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Coronavirus SARS-CoV-2 and Covid-19

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The causative agent of Covid-19, the severe acute respiratory syndrome (SARS), is a virus belonging to the *Coronaviridae* family, named SARS-CoV-2, which shows high homology with the virus that caused the SARS outbreak in 2003, SARS-CoV[1]. SARS-CoV-2 is a ribonucleic acid (RNA) virus, whose genetic material is a single positive RNA molecule (+RNA). Its entire genome contains less than 30,000 nucleotides, each one formed by a sugar molecule (ribose), a phosphoric acid and a nitrogenous base. Because it is an RNA virus, the nitrogenous bases are adenine, cytosine, guanine, and uracil. Approximately 29 different viral proteins are identified; among them, the most relevant are the spike (S) glycoprotein, and the nucleocapsid (N) protein[2]. The spike glycoprotein enables the virus entry into the host cell via binding to cell receptor and membrane fusion. The nucleocapsid protein, in its turn, regulates the process of viral replication.

SARS-CoV-2 is classified as +RNA because of its 5'-3' direction, meaning it can be directly read by cell structures. It is regarded as a type of messenger RNA that, when translated by cellular ribosomes, induces the production of viral proteins. Another characteristic of this type of RNA is the presence of the replicase enzyme (RNA polymerase), which accompanies the virus or is produced by the infected cells, when then the production of a negative (-) RNA molecule occurs from the +RNA molecule, typical of the virus. The -RNA molecule is transient, and based on it, innumerable +RNA molecules are produced that are identical to the original +RNA. Therefore, the transient -RNA molecule serves as a template for the production of +RNA molecules; each of them will descend from the virus that infected the cell; these descendants will parasitize the cell and reproduce inside it.

The viral structure is represented in **Figure 1**.

The synthesis of the new +RNA molecules occurs inside cellular vesicular structures, called endosomes; they become organized as soon as viral +RNA enters cellular inner space. The production of viral proteins, on its turn, takes place with the participation of ribosomes bound to the rough endoplasmic reticulum in the presence of the Golgi apparatus, where the assembly of virions occurs (they are so called because are still not really viruses).

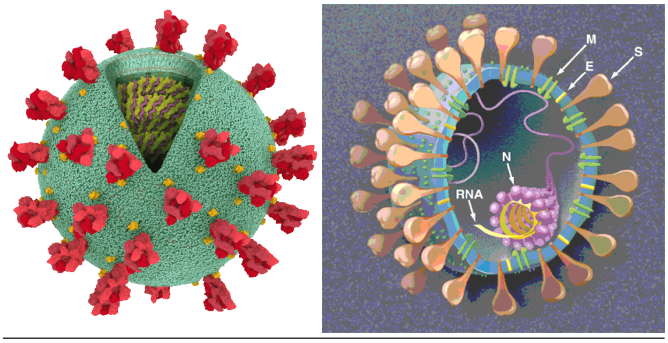

FIGURE 1 – *Structure of SARS-CoV-2*

Sources: A) Corum J, Zimmer C (2020)[3]; B) Holmes KV, Enjuanes L (2003)[4].

M: membrane protein; S: viral spike binding to cell receptors; E: envelope protein; N: nucleocapsid protein.

The virus attachment with the target cell happens by means of the S protein, which interacts with cell receptors – this process resembles a flash drive with an integrated USB interface. **Figure 2** represents the S protein.

That protein comes into contact with the protein cell receptor, the angiotensin-converting enzyme 2 (ACE2), which is especially present in lung cells. With the linking of both proteins, the virus can invade the cell. **Figure 3** illustrates the binding of both proteins.

FIGURE 2 – *S protein, responsible for viral binding to host cell*

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FIGURE 3 – *Binding of S protein to ACE2 receptor*

ACE2 is represented in yellow and corresponds to the USB port of a computer.

ACE2: angiotensin-converting enzyme 2.

After the fusion of the proteins – the viral and the ACE2 receptor –, the fusion of the lipid membrane of the virus occurs with the cell plasma membrane, and the virus gains the interior of the cell, as can be observed in **Figures 4** and **5**.

After the introduction of the viral genetic material in the host cell, a cellular vesicle is formed (a kind of bag, called endosome); inside it, the virus is retained and multiplies. Later, the +RNA molecules produced inside the endosomes are released, and the synthesis of viral proteins happens.

The production of new +RNA molecules of the virus occurs in the cell endosomes thanks to replicase enzymes already existing in the incoming virus. **Figure 6** briefly illustrates the procedure resulting in the production of new +RNA molecules of the virus.

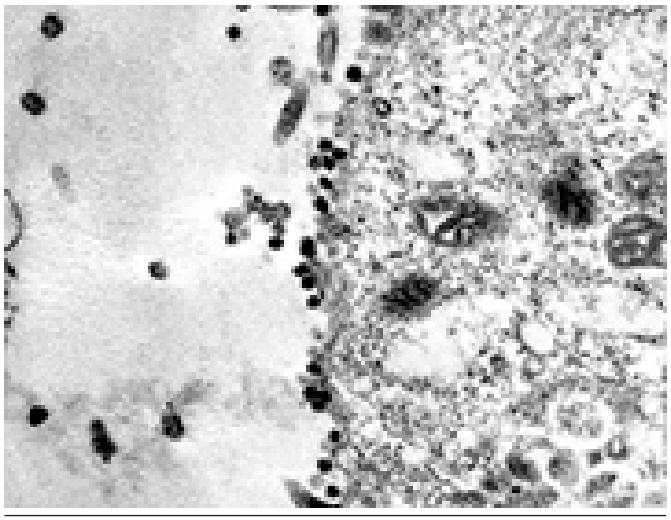

FIGURE 4 – *Adhesion between virus and cell membrane*

Source: Instituto Oswaldo Cruz (IOC/Fiocruz).

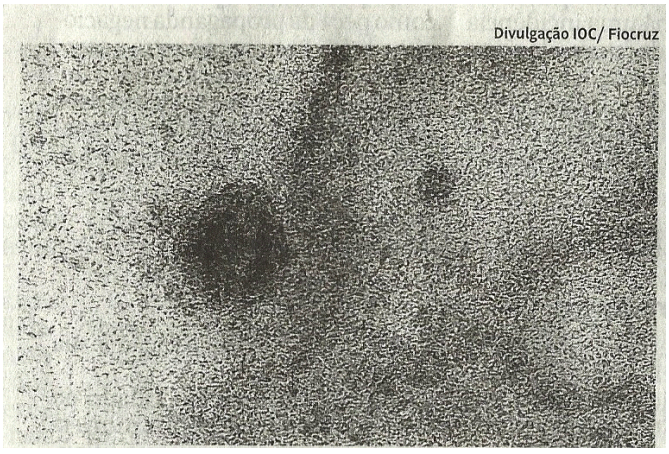

FIGURE 5 – *Virus internalization into the host cell*

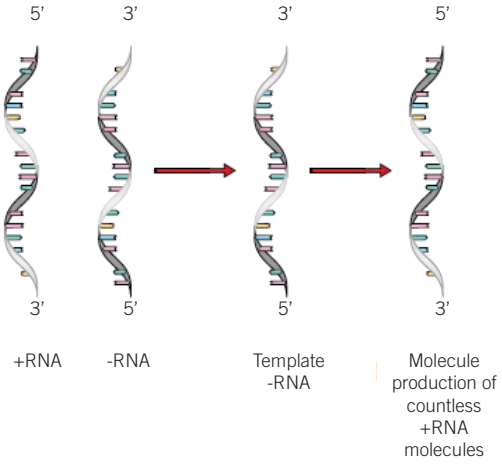

FIGURE 6 – *Scheme of virus replication*

+RNA: positive ribonucleic acid; -RNA: negative ribonucleic acid.

The -RNA molecule produced based on the +RNA molecule acts as a template for the production of countless +RNA copies, which will be components of the genetic material of the descendants of the virus that broke into the cell.

With the viral proteins already produced, the assembly of virions occurs by the inclusion of +RNA molecules in protein capsids. As they approach the cell membrane, already totally worn out, the virions – with their capsids and protein spikes – are surrounded by a lipid bilayer, probably originated from the cell membrane of the host cell, which will break open at the end of the process, releasing a great amount of viruses to the outside. In this case, they are already the descendants of the virus that invaded the cell. Some scientific studies showed that when new viruses try to escape a cell, the cell can capture them with tetherins (proteins). However, some researches suggest the existence of another viral protein, the ORF7 – required for viral release –; it would reduce

an infected cell's supply of tetherin, preventing viruses from being captured and allowing more viruses to escape. Researchers also discovered that the ORF7 protein can initiate apoptosis, that is, the infected cells commit suicide, what contributes to the damage that Covid-19 causes to the pulmonary alveoli cells.

To gain the interior of host cells, SARS-CoV-2 depends on a serine protease called transmembrane protease serine 2 (TMPRSS2), which has the ability to cleave and activate S protein, allowing the virus to bind to the ACE2 receptor. That action of the serine protease favors the virus adhesion to the cell plasm membrane and enables its entry into cells, that is, in the endosomes[5]. The nafamostat mesylate is a substance usually used in the treatment of acute pancreatitis and other diseases that lead to the formation of blood clots. According to studies conducted by Danish researchers, the mesylate acts on TMPRSS2 membrane protein, blocking its action, thus hindering the virus entry to the cell. Several studies are in progress, which aim to prove this blocking action on virus entry, what can result in a treatment against the microorganism[6].

Besides the clinical characteristics, represented by symptoms such as fever, headache, coughing, sneezing, general malaise, among others, there are two possibilities for the establishment of diagnosis. One of them is related with the recognition of the virus genetic material by the reverse transcriptase polymerase chain reaction (RT-PCR), from nasal and oropharyngeal swabs. The second possibility aims at finding and recognizing antibodies present in the serum of supposedly infected patients, a method known as serology for SARS-CoV-2.

The available treatment so far depends fundamentally upon the presented symptoms (high body temperature and typical symptoms similar to a strong flu). There is still no drug acting directly on the virus, as happens with oseltamivir, commercially known as Tamiflu, used in the treatment against the H1N1 influenza, which acts on the neuraminidase enzyme, favoring the exit of H1N1 from cells.

The most commented treatments for Covid-19 are chloroquine and its derivatives, hydroxychloroquine and chloroquine diphosphate. The action mechanism of those substances is reasonably known, and, presumably, it avoids the formation of cell endosomes and favors a discreet elevation of cell pH, a fact that hinders multiplication of the virus genetic material. In some hospitals of Brazil and other countries, the efficacy of these substances is the target of controlled experiments, whose aim is to verify if they are really effective. Traditionally, these substances, especially chloroquine, have been used in the treatment of rheumatoid arthritis, systemic erythematous lupus and malaria. However, their use, as authorities of public health and countless medical professional warn, can cause severe cardiac, hepatic and eye side effects. Therefore, the treatment of SARS-CoV-2 with chloroquine needs to be established by the assistant physician, the hospital where the treatment is carried out, and with the patient's consent. It is important to highlight that treatment with chloroquine, or its derivatives, has been associated with the concomitant use of the antibiotic azithromycin. According to known studies, besides acting as an efficient antibacterial agent, azithromycin has a property of keeping the integrity of epithelial lining of pulmonary alveoli.

Some other substances are also proposed in the treatment of this viral disease, all still in the phase of experimental proving. Among them, lopinavir-ritonavir can be cited, still in the trial phase. As for the medicines usually employed, both in the hospital setting and at home, there are pain-killers, antipyretics, and, especially, anti-inflammatory drugs, notably corticosteroids, as the virus acts upon the affected organs, especially the lungs, causing what is called an "inflammatory storm" or "cytokine storm", resulting from the mobilization of the body defense cells.

The possibility is discussed of using antibodies produced by convalescent patients, which could be employed in the treatment of other patients needing these antiviral defense molecules. Lately, the hypothesis that the vermifuge ivermectin can be used in the treatment was proposed, what will be verified by studies.

In hospitalized patients in serious condition, intubation with adequate equipment and the introduction of oxygen has been carried out to provide proper ventilation of pulmonary alveoli. There is still no vaccine to immunize people against this virus. In some countries (Israel, for example), the efficient preparation of a vaccine is at an advanced stage for prevention of Covid-19. It is necessary to wait for the results, what can take some time for disclosure and utilization.

Preventive measures are those habitually disseminated by public health authorities and media: avoid crowded environments, keep distance between people, stay indoors, go to establishments only in cases of extreme need, in other words, maintain social distancing. The use of masks and gloves is also recommended, especially when the subject needs to leave home. In many cities, establishments considered non-essential were closed, only essential ones remained open, such as supermarkets, pharmacies, hospitals, and treatment centers especially built and destined to this aim. Due to this scenario, delivery services have been used quite often in many places. As other measures to contain virus dissemination, traffic between cities has been blocked to reduce the possibility of spread.

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