and even by mineralocorticoid receptor (MR) antagonists [73,74].

In this context, a systematic review and meta-analysis of 52 studies evaluated the clinical outcomes among 101949 patients with COVID-19 who did and did not receive ACE-Is or ARBs [75]. The Authors demonstrated a significantly lower risk of multivariable-adjusted mortality and severe adverse events among patients who received ACE-inhibitors or ARBs compared with patients who did not [75].

Notably, a subgroup analysis of patients with hypertension indicated significant decreases in mortality and severe adverse events among patients receiving ACE-inhibitors or ARBs in both unadjusted and adjusted analyses [75].

6. Vaccines and ACE2 interactions

SARS-CoV-2 vaccination is now offering the opportunity to come out of the current phase of the pandemic. However, some concerns regarding the safety of SARS-CoV-2 vaccines have been recently raised, mostly based on scattered reports of thromboembolic events [76–79].

It has been recently suggested that free-floating Spike proteins released by the destroyed cells previously targeted by vaccines may interact with ACE2 of other cells, thereby promoting ACE2 internalization and degradation [80,81]. This mechanism may enhances the imbalance between Ang II overactivity and Ang_{1-7} deficiency through the loss of ACE2 receptor activity, which may contribute to trigger inflammation, thrombosis, and other adverse reactions [82,83].

Of note, spike proteins produced upon vaccination have the native-like mimicry of SARS-CoV-2 Spike protein's receptor binding functionality and prefusion structure [84].

7. Conclusions

At the beginning of the pandemic it was assumed that a mild or moderate deficiency of ACE2 could protect from viral infection. To date, this hypothesis appears to be rejected by the evidence that symptoms, clinical presentation and outcome of COVID-19 may be consistently related to molecular changes and dysregulation of the RAAS [13,15,85,86]. The interaction between ACE2 and SARS-CoV-2 Spike protein induces a substantial loss of ACE2 receptor activity from the external site of the cellular membrane. This phenomenon leads to less Ang II inactivation and less generation of Ang_{1-7} (imbalance between Ang II overactivity and Ang_{1-7} deficiency), which may ultimately trigger inflammation, thrombosis, and other severe adverse reactions influencing the outcome of COVID-19 [2,3,13,15].

The degree of ACE2 expression and the biological relevance of ACE2 deficiency may vary depending on the presence of specific characteristics and conditions (phenotypes of ACE2 deficiency). Important phenotypes including older age, hypertension, diabetes, cardiovascular disease and COPD [2,3,46] share a variable degree of ACE2 deficiency and are associated with more severe forms of COVID-19. Thus, it is very likely that is not the 'excess', but the deficiency of ACE2 receptor activity that complicates the outcome of COVID-19.

In the SARS-CoV-2 era, we may assume that ACE2 receptors are not dangerous Trojan horses, but useful defense weapons against the adverse mechanisms associated with the disease.

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