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SARS-CoV-2 genetic and immunology insights: what does the scientific community know so far?

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ABSTRACT. The challenge presented by the SARS-CoV-2 pathogen has changed the global perception about virus diseases. In Wuhan, China the first case of the disease called COVID-19 (Coronavirus Disease 2019) was reported in December 2019 and quickly reached 215 countries. The pathogenic SARS-CoV-2 virus has an RNA genome composed of a positive-sense single-strand, harboring 14 ORFs that encode 50 proteins composed of typical structural proteins. The spike protein, a surface glycoprotein, is essential for the invasion of the causal agent of COVID-19 into the host system. Several variants have specific mutations in protein S that affect transmission processes, diagnosis, and available therapies. Entry of SARS-CoV-2 into the host cell promotes immunological dysregulation with increased expression of interferon type 1 and an exaggerated proinflammatory cytokine event called "cytokine storm".

This event is often associated with deleterious outcomes such as acute respiratory distress syndrome. In addition, substantial immunological memory can be generated after initial SARS-CoV-2 infection, involving four major cell types, such as anti-spike protein memory B cells (RBD IgG, IgM), T cells (CD4+ and CD8+) and other molecules, such as antibodies. It is important to collect genetic and immunological information related to the SARS-CoV-2 virus to provide a global vision and high quality knowledge about the biology and this disease in order to develop effective control measures and treatments.

Key words: COVID-19; Variants; Pandemic; Immunologic response; Severe Acute Respiratory Syndrome

1. BACKGROUND

The recent SARS-CoV-2 outbreak in world changed the knowledge concerning viruses' infection, mainly those caused by coronavirus. In Wuhan - a Chinese city - was reported the first case of the illness named COVID-19 (Coronavirus Disease, 2019), in December 2019 (Zhu et al., 2020). Through the SARS-CoV-2 high transmissibility capacity, this causative agent of COVID-19 quickly spread to more than 215 countries. In accordance with Johns Hopkins University and WHO data, it is currently not possible to predict the size of the losses caused by covid-19; however, the numbers of confirmed cases and deaths are very high, 546 million and 6.33 million, respectively, data from June 2022. Countries like the United States, India and Brazil have the highest numbers of confirmed cases and deaths. (World Health Organization Clinical Management Report, 2020; World Health Organization Situation Report, 2020). Throughout the SARS-CoV-2 outbreak, it was observed that this virus could present some strategies (escape to the host's immune system) during the infection cycle, that allow it to persist and survive in the intracellular environment (Yuki et al., 2020). The ability to persist within the host and the rapid spread of the virus are important points to consider in future epidemics to propose effective surveillance programs to control outbreaks. However, a better understanding of the viral mechanisms that lead to SARS-CoV-2 infection in the host is necessary, considering the current scenario.

Historically, the first coronavirus discovered (back in the 1930s) was the avian coronavirus known as Infectious Bronchitis Virus, or IBV (Cavanagh, 2005). These viruses are characterized by having a positive RNA single-strand genome (Lauxmann et al., 2020). Additionally, they are part of the family called Coronaviridae and order known as Nidovirales (Kirtipal et al., 2020; Giovanetti et al., 2021). The Coronaviruses are genotypically and serologically linked to following genera: the Alphacoronavirus (AlphaCoV); Betacoronavirus (BetaCoV); Gammacoronavirus (GammaCoV) and Deltacoronavirus (DeltaCoV). The International Committee on Taxonomy of Viruses (Vance et al., 2021) provides this classification. The first two coronaviruses are related to human infection and the second pair affecting predominantly birds (Hu et al., 2020a). Most coronaviruses can cause a cold-like illness in mammals such as bats, camels, and cattle. A small percentage can cause infection in human, feline and canine species. The group of

coronaviruses that infect feline and canine animals, in particular, do not infect humans. (Hu et al., 2020a; Kirtipal et al., 2020). Experts reveal that high urbanization and domesticated birds are the main drivers and generators of these diseases, allowing a rapid exchange between species, resulting in a highly rate of viral recombination (Kirtipal et al., 2020; Lauxmann et al., 2020).

Bats for instance, have been responsible for about 400 new strains of CoV, according to the EcoHealth Alliance of China (Kirtipal et al., 2020). The bats are the unique mammals with a conditioned immune system able to resist a wide range of viruses. Although no directly scientific evidence exist about the transmission between bats to humans, it is suspected a vector-borne transmission via intermediate species, in this case, bats carrying coronaviruses strains (Kirtipal et al., 2020; Lauxmann et al., 2020). However, a suspicion focuses on pangolins: possibly these animals helped in the acquisition of a fragment of the spike protein for SARS-Cov-2. The similarities between the functional sites of the spike protein in the virus isolated from pangolins and those from SARS-CoV-2 could reinforce this theory (Lam et al., 2020; Wong et al., 2020).

HCoV-229E virus was the first coronavirus identified from human (HCoV), it was took place in Foshan, China in November 2002, causing a global concern with a lethal rate of 10% (Kirtipal et al., 2020). Over 8,096 people affected, across 28 countries and 774 fatalities. One decade later, the second HCoV was HCoV-OC43 appeared in Jeddah, Saudi Arabia in June 2012, reaching levels of 35% fatality worldwide (Kirtipal et al., 2020). The infection caused by this pathogen, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), reached 2,494 cases with 858 deaths, affecting 27 countries (Kirtipal et al., 2020). Probably MERS-CoV could originally come from bats, through dromedary camels as vectors for human transmission (Lauxmann et al., 2020). Currently, the causative agent of COVID-19 pandemic is the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Hu et al., 2020a; Kirtipal et al., 2020; Wang et al., 2020a; Giovanetti et al., 2021). The unusual and overwhelming spread of SARS-CoV-2 is related to a higher number of confirmed cases and the geographic spread of the disease (Wang et al., 2020a). The rapid circulation of SARS-CoV-2 between species that harbor the virus or in infected hosts could easily facilitate the emergence of new pathogenic variants through the appearance of viral mutations, either by selective pressures or by increased contact with humans. Finally, the anthropological actions that directly influences the environment causing dismantlement, habitat degradation of many animals, urbanization, illegal commerce of wild animals, including their consumption as food, in addition to the increase in global temperature, favors human-animal contact and thus, the emergence of many diseases never before reported such as COVID-19 (WHO, 2021).

2.1. Genetics basis of SARS-CoV-2

The SARS-CoV-2 is comprised of an RNA genome composed of a positive-sense single chain. In its genomes are present coronavirus genes that codify structural proteins, which are involved to viral membrane, envelope and nucleocapsid synthesis. Another important, the spike protein (protein S), is close related to the transmission of the virus (Tufan et al., 2020). Although phylopathogenesis of SARS-CoV-2 is not yet well determined (García-Salido, 2020), the genetic sequences of this virus are available in public databases such as GISAID (https://www.gisaid.org/). The scientific community can access

the reference sequence of the SARS-CoV-2 genome - NCBI reference number: NC_045512 (Hu et al., 2020a) and several other sequences, which allows comparing the sequences of the coronaviruses and monitoring the evolution of the pandemic in real time.

The Coronavirus genomes present a size from 26 to 32 Kb base pairs. SARS-CoV-2 is an enveloped virus, with a single-chain RNA genome and the size of its genome reaches almost 30 Kb, harboring 14 ORFs that encode 50 proteins (Khailany et al., 2020; Lu et al., 2020; Raskin, 2020; Wang et al., 2020d). Its genome architecture showed that genes present in the 3' portion of the genome, including surface protein (S), envelope (E), membrane (M), and nucleocapsid synthesis (N), encode structural proteins. This portion is located in only one third of the whole genome. The two-thirds portions of the genome located in the 5' region comprise coding sequences (ORF1a and ORF1ab) which encode polyproteins (PP1a and PP1ab), respectively. Others nine accessory proteins are found in SARS-CoV genomes (Khailany et al., 2020; Raskin, 2020) and are crucial for the production of an imbalance in the host immune response called cytokine storm. Sixteen non-structural proteins are identified are they are involved in process such as replication and proofreading activity, translation, defense against host proteins and immune system scape (Raskin, 2020). The surface glycoprotein S is one of the key factors in the invasion of SARS-CoV-2 into the host system and the transmission of the virus among humans (Zhu et al., 2018; Huang et al., 2020).

Mulato-Briones et al. (2020) performed out a genomic computational analysis, that compared several SARS-CoV-2 isolates from the United States, Brazil, Nepal, and China and the majority of genomes share high identity values in more than 99% in all cases (Mulato-Briones et al., 2020). Mulato-Briones et al. (2020) state that approximately four base pairs were different among the isolates analyzed, which is an indication that the SARS-CoV-2 genome has high stability (Mulato-Briones et al., 2020). Comparing the causative agent of COVID-19 with other coronaviruses, its genomic structure is closely related to the BatCoVRatG13 (found in a horseshoe bat, *Rhinolophus affinis*), sharing an identity of the 96% of its genomic sequence (Hu et al., 2020a; Pastrian-Soto, 2020; Wang et al., 2020d). This close genetic similarity with RaTG13 lead the scientific community to affirm that SARS-CoV-2 could have come from bats (García-Salido, 2020; Lauxamann et al., 2020). Furthermore, current works reported a greater similarity with another virus, the 'RmYN02', an emergent coronavirus isolated from the bat *Rhinolophus malayanus* (Hu et al., 2020a). This virus has been sampled in Yunnan, China reaching a 97.2% of similarity with SARS-CoV-2 (Hu et al., 2020a). Finally, the current human coronavirus shares 79% of genomic similarity with its predecessor SARS-CoV and 50% of genomic similarity with MERS-CoV (Hu et al., 2020a). The Coronavirus Study Group (CSG) has recognized the SARS-CoV-2 closely related to SARS-CoV (Lauxamann et al., 2020). In phylogenetic arrangements, SARS-CoV-2 is grouped parallel to SARSr-CoVs and SARS-CoV. However, it was considered part of a distinct group lineage consisting of four horseshoe bat coronaviruses (RaTG13, RmYN02, ZC45 and ZXC21) (Hu et al., 2020a).

2.2. Current variants of SARS-CoV-2 across the countries

Generally, genetic material of living beings and viruses is subject to changes in its either content, by mutations, recombination, gain or loss of part of its genomic sequence (Fleischmann, 1996; Lauring and Hodcroft, 2021; Mohammadi et al., 2021). These changes

can be the result of interaction with internal factors (i.e., resulting of cellular processes) as well as external factors (for example, chemical compounds) (Fleischmann, 1996). In viruses, mutations are usually present and can occur as result of their replication process, which can generate specific and punctual changes in their original genomic sequence (Fleischmann, 1996; Callaway, 2020; Lauring and Hodcroft, 2021; Mohammadi et al., 2021). Coronaviruses also tend to present frequent genetic recombination events (Singh and Yi, 2021).

Mutations in viruses' genomes can arise resulting of the replication process of their genetic material and can be associated with certain enzymes involved in replication (Callaway, 2020; Lauring and Hodcroft, 2021; Mohammadi et al., 2021). However, they can also arise from the action of enzymes present in the host, for example, and even from spontaneous damage to nucleic acids. Generally, RNA viruses, including coronaviruses, have a higher mutation rate, which is not observed for DNA viruses (Callaway, 2020; Lauring and Hodcroft, 2021). For coronaviruses, a lower mutation rate is observed and this is probably due to the action of an enzyme that could correct the errors produced in the replication processes (Fleischmann, 1996; Callaway, 2020; Lauring and Hodcroft, 2021; Mohammadi et al., 2021). In addition, the size of the genome, the degree of fidelity of this enzyme that corrects post-replication errors, among other factors, can also influence the rate of viral mutation (Mohammadi et al., 2021).

The WHO classified the virus variants based on changes in their genomic content through one or more mutations, which can lead to changes in the infection and transmission of that virus with respect to the original virus. According to Center for Disease Control and Prevention (CDC), during the last pandemic months have been emerged several genetics variants of SARS-CoV-2. Comparative analysis genomic sequences among these viruses isolated from distinct countries have allowed identifying genetic differences, most commonly, somatic mutations. Two types of variants are of interesting in public health: the variant of interest and variant of concern. To classify one variant such as interest some criteria are take into account: specific genetic marker that could affect the transmission processes, the diagnosis, and the therapies available, as well as, to be able to escape of host immune system. Furthermore, these variants can lead to an increase in the number of specific cases or outbreaks and be locally restricted to a specific location. In accordance with CDC, currently there are not any variant of interest, all of them were formed variant being monitoring (VBM). Among them are Alpha (B.1.1.7) from United Kingdom (September 2020); Beta (B.1.351, B.1.351.2, B.1.351.3) from South Africa (May 2020); Gamma (P.1, P.1.1, P.1.2) from Japan/Brazil (November 2020); Delta (B.1.617.2, AY.1, AY.2, AY.3, AY.4, AY.5, AY.6, AY.7, AY.8, AY.9, AY.10, AY.11, AY.12) from India (Octuber 2020); Epsilon (B.1.427 and B.1.429) from United States of America (March 2020); Eta (B.1.525) from several countries (December 2020); Iota (B.1.526) from United States of America (November 2020); Kappa (B.1.617.1) from India (Octuber 2020); 1.617.3 from India (Octuber 02020), Zeta (P.2) from Brazil (April 2020) and Mu (B.1.621) from Colombia was declared by the World Health Organization (WHO) (June, 2022). The second classification of variants (variants of concern), considers an increase of transmissibility, the emerging of severe disease with increase of death cases, inefficacy of treatment and immunity control through vaccination and failure in the diagnosis methods available. In these classification is Omicron (B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4 and BA.5 lineages) from South Africa (June, 2022) according to World Health Organization (WHO).

Supplementary 1 summarizes the SARS-CoV-2 variants found to date and their characteristics related to genetic and immunological changes found.

Globally, an increase in the number of countries and territories reporting continues to be observed. However, this increase must take into account the limitations related to surveillance systems or surveillance mechanisms, as well as the capacity of the countries and territories to sequence samples. According to the COVID-19 WHO Weekly Epidemiological Update published on 26th June 2022, the Omicron variant has been worldwide reported. This variant remains the dominant variant circulating in the world, reaching 94% of the sequences generated. The rates linked to each lineage are BA.2 (25%), BA.2.12.1 (11%), BA.4 (12%) and BA.5 represents 43%.

2.3. Transmission and infectivity of SARS-CoV-2

Depending on the variant, the transmission and infectivity can change. However, in this section we will describe this topic focusing on the generalities of the transmission and infectivity of SARS-CoV-2. The common transmission route of the virus is via airborne droplets (Pastrian-Soto, 2020), either directly through exposure to cough or sneeze at around 1.8 meters or less (Lauxamann et al., 2020; Sepúlveda et al., 2020) or entering through the mouth, nose and eyes mucosa due to physical contact (Lauxamann et al., 2020). However, airborne transmission mechanism still need a better understanding (Sepúlveda et al., 2020). Although evidence suggests that oral-fecal or vertical mother-fetus routes are still not ruled out (Quintero et al., 2020). Recent studies reveal that in 20% of patients with COVID-19, the viral RNA was found positive in faecal matter, opening the possibility of indirect transmission via faecal-oral transmission (Hu et al., 2020a; Lauxamann et al., 2020). Another source of contagion is during dental or medical procedures with patients' saliva or contaminated blood exposure (Lauxamann et al., 2020; Sepúlveda et al., 2020). In addition, contaminated surfaces are also a great source of COVID-19 transmission (Lauxamann et al., 2020).

Studies also reveal that estimated average incubation period is between 3 to 9 days, with a range oscillating between 0 to 24 days (Sepúlveda et al., 2020). This study also suggest that the highest viral risk (44%) of transmission occurs prior to symptoms onset, whilst 18% of the SARS-CoV-2 positive cases remain asymptomatic, most of them being young patients. Asymptomatic infections refer to the positive detection of nucleic acid of SARS-CoV-2 in patient samples by reverse transcriptase polymerase chain reaction (RT-PCR), but have no typical clinical symptoms or signs, and no apparent abnormalities in images, including lung computed tomography (CT). Although patients tend to overcome after 10 to 15 days in most of the cases, severe cases continue to contagious for up to 25 days from the initial onset of symptoms (Sepúlveda et al., 2020). The asymptomatic patients are fully capable of transmitting the virus (Vardhana and Wolchok, 2020). In addition, the prolonged human contact may play a role in virus adaptation to humans.

2.4. Clinical aspects of the COVID-19 pandemic

According to the progression of COVID-19, the clinical variation range from asymptomatic, mild clinical symptoms to acute respiratory-distress syndrome (ARDS) and even death in some of the cases (Ahmadpoor and Rostaing, 2020; García-Salido, 2020;

Tufan et al., 2020). The first clinical and epidemiological characteristics of COVID-19 show symptoms of fever, dry cough, chest malaise, headache, gastrointestinal infection, and viral pneumonia (Lauxamann et al., 2020). However, the most critical cases presented dyspnea and infiltration in both lungs (Hu et al., 2020b). In addition, digestive manifestations include weight loss, ranged from 39.9%-50.2% of cases, diarrhea, nausea, vomiting, abdominal pain and gastrointestinal bleeding (4.0%) during critical events (Hu et al., 2020b). Case guideline of COVID-19 also point out a decrease in lymphocytes as well as reduction of white blood cells in infected patients (Lauxamann et al., 2020). Moreover, SARS-CoV-2 might produce serious cardiac and renal injury, especially in elderly people or patients with comorbidities (Pastrian-Soto, 2020; Sepúlveda et al., 2020; Tufan et al., 2020; Vieira et al., 2020). Those prevalent comorbidities for COVID-19 cases have been classified as hypertension (30.7%), diabetes mellitus (14.3%), cardiovascular disease (11.9%), cerebrovascular disease (6.6%), malignant neoplasm (4.3%), chronic liver disease (2.8%), chronic pulmonary disease (2.4%), chronic kidney disease (2.1%) and HIV (1.4%) (Sepúlveda et al., 2020). Although most of the SARS-CoV-2 -infected individuals recover without requiring hospitalization, many others remain asymptomatic due to strong immune responses (Laing et al., 2020).

There is limited information about the clinical course and viral load in asymptomatic patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, current studies indicate that, approximately 80% of COVID-19 cases are asymptomatic or mild, 15% are severe and require oxygen, and 5% are critical infections that require ventilation (WHO 2020). A notable feature of SARS-CoV-2 is asymptomatic infection, where a fraction of the population infected with the virus do not experience, develop and report symptoms (Champredon and Moghadas, 2017). The asymptomatic infection, show the importance of "unknown" carriers of the virus. The transmission probability relative starts rising just over two days before symptom onset, and that ~ 44% of transmission may occur prior to symptom onset (He et al., 2021). The transmission and viral load data the study from He et al. (2020) would suggest that prior to 2.4 days before symptom onset, infected people may not have sufficient virus to be diagnostic by a test (Jarvis et al., 2020; Lucirka et al., 2020; He et al., 2021). The findings emphasize that control measures should be adjusted to account for probable substantial presymptomatic transmission.

2.5. What is happening with host immune system during SARV-CoV-2 infection?

The human immune system is a powerful machinery in which cells and molecules of the innate and adaptive system act against a pathogen, in that case, the SARS-CoV-2 (de León Delgado et al., 2020). The immune system has the capacity to produce antibodies that neutralize the interaction between the pathogens and cell receptors, by limiting their ability to access the host cell structure (de León Delgado et al., 2020). It is also responsible for activating the natural killer (NK) lymphocytes by producing a cytokine effect over the infected cells through the mechanism of apoptosis or cell death (de León Delgado et al., 2020). Scientific evidence shows that ancestors of SARS-CoV-2 produce a high immune response (IR) through pro-inflammatory cytokines (García-Salido, 2020) produced by the white blood cells of the host. Recent advances suggest that the innate IR inhibits virus

replication, promote viral clearance, limit viral spread throughout the organism, while facilitating a prolonged adaptive response responsible for controlling viral infection and clinical recovery (Ahmadpoor and Rostaing, 2020; Lauxamann et al., 2020). However, a high increase in the immune response may play an opposite role such as the risk of attacking not only the virus but also lung cells, leading to secondary complications of immunopathogenesis. These secondary complications include pulmonary tissue damage, reduced lung capacity, respiratory distress syndrome (Lauxamann et al., 2020) or even heart, liver or brain damage (de León Delgado et al., 2020). Therefore, the lack of an adequate adaptive response could lead to persistent innate-induced inflammation driven by a "cytokine storm" (Ahmadpoor and Rostaing, 2020). Some studies reveal a significant association between severity of COVID-19 and levels of proinflammatory cytokines provided by the immune cells (Tufan et al., 2020).

Although an efficient immune response may be considered fundamental in the fight against COVID-19 (García-Salido, 2020; Tufan et al., 2020; Vieira et al., 2020), the understanding of the reasons for which the immune system fails or weakens facing the infection of SARS-CoV-2 becomes the main objective of many researches. Some studies report that clinical severity of COVID-19 is a result of the excessive activation of host immune response (García et al., 2020).

2.5.1. Entry into human host cells

The initial pathway of coronavirus infection is mediated by binding of virus particles to host receptor cells following by fusion of their membranes (Ashraf et al., 2021). The virus needs entries in the host intracellular environment to use the protein synthesis machinery from host to translate its own proteins (Ashraf et al., 2021). The glycoprotein called spike (S), play an important role in initial attachment process and it present several levels of amino acid conservation across members of *Coronaviridae* family (Casalino et al., 2020). This homo-trimeric protein, as known as class I fusion protein, is inserted in the virion envelope and present each monomer subdivided in two subunits (S1 and S2) (Casalino et al., 2020; Conceicao et al., 2020; Ashraf et al., 2021). Each subunit perform a function in binding viral particle to host cell receptors. The S2 subunit participates of viral and host membranes fusion (Casalino et al., 2020).

The S1 subunit through of its receptor-binding motif (RBM) interacts with N-terminal helix of angiotensin-converting enzyme 2 (ACE2) receptor at host environment and finally can start the entry process (Casalino et al., 2020; Chowdhury et al., 2020; Ashraf et al., 2021). The ACE2 receptor, an zinc-binding carboxypeptidase, are located in heart, lung, kidney, vasculature, nasal and oral mucosa, gastrointestinal tract, pancreas and brain cells surface of human body. Generally, this enzyme controls the maturation of angiotensin hormone, which in turn, plays a role in pressure blood and vasoconstriction regulation (Yan et al., 2020; Ashraf et al., 2021). Two proteins spike through their receptor-binding motif (RBM) can make contact with the N-terminal helix peptidase domain (PD) of ACE2 receptor in host cells, which have higher affinity and thus, is formed the SARS-CoV-2 /ACE2 complex. The transmembrane serine protease 2 is activated and this complex undergoes endocytosis to host intracellular environment. After, the endosome is acidified and the genetic material of virus available in the cytoplasm for replication and translation. However, SARS-CoV-2 could use others receptors to entry in host cell such as the

transmembrane protease serine 2 (TMPRSS2) and Glucose regulated protein 78 (GRP78) (Hoffmann et al., 2020; Gadanec et al., 2021), Figure 1.

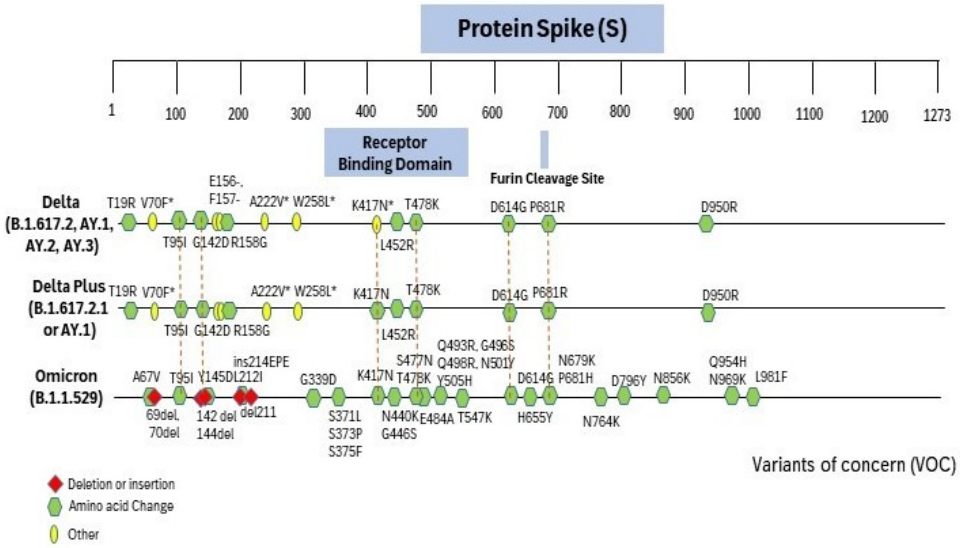

Figure 1. Schematic representation of Immune Response during SARS-CoV-2 Infection. A) The binding of SARS-CoV2 particles to host receptor cells (ACE2/TMPRSS2) followed by fusion of their membranes is the initial pathway of COVID-19 infection. B) A variety of pattern-recognition receptors (PRRs) detect the released pathogen-associated molecular patterns (PAMPs); once the virus enters, it actively replicates causing pyroptosis in the cell, resulting in an increase the production of pro-inflammatory cytokines "cytokine storm". C) Immunological memory consists of memory CD4+ T cells, memory CD8+ T cells, memory B cells and IgA, IgM and IgG antibodies in its receptor-binding domain (RBD).

The infectivity and the rate of transmissibility of the SARS-CoV-2 variants could be linked to the route of entry that SARS-CoV-2 takes to infect the host cell. According to the CDC, the Omicron variant is highly contagious, more so than other variants, making the Omicron variant dangerous. This variant has several mutations in the spike protein and in its RBD domain, which could turn some vaccines ineffective, hindering the acquired immunity response against SARS-CoV-2. Specific variations (K417N and T478K) in different spike protein sites make this variant have a higher affinity for binding to ACE2 receptors found on the surface of certain human cell types, which allow the immune escape to the host's cellular environment as well as increasing transmission capacity and infectivity (Hagen, 2021).

Finally, despite the fact that SARS-CoV-2 has high affinity for ACE2 receptor, the of trimer structure formation into spike protein reduces the accessibility of SARS-CoV-2 to receptor, so the virus is less exposed to immune system of host. Additionally, SARS-CoV-2 has a polybasic site, a genetic sequence 12 base pairs long (four amino acids), within of the spike protein. The polybasic site forms an exposed loop and, therefore, facilitates the cleavage of protein S by host proteases (furin) aiding to process of SARS-CoV-2 infection (Raskin, 2020).

The variant Omicron (B.1.1.529) from South Africa present several mutations that lead to amino acid substitution into spike protein (A67V, del69-70, T95I, del142-144,

Y145D, del211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F), World Health Organization (WHO). Additionally, can lead an increasing of transmissibility, can reduce the effectivity of treatments with monoclonal antibodies and affects the neutralization by post-vaccination. The map of important mutations in the two most important variants of SARS-CoV-2 are represented in Figure 2.

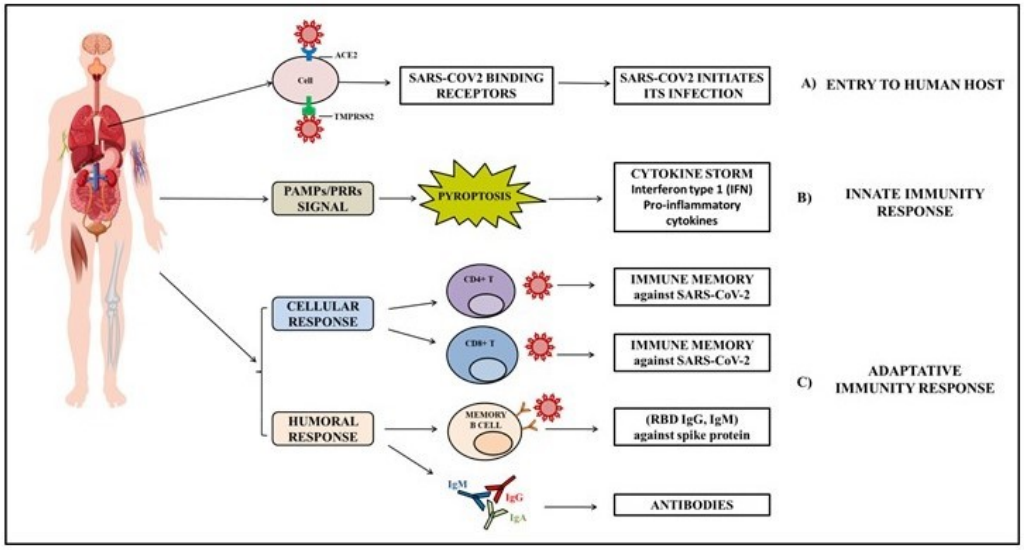

Figure 2. Schematic representation of important mutations in the spike protein of the SARS-CoV-2 virus. Main SARS-Cov2 variants during the COVID-19 pandemic according to the Centers for Disease Control and Prevention (CDC).

2.5.2. Innate immunity response generated by SARS-CoV-2

Vertebrates show two general types of immune response against viral infections. One is a rapid "innate" response to eliminate the infection. In some cases, innate response could be sufficient to eliminate an infection (de León Delgado et al., 2020). Innate immune response to combat SARS-CoV-2 infection, similar to that of other Coronaviruses and microorganisms, starts with activation of pattern recognition receptors (PRRs). During the binding of SARS-CoV-2 to the membrane surface, its internalization, endosomal transport, removal of the cytosolic coating, polymerization of its genomic material (RNA), and translation of viral proteins inside host environment are active by many pathogen-associated molecular patterns (PAMPs), such as cell membrane, endosomes and cytoplasm (Chen et al., 2020a). In turn, it triggers an increased expression of interferon type 1 (IFN) and other pro-inflammatory cytokines, Figure 1. This immune dysregulation, called hypercytokinemia or cytokine storm," is often associated with adverse outcomes such as respiratory disorders (Chen et al., 2020b). The genetic mutations of SARS-CoV-2, in comparison to the Pangolin-CoV, MERS-CoV, SARS-CoV and RaTG13 might provide information concerning to deactivation or overexpression of the IFN pathway in COVID-19 (Kumar et al., 2021; Zhang et. al, 2022). Once the virus enters, it actively replicates causing

pyropoptosis in the cell, resulting in the release of harmful compounds and consequently the growth of pro-inflammatory cytokines production around the surrounding cells, including the endothelial, epithelial, and macrophages. Pyroptosis is defined as a highly inflammatory form of programmed cell death, which is commonly related to infectious and autoimmune diseases (Yu et al., 2021). During the initial inflammation process, macrophages attracted by interleukin 8 (IL-8) are responsible for recognizing SARS-CoV-2 viral particles that have been neutralized. Apoptotic cells and pathogen-associated pattern molecules (PAMPs) are removed by phagocytosis (Poon et al., 2014). Therefore, it prevents their binding to PRRs and induces more inflammation. (Tay et al., 2020). The associated lymphopenia might be mainly explained due to the pulmonary recruitment of immune cells from the blood and the lymphocyte infiltration into the airways, in addition to the increased neutrophil-lymphocyte ratio observed in approximately 80% of the SARS-CoV-2 patients (Wang et al., 2020b). The reduction in mobility of COVID-19 infection is determined, almost exclusively, by an adequate balance of innate immune responses between resistance and tolerance, such as occurs against a coronavirus infection in bats (Barnejee et al., 2019).

In addition, after an asymptomatic SARS-CoV-2 infection, people with unhealthy immune systems may exhibit a pattern of illness by suffering an over-immune reaction commonly named as cytokine storm, and consequently an increased respiratory distress that could potentially lead to death (McGonagle et al., 2020). This uncontrolled increase of cytokines due to COVID-19 might suggests, in most of the cases, a failure in the pattern recognition mechanism of the immune system. The role of the interferon (IFN) inducible genes such as Interferon stimulated gene (ISG) and MxA proteins are vital to prevent the formation of progeny virions through the inactivation of the nucleocapsid protein (NC) or the ribonucleoprotein inhibition (Kumar et al., 2021). Controlling cytokine storm in the very early stage of COVID-19, via immunomodulatory drugs and cytokine antagonists, as well as reducing the infiltration of pulmonary inflammatory cells, are the key components for improving the success rate of treatments, as well as reducing the mortality rate of patients with COVID-19 (Ye et al., 2020). Previous studies have revealed that serum levels of cytokines are significantly high in patients with acute respiratory distress syndrome (ARDS), being positively correlated the level of cytokine increase, with the mortality rate (Parsons et al., 2005). In addition, the elevation of the cytokines rates might contribute to a clinical course of extrapulmonary multi-organ failure in patients (Wang and Ma, 2008). Some studies have found that germline variants of genes related to primary immunodeficiency (PID), including anomalies of innate immunity response, are strongly attached to hereditary factors which deregulate the inflammatory responses of the host during the COVID-19 infection (Cunningham et al., 2020). A set of twelve (12) primary immunodeficiency's (PID) genes, listed as follows: PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, AP3B1, SH2D1A, BIRC4, ITK, CD27 and MAGT1 have been recognized as triggers of cytokine storms, being called hemophagocytic lymphohistiocytosis (HLH) genes (Al-Samkari and Berliner, 2018). A recent study performed by Luo (2021) and colleagues, focused on the whole exome sequencing, using 233 COVID-19 hospitalized patients, it provided the identification of four variants of the PID genes called: UNC13D; AP3B1; RNF168; DHX58 which were significantly enriched in patients with COVID-19, who also experienced serious cytokine storms. The germline variants of NC13D and AP3B1 for instance, were found associated with severe cytokine storms, causing fatal outcomes to the patients. Those genetic implications provide relevant information for a better

understanding of the optimal individual conditions to develop severe cytokine storms in certain patients (Luo et al., 2021).

SARS-CoV-2, also activates a complex and powerful proinflammatory leukocytes based on eosinophil cells, characterized by expression of preformed cationic granules comprised by cytotoxic proteins, such as: eosinophil peroxidase (EPO) and RNase 2 (neurotoxin eosinophilic and cationic eosinophilic proteins) (Fulkerson and Rothenberg, 2013; Ramirez et al., 2018; Flores-Torres et al., 2019). As a result, those cells produce reactive nitrogen substance for antiviral purposes (Flores-Torres et al., 2019). At the early stage of the infection, the activation of eosinophil cells could be beneficial, as they facilitate the removal of viral replication (Fulkerson and Rothenberg, 2013). However, Ho (2021) and colleagues suggest this response might also provide protection against COVID-19 infection. According to the authors, blocking eosinophil activation, in critical COVID-19 patients, could lead to a harmful immunity process. This study emphasizes the importance of eosinophils cells, as key components for antiviral immunity response, particularly in relation to SARS-CoV-2 (Ho et al., 2021). Further studies are necessary to better understand those mechanisms of activation. Furthermore, a special attention should be put to clinical studies for the prognostic implication of eosinophilic inflammatory disorders produced by COVID-19, and the eosinophilia as a key diagnostic parameter of COVID-19 infection.

2.5.3. Adaptive immunity response generated by SARS-CoV-2

The "adaptive immune response" is activated once the virus infection progresses beyond the first round of viral replication (Klimpel, 1996; Mueller and Rouse, 2008; Rouse and Sehrawat, 2010). The adaptive immune response itself is composed by two different classes of reactions: the humoral response (mediated by the synthesis of the virus-specific antibodies B-lymphocytes) and the T cell-response (mediated by the synthesis of specific cytotoxic T lymphocytes able to destroy the infected cells). Such components or reactions of the adaptive immune response are also responsible for the production of long-lived “memory cells" for more rapid response (immunity) to subsequent viral infection, caused by the same virus. The adaptive immune responses strongly depend on the major histocompatibility complex (MHC) class I and class II proteins (Iwasaki and Medzhitov, 2015).

SARS-CoV-2 generates specific immune memory induced by B and T cells, over a period of at least 6 to 8 months longer, after the onset of the infection (Hartley et al., 2020; Zuo et al., 2021). Substantial immune memory generated after initial SARS-CoV-2 infection four main types of cells: memory B cells, against spike protein (RBD IgG, IgM), $CD8^+$ T, $CD4^+$ T and antibodies, (see Figure 1). According to previous studies, the immune memory in 95% of SARS-CoV-2 infected subjects, measured in at least three of these immune compartments, reaches an average lifetime in a range of 5 to 8 months. In addition, these studies demonstrated the potential of that immune memory developed over time to face a possible secondary illness of COVID-19 in most of the population (Dan et. al, 2021; Sandberg et al., 2021). Long-lasting IgG peaks were identified in subject post infection, showing a slight decline of about 6 to 8 months in the entire population (Dan et al., 2021). On the contrary, the study carried out by Carsetti and colleagues (2020), included asymptomatic cases, highlighting the early and transient increase of IgA and IgM in a less

expressive way. It was found that specific IgG is associated with asymptomatic SARS-CoV-2 infection (Carsetti et al., 2020).

It is necessary to know the factors related to the host-pathogen relationship, particularly those that generate the virus-specific antibodies, which limit the spread of the causative agent of the disease or the overproduction of cytokines produced by host to combat the infection (Laing et al., 2020). One of the biggest issues for the control of SARS-CoV-2 is the ability to track the circulating variants and understand the diversity of major histocompatibility complex (MHC) class I and class II in the human population worldwide (Dearlove et al., 2020). According to the authors, MHCI and MHCII play a key role to activate the adaptive immune system and consequently establish a strong and long-lasting immune response. Experts also affirm that the MHC system is placed on the short arm of chromosome 6, considered the most complex and diverse genetic system within the human genome, because of the inclusion of HLA (human leukocyte antigen) genes (Breuning et al., 1977). The HLA gene variants have been implicated in the host susceptibility or resistance to a wide range of diseases, including those derived from SARS and MERS viruses (Lin et al., 2003; Hajeer et al., 2016). Concerning the relation between ethnicity and COVID-19 infection reveal that blacks (African Americans (8%) and Asians (5%) compared to Caucasians people (33%) are at higher risk of contracting COVID-19 (Koper J., et al., 2020). Studies carried out by Liang and colleagues (2021) show substantial differences in the presentation of the epitope of the SARS-CoV-2 proteins into the Major Histocompatibility Complex II (MHCII) based on different ethnic groups and slight differences in the presentation of epitope of the MHCI. These results indicate that the high level of COVID-19 mortality rates observed in some countries appear to be linked to a poor presentation of MHCII class and consequently a weak adaptive immune response against these viral envelope proteins (Liang et al., 2021). Nevertheless, patient heterogeneity, including not only ethnicity but also age, gender, clinical conditions and underlying illness are important drivers to be considered for COVID-19 transmission (Laing AG et al., 2020). Sepúlveda and colleagues (2020) states that the most vulnerable stratus of the population to be infected with COVID-19 are adults aged 30-79 years old (reaching 87% of the population). This rate are followed by the group aged 20-29 (8%), then those over 80 years old (3%) and finally the group of people under 19 years (2%) (Sepúlveda et al., 2019).

Langton and colleagues (2021) provided evidence that demonstrated that the HLA alleles could interact with patient conditions to increase the susceptibility to the development of serious complications of the disease. HLA alleles associated with asymptomatic disease are commonly seen in European descent, mainly coming from higher latitudes (Langton et al., 2021). However, the most remarkable finding of this study is related to the protection provided by the presence of the DRB1 *04:01 allele, which mainly occurs in 57% of the cases (Chen et al., 2002). According to the authors, some HLA alleles provide stronger protection because of the presence of multiple epitopes able to activate T cells, in response to invasive pathogens. Because both, genetic and medical conditions of patients play a key role either for disease severity, or for mortality rate of COVID-19, a better understanding regarding the connection between HLA alleles and SARS- CoV-2 proteins presentation could provide a broader perspective to understand the behavior of the virus. This understanding could strongly contribute to the development of more effective vaccines in the fight against COVID-19 (Noorimotlagh et al., 2020; Ramírez-Salinas et al., 2020; Smith et al., 2021). Up to date, the WHO has authorized clinical and preclinical

studies based on 238 candidate vaccines (WHO list as of February 2, 2021), being six of them, widely implemented worldwide nowadays (Oxford / AstraZeneca, Pfizer / BioNTech, Sinovac, Sinopharm, Modern and Sputnik).

Finally, the humoral immunity process against COVID-19 is crucial to provide a long-term prevention for the current pandemic. Despite the different studies, regarding acquired immune memory against SARS-CoV-2, Wu et al. (2021) stated that more studies are needed to better understand the long-term antibody responses with neutralized activities against SARS-CoV-2 (Wu and McGoogan, 2021). These authors conducted a clinical study with 349 symptomatic COVID-19 patients (585 samples) and found that human body produces immunoglobulins (IgM-S and IgG-S) commonly known as antibodies, in charge of recognizing the receptor-binding domain (RBD) of the Spike protein (S) from SARS-CoV-2. Furthermore, those molecules are able to recognize the nucleocapsid proteins (N) (IgM-N and IgG-N). The authors also found that during the first week after the onset of SARS-CoV-2, the immunoglobulin frequencies were IgM-S (67%)> IgG-N (33%)> IgM-N (22%)> IgG-S (11%) in those 349 patients. Although the main rate of IgM-S reached a peak of 95% in week 5, a significant decrease to 0% was observed in week 13. In the case of IgM-N, its rate was found around 72% in patients at week 3 and during the weeks 10 to 12 their rates were undetectable. Those patterns were not observed for IgG-S and IgG-N. Instead of that, during the first week, IgG-S reached a rate level of 98% and IgG-N, 88% and both indicators remained over time (26 weeks) (Wu et al., 2020).

In addition, the seroconversion incidence in patients with SARS-CoV-2 reached up to 90% between the 10 and 14 days (Zhao et al., 2019; Lou et al., 2020; Carrillo et al., 2021; Wu et al., 2021). As reported by Wu and colleagues (2021), the response against SARS-CoV-2 in several cases is transient, and the typical composition of IgA, IgG and IgM decrease after the 2 or 3 weeks (Zhao et al., 2019; Carrillo et al., 2021). However, IgA and IgG can be detected before the presence of IgM (during the 4 to 6 days after the onset of symptoms). The presence of a particular antibody appears to be conditioned by the severity of COVID-19 infection. According to previous studies, higher levels of IgG and IgA are observed during severe cases, compared to mild cases, in which the presence of those immunoglobulins are almost undetectable (Zhao et al., 2019; Long et al., 2020; Lou et al., 2020; Carrillo et al., 2021).

CONCLUSIONS

The current pandemic calls for action on many fronts that could play a key role in controlling the disease globally. The rapid growth of the world population; the huge increase in people living in urban areas; the transfer of pathogens from animals to humans; and a hyperconnected world facilitate the spread of the COVID-19 disease.

A real scenario of the COVID-19 pandemic shows disproportionate damage to poor countries. It has also been said that SARS-CoV-2 will probably never be globally eradicated due to zoonotic presence and attachment to the environment. Insights from this work revealed three critical factors shaping the course of the COVID-19 epidemic, summarized below: (i) population immunity and effective vaccination; (ii) VOC variants of concern and (iii) public response to the pandemic. Therefore, global strategies are urgently required to deal with the endemic presence of COVID-19 over a long period. In this sense, it is essential to generate more knowledge about how the human body responds to SARS-CoV-2

infection and how the genetic bases of this infectious agent interfere with this response in order to develop more protective vaccines and effective antiviral drugs.

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AUTHORS' CONTRIBUTIONS

MC, MSV, VD: Research design, database organization, data analysis, manuscript writing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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