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SARS-COV-2 VARIANTS AND THE SO-CALLED *RESISTANCE TO VACCINES*

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Abstract RNA viruses (except retroviruses) replicate by the action of an RNA-dependent RNA polymerase, which lacks a proofreading exonuclease and, consequently, errors may occur in each replication giving place to viral mutants. Depending on their fitness, these mutants either become extinct or thrive, spawning variants that escape the immune system. The most important SARS-CoV-2 mutations are those that alter the amino acid sequence in the viral S protein because this protein holds the key for the virus to enter the human cell. The more viruses replicate, the more they mutate, and the more likely it is that dominant *resistant* variants will appear. In such cases, more stringent measures for community protection will be required. Vaccines and polyclonal antibodies, which induce a response directed towards several sites along the S protein, would maintain effective protection against SARS-CoV-2 variants. Furthermore, vaccines appear to induce an increased helper and cytotoxic T-cell response, which may also be a biomarker of protection. In densely populated areas with insufficient protection measures, the virus spreads freely, thus increasing the likelihood of generating escape mutants. India and Manaus exemplify this situation. Natural evolution selects the mutants that multiply most efficiently without eliminating the host, thus facilitating their spread. Contrastingly, the circulation of viruses of high virulence and lethality (Ebola, hantavirus) that eliminate the host remain limited to certain geographic areas, without further dissemination. Therefore, it would be expected that SARS-CoV-2 will evolve into more infectious and less virulent variants.

Key words: coronavirus, SARS-CoV-2, viral resistance, escape variants, RNA polymerase, virulence

Resumen ***Las variantes de SARS-CoV-2 y la llamada* resistencia a las vacunas.** Los virus ARN, excepto los retrovirus, se replican por acción de una ARN polimerasa ARN-dependiente que carece de exonucleasa correctora y, en consecuencia, en cada replicación puede cometer errores. Así se originan mutantes que, según su menor o mayor *fitness*, se extinguen o bien prosperan y originan variantes que escapan al sistema inmune. Las mutaciones de SARS-CoV-2 más importantes son las que alteran la proteína viral S, porque ella tiene la llave de ingreso del virus a la célula humana. Cuanto más se replican los virus, más mutan, y se hace más probable que aparezcan variantes *resistentes* dominantes. En esos casos, se requerirá una aplicación más estricta de las medidas de protección de la comunidad. Las vacunas y los anticuerpos policlonales, que inducen una respuesta dirigida hacia toda la proteína S, mantendrían protección efectiva contra las variantes del SARS-CoV-2. Además, las vacunas inducirían una mayor respuesta de células T *helper* y citotóxicas, lo que puede ser un biomarcador de protección. En áreas densamente pobladas con escasas medidas de protección, el virus se difunde libremente y aumenta la probabilidad de mutaciones de escape. India y Manaos ejemplifican esa situación. La evolución natural selecciona las mutantes que se reproducen con mayor eficiencia sin eliminar al huésped, lo que facilita la propagación. En cambio, la circulación de virus de alta virulencia y letalidad (Ebola, hantavirus), que eliminan al huésped, se circunscribe a determinadas áreas geográficas, sin mayor difusión. Por lo tanto, sería esperable que SARS-CoV-2 evolucione a variantes más infecciosas y menos virulentas.

Palabras clave: coronavirus, SARS-CoV-2, resistencia a la vacuna, variantes de escape, ARN polimerasa, virulencia

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KEY POINTS

- RNA viruses replicate by the action of an RNA polymerase that lacks a proofreading exonuclease. In each replication, errors occur that originate mutants, some of which alter the S protein (spike protein on the outer surface of the virus), the key to entry of the virus into the human cell.
- The more viruses replicate, the more they mutate, the more likely varieties that evade the immune system will emerge.
- Vaccines and neutralizing antibodies would continue to protect against SARS-CoV-2 variants because they act on many antigenic determinants on the S protein rather than a single one.
- Furthermore, the vaccines appear to induce an increase in helper and cytotoxic T cells which, besides neutralizing antibodies, can induce protection.

The term resistance has been used to describe vaccination´s reduced effectiveness against some SARS-CoV-2 viral strains. By analogy with the idea of microbiological resistance, we refer to SARS-CoV-2 "resistance to vaccines and antibodies". However, resistance of microorganisms to antibiotics and chemotherapeutic agents emerges during treatment by selective pressure, while "resistant" variants of SARS-CoV-2 spread in the population regardless of the use of vaccines. In this article we draw some conclusions based on the analysis of similarities and differences between both types of resistance.

Resistance of bacteria and viruses to antibiotics

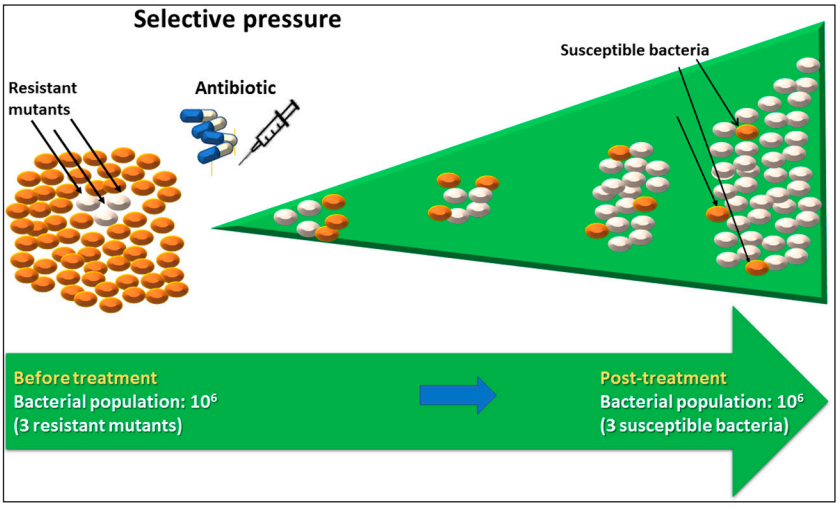
Bacteria and viruses can display natural or acquired resistance to the drugs used for their treatment, be they antibiotics or chemotherapeutic agents. For example, Gram-negative bacteria are naturally resistant to penicillin because a layer of lipopolysaccharides and proteins surrounding their cell wall prevents its penetration.

On the other hand, acquired resistance emerges in patients treated with a drug (be it antibiotic or chemotherapy) from spontaneous mutants produced at random before contact with that drug. Once in its presence, those resistant mutants continue to reproduce while the rest are eliminated. If just this medicine is given for a long time, the mutants might develop a population as large as the original, but made up completely of bacteria or viruses that are resistant to it. For this reason, two or more drugs are used in antiretroviral treatment for HIV-Aids, and four in the initial treatment of tuberculosis. The aim is to eliminate mutants resistant to an agent by simultaneously administering another, or others, with different mechanisms of action (Fig. 1).

Mutations and RNA virus

The genetic material of coronaviruses (like SARS-CoV-2) and orthomyxoviruses (like influenza) is RNA and not

Fig. 1.– Generation of secondary (acquired) bacterial resistance. *Image inspired by: Mutations and selection, with permission from ReAct- Action on antibiotics resistance (copyright). Original figure available in: https://www.reactgroup.org/toolbox/understand/antibiotic-resistance/mutation-and-selection/*



This Figure illustrates the case of a tuberculous lung cavern, with an initial population of 10^6 bacilli, among which there are 3 naturally resistant mutants to the drug (e.g. streptomycin). When a treatment is applied with this single bactericidal drug, susceptible bacilli disappear, but resistant mutants continue to reproduce, and can constitute a population of a magnitude similar to the original, but mainly composed of drug-resistant mutants

DNA. RNA viruses replicate and transcribe by the action of an RNA-dependent RNA polymerase (RdRp). Unlike DNA polymerases, which copy DNA with high fidelity because they possess a proofreading exonuclease domain, RdRp enzymes lack this domain, therefore fidelity is not guaranteed, and errors in genome replication with nucleotide substitutions occur frequently. This is the origin of mutants that, depending on their biological fitness, can either fade away if the mutation produces an adaptive handicap or else, thrive in the community if the mutation provides an advantage. This is how "escape variants" originate that can evade the immune system.

The case of influenza viruses

Influenza A and B viruses that cause the flu are constantly mutating. Most genetic changes occur slowly (antigenic drift) with a measurable annual frequency. The immune system does not recognize these new escape variants and the vaccine needs to be reformulated accordingly. Furthermore, six months following vaccination, levels of antibodies against influenza virus types A and B drop considerably and are no longer detectable after one year. The efficacy of vaccination then varies according to the population immunity, the differences between the circulating strains and those included in the vaccine, and the eventual appearance of new escape variants[1]. Major changes can also occur due to abrupt antigenic shifts, and generate a new subtype or genetic mixture that includes genes from different animal viruses. This happened in 2009 when a new virus A (H1N1) emerged through a combination of genes from the swine, avian and human influenza viruses. And this virus is highly related to the one responsible for the previous pandemic, the "Spanish" flu, which caused some 50 million deaths in 1918-1920[2].

Coronaviruses and mutations

Coronaviruses have certain characteristics that differentiate them from other RNA viruses: their genome is remarkably large (26-32 kb) and, in the absence of an exonuclease domain in their RdRp, they possess an exoribonuclease (ExoN) that helps to correct errors occurring during replication. As a result, the frequency of mutations is lower in coronaviruses (including SARS-CoV-2) than in other RNA viruses that lack the ExoN enzyme.

Mutants, variants, strains, and lineages

Although the terms "mutant", "variant" and "strain" are often used interchangeably in epidemiological descriptions of SARS-CoV-2, they do not have the same meaning. Mutations are changes in the nucleotide sequence of the genome and viruses carrying such changes are called "mutants". When a mutant is selected through numerous cycles of viral replication, a viral "variant" emerges[3]. If the variation results in a virus with distinct phenotypic characteristics, the variant is called a "strain". When, by genetic sequencing, a new variant is found to represent a different branch in the phylogenetic tree, we are in the presence of a new "lineage"[3].

The reason why some variants predominate over others is not well understood. But the most interesting questions concern their medical potential: are these variants or strains more widely transmitted? Are they more virulent? Above all, can they evade the immunity induced by vaccination or previous infection? If they do, they are called "escape variants" and sometimes also "resistant variants".

Mutations that alter the amino acid sequence of the S protein (spike protein) are of particular interest because it is the gateway of SARS-CoV-2 to the host cell and, as such, the vaccine target. The S protein binds the virus to its cellular receptor ACE2, the type 2 angiotensin-converting enzyme. This binding is mediated by the receptor-binding domain (RBD) located in S (Fig. 2).

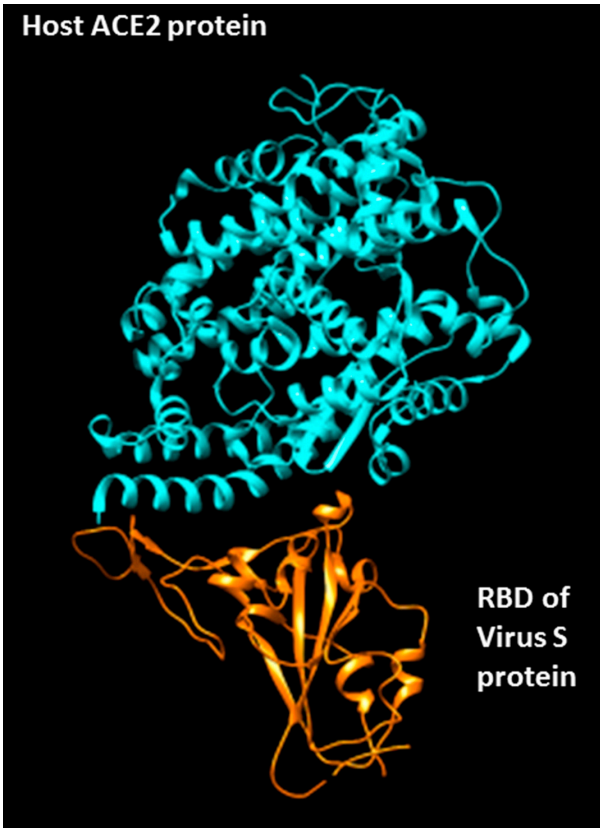
As of March 2020, SARS-CoV-2 variants containing the D614G substitution (from aspartic acid to glycine at position 614) of the S protein spread simultaneously in different regions and, within a few months, they became the dominant form of the virus worldwide. What was this due to? Could it be random? Several investigations, in humans and animal models, have shown that these D614G mutants can locate their RBD to interact more efficiently with the ACE2 receptor. This mutation is associated with a higher viral RNA load in the nasopharynx, which increases transmissibility[4, 5].

What variants of SARS-CoV-2 are the most important?

Once the SARS-CoV-2 sequence became known, the efforts of pharmaceutical companies and academia were directed, and with remarkable success, to the swift development and production of vaccines. But variants of the virus started also to appear.

Variant B.1.1.7, identified in the UK in October 2020, harbors 9 changes (deletions and substitutions) in the S protein compared to the original sequence. Variant B.1.351, discovered in South Africa the same month, harbors 10 changes. Variant P.1, from Manaus, Brazil, was identified in December 2020 and harbors 12 changes. Several of these changes are mutations in the RBD, located in the S1 subunit of the S protein, which is the region that interacts with ACE2, its receptor in the human cell membrane. The viral interaction surface is a small sequence of 25 amino acids located at the tip

Fig. 2.– Structure of the ACE2 receptor bound to the receptor-binding domain (RBD) of the S protein of the SARS-Cov2 virus. The crystallographic structure was created from a chimeric protein between the RBD of SARS-CoV-2 together with the human ACE2 receptor (PDB_ID: 6VW1); the visualization of the structure was done using the Chimera 1.10.2 program. Original source of the PDB: Shang J, Ye G, Shi K, et al. Structural basis of receptor recognition by SARS-CoV-2, *Nature* 2020; 581: 221-4



of RBD and is also the binding site of many powerful neutralizing antibodies.

The RBD mutations in these three SARS-CoV-2 variants are N501Y (substitution of asparagine for tyrosine at position 501) in B.1.1.7; K417N (from lysine to asparagine at position 417) and E484K (glutamic acid to lysine at position 484) in B.1.351; and finally three mutations in P.1: K417T (lysine for threonine at position 417), E484K, and N501Y[6].

B.1.1.7, B.1.351, and P.1 were classified by the WHO as worrying (of concern) in February 2021, based on their greater transmission and the reduced neutralizing capacity of monoclonal antibodies, convalescent sera, and sera of vaccinated subjects[7-9]. However, differences among variants appear to exist. Because the lineage variant B.1.1.7 appears to be sufficiently susceptible to the antibodies generated by the vaccinations (at least by the two mRNA vaccines), a considerable loss in the efficiency of vaccination against it seems unlikely. In contrast, the lineage variant B.1.351 is up to six times less sensitive to vaccination-induced neutralizing antibodies.

In addition to those three variants, another one, B.1.617, just emerged in India, has spread rapidly, and was declared a public health concern by the WHO in May. It possesses 13 mutations in its genome, three of which code for the S protein[10]. Sera from convalescents and vaccinated people would partially neutralize this variant.

It should be noted, however, that in vitro neutralization titers do not necessarily mirror the situation in vivo. Vaccines induce the production of high levels of antibodies, hence a decrease in neutralizing power may not compromise host protection. T-cell-dependent cellular immune responses might be unaffected.

In its report of 5/9/2021, the PAIS Project Consortium has observed in the city of Buenos Aires (CABA) and Greater Buenos Aires (GBA), a higher frequency of detection of B.1.1.7 variants (United Kingdom), P.1 (Manaus), and the so-called Andean variant (lineage C.37, L452Q, leucine to glutamine at position 425 of S protein), in cases with no epidemiological link with tourism abroad. The frequencies are: 27% in CABA and 13% in GBA for the UK variant; about 32% for the Manaus variant in both districts, and 33% (CABA) and 49% (GBA) for the Andean variant. Until that date, the South African variant had not been detected[11].

It was also shown that more than 90% of the SARS-CoV-2 viruses currently circulating in these areas contain the D614G mutation in the S protein, which distinguishes them from the SARS-CoV-2 viruses of the first wave.

How do SARS-CoV-2 variants emerge?

There is little evidence as to why the variants that arose in the United Kingdom, South Africa, or the other more recent variants were successful. Some theories attribute their dramatic expansion to the selective pressure exerted by intermittent antiviral therapy, monoclonal antibodies, convalescent plasma, or the persistence of huge levels of virus in the body, as seen in immunocompromised COVID-19 patients with a lengthy course of disease[12-14].

In densely populated areas with few prevention or protection measures, the virus spreads freely during the pandemic and finds new hosts to replicate. This also increases the probability of generating spontaneous mutations with higher fitness, which become dominant. India and Manaus exemplify this epidemiological situation.

Antibodies, sera, and vaccines

The S protein is the target of neutralizing antibodies which, by binding to its RBD, prevent the virus from binding to the ACE2 receptor on host cells. It has been shown that mutations in S can decrease or suppress the neutralizing activity of monoclonal antibodies, but that neutralization by polyclonal immune serum is less affected. Since a polyclonal serum, such as convalescent serum (or hyperimmune equine serum) contains multiple antibodies against different parts of the S protein, its action is less affected by point mutations than that of monoclonal antibodies that are directed against a single antigenic determinant[15-18].

Role of cellular immunity

Immunity against SARS-CoV-2 is not based only on antibodies; cellular immunity also plays an important role. In addition to inducing the production of neutralizing antibodies, vaccines induce an increase in T helper and cytotoxic T cells. Serological tests, it should be noted, detect circulating antibodies rather than T cell or memory B cell activity. Therefore, although the presence of neutralizing antibodies correlates with the protection conferred by the vaccine, it is not its only biomarker[19].

Final considerations

The more a virus replicates, the more it mutates. And the more it mutates, the more likely it is that a dominant variant will appear posing a public health threat. The best we can do is to reduce the number of infected people in circulation and thus the number of circulating viruses[20-22].

By natural evolution, some viruses select for mutations that allow them to infect, replicate, and transmit more efficiently without eliminating the host, which makes it easier for them to spread. On the other hand, the circulation of highly lethal viruses (such as Ebola or hantavirus) is restricted to certain geographical areas. According to this, it would be expected that SARS-CoV-2 will tend to evolve into more infectious, but less virulent variants.

Public health interventions such as masks, physical distancing, and limitations to large gatherings remain effective, but control of a more communicable variant will require stricter and more widespread application of these measures.

Regarding the role of vaccination, since current vaccines induce a polyclonal immune response to multiple sites of the S protein, it is expected that they maintain reasonable and effective protection power despite the changes in the antigenic sites present in the SARS-CoV-2 variants.

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