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Genetic variability of SARS-CoV-2: an analysis of mutations in MHC-I and II epitopes and impacts on vaccination by AZD1222 in the brazilian population

Variabilidade genética do SARS-CoV-2: uma análise de mutações em epítopos de MHC-I e II e impactos frente à vacinação por AZD1222 na população brasileira

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ABSTRACT
The study aimed to analyze mutations in MHC-I and II epitopes in the face of vaccination with AZD1222 in Brazil. Mutations were identified in six epitopes, some of which decreased their immunogenicity, such as NYNYRYRLF of MHC-I, from 783 to 171. This suggests a possible reduction in the vaccine's efficacy against mutant variants of the virus. This research highlights the importance of adapting immunization strategies to deal with emerging SARS-CoV-2 mutations. It also highlights the need to monitor the genetic variability of the virus and develop updated vaccines to combat the pandemic. These results also emphasize the importance of understanding the mutations of the virus to correlate with its evolution and virulence, providing valuable insights for the control of COVID-19.

Keywords: Epitopes. SARS-CoV-2. Vaccination. Variability.

RESUMO
O estudo se propôs a analisar mutações em epítopos de MHC-I e II frente à vacinação com AZD1222 no Brasil. Foram identificadas mutações em seis epítopos, algumas diminuindo sua imunogenicidade, como o NYNYRYRLF de MHC-I, de 783 para 171. Isso sugere uma possível redução na eficácia da vacina contra variantes mutantes do vírus. A pesquisa destaca a importância de adaptar estratégias de imunização para lidar com as mutações emergentes do SARS-CoV-2. Além disso, ressalta a necessidade de monitorar a variabilidade genética do vírus e desenvolver vacinas atualizadas para combater a pandemia. Esses resultados também enfatizam a importância de entender as mutações do vírus para correlacionar com sua evolução e virulência, fornecendo insights valiosos para o controle da COVID-19.

Palavras-chave: Epítopos. SARS-CoV-2. Vacinação. Variabilidade.

INTRODUCTION

SARS-CoV-2 is a single-stranded RNA virus that has afflicted over a billion people worldwide, and in Brazil alone had led to more than 660,000 deaths by the end of July 2022[1] [2]. In 2021, the first vaccines were made available in Brazil on the Unified Health System - SUS, and currently the AZD1222 (Covisshield) vaccine, from Oxford-AstraZeneca, is one of those made available. It is a viral vector vaccine, based on the spike protein, with an estimated efficacy of around 81% [3].

It is true that making the virus available is an important step towards reducing the number of deaths and severe cases of COVID-19, as well as controlling the pandemic in general. However, as it is an RNA virus and because of its transmission rate, SARS-CoV-2 has a high degree of mutation, and there is a diversity of variants that are constantly emerging [4]. It is recognized that these variants can lead to reduced neutralization by antibodies generated by previous infection or vaccination, widespread interference with diagnostic test targets, potential impact on clinical diagnosis, increased transmission, and severity of the disease, among others [3]. Given these mutations, there is concern about the continued effectiveness of vaccination, since the variations can cause reduced neutralization by antibodies generated by previous infection or vaccination.

The concern over ongoing vaccine efficacy is warranted due to these mutations, understanding the impact of the genetic variability of SARS-CoV-2 in relation to vaccination in the Brazilian population is a useful effort both for a general understanding of the evolution of SARS-CoV-2 in relation to the human immune system and, consequently, for the development of vaccines, considering the importance of MHC-I and II epitope regions. In addition, this research focused on the selection of Brazilian sequences, an approach that has not been found in any research to date.

Thus, considering that the vaccination currently available for SARS-CoV-2 induces not only neutralizing antibodies, but also CD4+ and CD8+ T-cell responses specific to SARS-CoV-2 [5], this study aimed to analyze the genetic variability of SARS-CoV-2 and its possible impact on vaccination in the Brazilian population by investigating mutations in MHC I and II recognition epitopes located in the region of the gene in which the AZD1222 vaccine was designed.

METHODOLOGIES

EPITOPE SELECTION

The MHC-I and II epitopes subjected to analysis underwent a meticulous selection process involving a comprehensive review of scholarly literature and curation from the Immune Epitope Database [6]. which is a freely available database that catalogs experimental data on antibodies and epitopes studied in humans, non-human primates, and other animal species in the context of infectious diseases, allergy, autoimmunity, and transplantation. The selection in the database [6] was based on the epitopes located within the S Gene that have been most frequently addressed in studies, with experiments obtaining positive results. The choice of epitopes located in Gene S is since the AZD1222 vaccine is based on it.

SELECTION AND COLLECTION OF SEQUENCES

Genomic sequences of SARS-CoV-2 samples sequenced in Brazil were collected for evaluation. These sequences were selected from the National Center for Biotechnology Information (NCBI) available on the following website: https://www.ncbi.nlm.nih.gov/, which is a homepage that includes various resources and data, and the selection criterion will be preference for sequences that do not have unidentified bases. The sequences were collected from Genbank https://www.ncbi.nlm.nih.gov/genbank/ and GISAID https://gisaid.org/, two platforms that function as a database in which all the world's sequences are deposited to share knowledge about the genetics of organisms.

SEQUENCE ALIGNMENT

After the selection of the sequences, multiple alignments for nucleotides and proteins were carried out using the MEGA program version 10 (https://www.megasoftware.net/) and compared with the reference sequence used in the development of the AZD1222 vaccine, identity NC_045512.2, collected from Genbank, to

identify possible mutations in selected epitope regions.

EPITOPE ANALYSIS FOR MHC

The analysis of epitopes to determine the possible impact of changes in the amino acids of epitopes for MHC I and II was carried out in the Immune Epitope Database [6]. As well as being a database, the IEDB offers useful resources for analyzing epitopes, including the one used here for immunogenicity analysis.

RESULTS

In the initial phase of this study, more than 20 MHC I and II epitopes were selected. To do this, the best-placed epitopes were considered, considering the largest number of positive assays. After this selection, the epitopes were located within the protein sequence of the AZD1222 vaccine. Epitopes that were not found, i.e. were part of another region, were excluded. Table 1 shows the epitopes selected and their characteristics.

Table 1. Selected epitopes

MHC- I	MHC-II
YLQPRTFLL	NLLLQYGSFCTQLNR
QYIKWPWYI	SFIEDLLFNKVTLAD
LTDEMIAQY	CTFEYVSQPFLMDLE
NYNYLYRLF	TDEMIAQYTSALLAG
KCYGVSPTK	YAWNRKRISNCVADY
KIADYNYKL	LKPFERDISTEIYQA
TNFTISVTT	HWFVTQRNFYEPQII
VVVLSFELL	LQPELDSFKEELDKY
TLDSKTQSL	RFASVYAWNRKRISN
	SASFSTFKCYGVSPT

Source: Own elaboration (2024)

This was followed by the stage of selecting and aligning the sequences that made it possible to analyze Gene S, then converting them to amino acids and analyzing Protein S. A total of 500 sequences were aligned. The selection of sequences followed the criteria defined in the methodology, such as preference for sequences that did not have unidentified bases. In addition, given the availability of information on the most frequent variants per period in GISAID [8], sequences were selected from the 5 most prevalent variants per month, from February to June 2023:

Table 2. Most prevalent variants according to month

February	March	April	May	June
XBB.2.3	XBB.1.5	XBB.1.5	XBB.1.5	XBB.1.5
XBB.1.5.102	XBB.1.9.1	XBB.1.9.1	XBB.1.9.1	XBB.1.9.1
XBB.1.18.1	BQ.1.1	XBB.1.16	XBB.1.16	XBB1.16
XBB.1.5	BA.5	XBB.1	XBB.2.3	XBB.2.3
XBB.1.5.86	CH.1.1	XBB.2.3	XBB.1.16.1	XBB.1.16.1

Source: Own elaboration based on GISAID data (2023)

The analysis resulted in the identification of mutations in 6 of the selected epitopes. The epitope with the most mutation variations was SASFSTFKCYGVSPT, as shown in Table 3.

Table 3. Mutations in selected epitopes
Note: I= Isoleucine, T= Threonine, A = Alanine, K = Lysine, M = Methionine, V= Valine, N = Asparagine, D = Aspartate, L = Leucine Y= Tyrosine, Q= Glutamine, F= Phenylalanine, R= Arginine, P= Proline, E= Glutamic Acid

Epitopes		Mutations	
MHC -I	YLQPRTFLL	YLQPRTFL-	
MHC -I	QYIKWPWYI	Q-IKWPWYI	
MHC -I	NYNYLYRLF	NYNY**R**YRLF	
MHC -I	KIADYNYKL	**N**IADYNYKL	
MHC-II	RFASVYAWNRKRISN	**K**FASVYAWNRKRISN	**T**FASVYAWNRKRISN
MHC-II	SASFSTFKCYGVSPT	**FAPFFA**FKCYGVSPT	**LAPFF**TFKCYGVSPT

Source: Own elaboration (2024)

Proceeding to the subsequent phase, after analysis as described in the methodology, some of the selected epitopes showed a difference in immunogenicity when mutated. A table was therefore drawn up with the epitopes that showed a difference in immunogenicity.

Tabela 4. Immunogenicity of mutated epitopes

Epitopes		Immunogenicity
Not mutated	**NYNYRYRLF**	**783**
Mutated	NYNYLYRLF	171
Not mutated	**FAPFFAFKCYGVSPT**	**3.996**
Mutated	LAPFFTFKCYGVSPT	3.966

Source: Own elaboration (2024)

Derived from this outcome, it was hypothesized that the epitopes that remained with the same immunogenicity values were epitopes that had their amino acids exchanged for amino acids from the same functional group. However, it was observed that some epitopes remained with the same immunogenicity values, even when their amino acids had been exchanged for those of a different functional group. For example, the KIADYNYKL epitope, which had a basic polar Lysine exchanged for an acid polar Asparagine.

In each instance, the epitopes became less immunogenic, or remained the same, with no epitopes becoming more immunogenic.

DISCUSSION

CoVs belong to the coronavirinae subfamily of the coronaviridae family, and are subdivided into four genera: alpha, beta, gamma, and delta - CoVs [9]. They are enveloped viruses capable of infecting the respiratory, gastrointestinal, hepatic, and central nervous systems of humans [10]. In addition, the variation in symptoms according to the genetic and clinical profile of individuals is widely investigated [11].

Here, we conducted an analysis of MHC-I and MHC-II epitopes located within the S gene of SARS-CoV-2 that synthesizes the Spike protein [12]. We can characterize epitopes as specific stretches of antigens that are recognized by

antibodies, because from the structural complementarity and affinity between residues of the epitope of the antigen and the antibody, the recognition that triggers the immune response occurs [13].

The choice of epitopes located within the Spike protein was made because the vaccine selected for epitope analysis in this study is a vaccine which applied technology is a viral vector, the adenovirus, which infects chimpanzees, being genetically manipulated and precisely inserting the gene of the "Spike" protein ("S" protein). The vaccine in question, AZD1222, was one of the first four to be authorized by the National Health Surveillance Agency (Anvisa) for immunization of the Brazilian population and was developed by AstraZeneca in partnership with the University of Oxford, and in Brazil is produced by the Oswaldo Cruz Foundation [14].

Our study identified a reduction in the immunogenicity of some epitopes after mutation. Immunogenicity is related to the responsiveness to the recombinant protein, the reactivity of specific antibodies against the peptide. Therefore, vaccines based on the epitopes of these new strains would be no more immunogenic than those based on the first. This demonstrates that the virus has lost its immunogenic capacity, and it is believed that this may make it easier for human immune responses to escape recognition, as described by Tarke et al. (2021) in a study similar to the one carried out here [15].

RNA viruses are susceptible to high mutation rates during genome replication [16]. In addition, the rapid spread of SARS-COV-2 on a global scale has raised questions, such as whether its evolution is driven by mutations [17]. In general, it is known that mutations help the virus to evolve into a better version of itself, and can make it fit better into its environment, for example by escaping the immune surveillance of host cells.

Saha et al. (2020) also points out that obtaining information about mutation sites in the sequences and their frequency helps to correlate the significance of these mutations with the evolution and virulence of the virus. Our results contribute to the general understanding of the evolution of SARS-CoV-2 against the human immune system [4]. The ability of the virus to become less immunogenic reinforces that a first infection is not enough to immunize individuals over time, and that these changes may decrease the recognition of these epitopes.

Furthermore, over the years, vaccine hesitancy has arisen in Brazil due to the idea that infection with the virus itself would be enough to make the individual immunized [18]. Our results go against this and reinforce the need for continued efforts to raise awareness about vaccination, as well as encouraging research and the construction of new vaccines with new technologies, allowing for the development of new and better vaccine constructs.

In our research, we selected only sequences collected in Brazil, considering this global context of scarcity of these data, and although we found no association between the diversity of functional groups of amino acids and the variation in immunogenicity of epitopes, we found a study by Tarke et al. (2021) which identified through bioinformatic analysis that 93% of epitopes remain conserved in function even in the face of mutations. Although their study did not focus on Brazilian sequences, their results are similar to ours, since only 4% of the epitopes we analyzed suffered any change in their immunogenicity [15].

It is important to note that the pertinent study by Tarke et al. (2021) also used the same resource to carry out the immunogenicity analysis, the IEDB [15]. In addition, Ramesh (2021), when giving an overview of the variants and the implications for the human immune response, points out that, as the infection spread around the world, there was an increase in the number of multiple variants of SARS-CoV-2, and that until the year of his research, four variants remained a major concern: Alpha, Beta, Gamma and Delta. They increased the infection rate, modified the potency of the neutralizing antibodies, and thus compromised the effectiveness of the vaccine. It also reinforces that an effective vaccine against COVID-19 requires neutralizing antibodies and a Th1-triggered cellular component [3].

CONCLUSION

In the context of this research our

investigation focused on analyze the genetic variability of SARS-CoV-2 and its possible impact on vaccination in the Brazilian population by investigating mutations in MHC I and II recognition epitopes located in the region of the gene in which the AZD1222 vaccine was designed, and identifying possible changes in epitope recognition links, mutations were found in two epitopes that had an impact on their immunogenicity. The MHC - I epitope, NYNYRYRLF had its immunogenicity reduced from 783 to 171, while the MHC - II epitope FAPFFAFKCYGVSPT had its immunogenicity reduced from 3,996 to 3,966. These results show the importance of efforts to produce new vaccines with new technologies, especially those aimed at infections caused by highly mutated viruses, such as SARS-CoV-2, as well as raising awareness among the population about the importance of continuing immunization with new doses of the vaccine.

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