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A RATIONAL STRATEGY FOR THE MAINTENANCE OF ANTIVIRAL IMMUNITY TO NEW SARS-CoV-2 STRAINS

© **V.P. Baklaushev[1, 2, 3], G.M. Yusubalieva[1, 2], M.V. Bychinin[1], S.M. Yusubalieva[4], V.A. Kalsin[1, 2], A.V. Troitskiy[1]**

[1] Federal Scientific and Clinical Center for Specialized Medical Assistance and Medical Technologies of the Federal Medical Biological Agency, Moscow, Russian Federation

[2] Pulmonology Scientific Research Institute under Federal Medical and Biological Agency of Russian Federation, Moscow, Russian Federation

[3] Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow, Russian Federation

[4] Astana Medical University, Astana, Kazakhstan

New variants of SARS-CoV-2 such as Omicron BA.2, BA.4/5, BA.2.12.1 and BA 2.75 are characterized by higher infectivity and the ability to escape virus-neutralizing antibodies against previous coronavirus variants. The S-trimer of BA.2 and its phylogenetic derivatives are characterized by a predominant «Up»-conformation, which facilitates the interaction with ACE2 on target cells and promotes the resistance to neutralizing antibodies. The immunity acquired from the infection with earlier strains is non-sterile for both early and later strains; the booster systemic immunization does not significantly affect the effectiveness of antiviral immunity, and its feasibility is currently being questioned. Studies of the mucosal immune response have shown that intranasal immunization with adenovirus vaccines provides more pronounced protective immunity than systemic reimmunization does. A promising approach is the creation of multivalent inhaled next generation vaccines containing immunoadjuvants that activate B- and T-cell mucosal immunity. Currently, a large number of intranasal vaccines are undergoing phase I/II trials, while the preclinical and preliminary clinical results indicate that this method of vaccination provides a better mucosal immune response at the entry site of the virus than systemic immunization does. This strategy may provide a long-term immune protection against the currently existing and yet unknown new strains of SARS-CoV-2.

Keywords: *COVID-19; SARS-CoV-2; Omicron BA.1, BA.2, BA.4, BA.5, BA.2.75; neutralizing antibodies; mucosal immune response; intranasal immunization; nasal vaccines; next-generation vaccines.*

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BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic, which began in late 2019, globally manifested itself as six distinct waves by the end of summer 2022, with each successive wave driven by the emergence of a new variant of SARS-CoV-2 with unique features. The most significant one was wave 5 at the beginning of 2022, which at its peak gave a daily increase of more than 4 million cases worldwide and more than 200 thousand cases in Russia; it arose as a result of the emergence of the omicron variant that has more than 30 mutations in the S-protein, first in the form of strain BA.1.1.529, which was then replaced by its phylogenetic descendant BA.2 [1–3]. At present, after the BA.1/BA.2 wave, wave 6 is growing in Russia due to omicron variants BA2.12.1, BA.4, BA.5, and BA.2.75 (Centaur) that arose on the basis of BA.2 and are widely spread, characterized by an even more pronounced ability to avoid the immune response of neutralizing antibodies developed against previous strains [4–6]. Although the number of hospitalizations and mortality in the case of infection with omicron strains is significantly lower than with the delta variant (B.1.617.2), the total number of cases increased many times over, including among those who had previously been ill with other strains of SARS-CoV-2 and those vaccinated by all existing types of vaccines cannot but cause concern.

The strategy of vaccination and revaccination, which was quite obvious before the emergence of the omicron variant, enabled the reduction to null the pandemic wave caused by the most pathogenic and lethal delta variant [7]. With the advent of omicron strains that evade effectively vaccine immunity, the strategy of regular revaccination, at first glance, ceased

to be so obvious [8], even if a meta-analysis showed the absence of allergic reactions in response to repeated vaccinations [9].

The vaccination with mRNA and adenoviral vaccines protects against severe COVID-19; however, no obvious data on protection against asymptomatic or paucisymptomatic infection with SARS-CoV-2 have been obtained either in the case of infection with the delta variant, or especially omicron [10]. The reason is that the existing vaccine immunity is not sterilizing (i.e., does not prevent infection and virus spread). An outbreak of a pandemic and infection spread among the vaccinated population was first recorded when the delta variant appeared in the summer of 2021 [11]. Nearly 100% vaccination coverage in Europe and the USA did not prevent another wave caused by omicron, which suggests primarily the ability it acquired to avoid virus neutralization by antibodies to the S-protein. Moreover, in the vast majority of cases, the disease was asymptomatic or paucisymptomatic, which is usually associated with the presence of immunity in most of the patients infected, which prevents a severe course with systemic damage to the lungs and other organs [1]. In addition, such an enormous outbreak confirmed once again that the systemic immune response to SARS-CoV-2 (both post-vaccination and convalescent) is not sterilizing, and people with humoral immunity spread the virus in the same way as those naive in relation to new variants of the virus [12].

The high infectivity and extremely low efficiency of immune protection against new omicron strains cannot but inspire concern despite the significant decrease in the incidence of severe disease. Concerns are primarily associated with the possible emergence of new strains characterized by increased pathogenicity, based on existing highly virulent variants. In addition, a relatively low proportion of various severe post-COVID complications associated with damage to the nervous and cardiovascular systems can become a significant absolute number with a large number of patients infected worldwide. Post-COVID syndrome, or the so-called long COVID, is characterized by psychoneurological, autonomic, pulmonary, vascular, endocrine, immune, and other disorders lasting for several months [13–17]; along with an increase in the number of people who have been ill, it is becoming an urgent medical and social problem. All of these concerns require a more modern strategy for creating and maintaining an immune defense against new strains of SARS-CoV-2.

This study aimed to analyze current studies of antiviral immunity to new strains of SARS-CoV-2 and develop a rational strategy for creating and maintaining immunity to the omicron variant and new variants that have not yet emerged.

PHYLOGENESIS OF NEW SARS-CoV-2 STRAINS

By the end of 2021, the World Health Organization (WHO) identified five variants, or clades (phylogenetic groups), of SARS-CoV-2, which were variants-of-concern (VOC) (WHO, 2022), namely, alpha (B. 1.1.7 according to PANGO[1] [18], or clade 20I according to Nextstrain[2] [19]), beta (B.1.351; clade 20H), gamma (P.1; clade 20J), delta (B.1.617.2, AY; clades 21I and 21J), and omicron (B.1.1.529, BA.1-5; clades 21K, 22C, and 22B) (Table 1) [20].

By the beginning of 2022, B.1.617.2 delta, the most virulent and lethal variant of SARS-CoV-2, prevailing worldwide throughout 2021, was almost completely replaced by the new omicron variant BA.1.1.529 (clade 21K) (Fig. 1). Then, by mid-2022, BA.1 was replaced by BA.2 (clade 21L) and then by BA.4/5 (clades 22A and 22B), which subsequently emerged in the Republic of South Africa (South Africa) and spread to other countries concurrently with variant BA.2.12.1 (clade 22C) originating in the USA. As of late August and early September 2022, omicron BA.5 (clade 22B) is the most common strain worldwide [1, 8] (Fig. 1). BA.5, along with strains BA.2.12.1 and BA.4 and some other derivatives of variant BA.2, caused wave 6 of COVID-19 in Russia, which, as of September 21, 2022, causes an official increase of more than 50,000 cases per day.

In July 2022, a new omicron strain, BA.2.75, began to spread globally, which was named "Centaur" because of the combination of the beneficial mutations of the BA1/2 and BA4/5 variants. This strain, which was first discovered in India, showed higher distribution dynamics than BA.5 and other phylogenetic descendants of strain BA.2. Currently, BA.2.75 centaur is rapidly spreading worldwide and may be the cause of another wave of increased incidence [4–6].

New SARS-CoV-2 strains are evaluated by their efficiency to spread in humans (disease contagiousness characterizes the so-called reproduction index [R_0] defined as the number of individuals to be infected by one typical affected patient) and ability to avoid humoral antiviral immunity and pathogenicity. The latter is directly due to amino acid substitutions in the S-protein, which modify the affinity of the receptor-binding domain for angiotensin-converting enzyme 2

[1] Access mode: https://cov-lineages.org
[2] Access mode: https://nextstrain.org

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Table 1

Characteristics of the main variants of SARS-CoV-2 that caused significant morbidity during the pandemic

Name according to WHO	Nomenclature		Country of occurrence	Date of occurrence	Mutations in the S-protein
	PANGO	NextStrain			
Hu-1 (Wuhan isolate)	-	19A	China	November 2019	-
-	B	20	-	-	D614G
-	B.1	20A	-	-	D614G
-	B.1	20B	-	-	D614G
Alpha	B.1.1.7	20I	UK	September 2020	D614G, 69/70del, 144/5del, P618H, T716I, N601Y, S982A, A570D, D1118H
Beta	B.1.351	20H	South Africa	May 2020	D614G, L18F, D80A, D215G, 242-4del, R246I, K417N, E485K, N501Y, A701V
Gamma	P.1	20J	Brazil	November 2020	D614G, L18F, T20N, P26S, D138Y, R190S, K417T, E485K, N501Y, H655Y, T1027I, V1116F
Delta	B.1.617.2	21I, 21J (Delta)	India	Oktober 2020	D614G, T19R, E156G, F157-, R158-, L452R, T478K, P681R, D950N
Omicron	BA.1	21K (Omicron)	South Africa	November 2021	A67V, H69-, V70-, T95I, G142D, V143-, Y144-, Y145-, N211-, 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
Omicron	BA.2	21L (Omicron)	South Africa	November 2021	T19I, L24del, P25del, P26del, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron	BA.2.12.1	22C (Omicron)	USA/Canada	December 2021	T19I, L24del, P25del, P26del, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452Q, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K
Omicron	BA.3	22K (Omicron)	South Africa	November 2021	A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron	BA.4	22A (Omicron)	South Africa	Yanuary 2022	T19I, L24del, L24del, P25del, P25del, P25del, P26del, P26del, P26del, A27S, H69del, V70del, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, R452K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

Table 1

Continued

Name according to WHO	Nomenclature		Country of occurrence	Date of occurrence	Mutations in the S-protein
	PANGO	NextStrain			
Omicron	BA.5	22B (Omicron)	South Africa	Yanuary 2022	T19I, L24del, L24del, P25del, P25del, P25del, P26del, P26del, P26del, A27S, H69del, V70del, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, R452K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron	BA.2.75	22D (Omicron)	India	June 2022	T19I, L24del, P25del, P26del, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, R493Q, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

Note: Revised from [20]. WHO — World Health Organization.

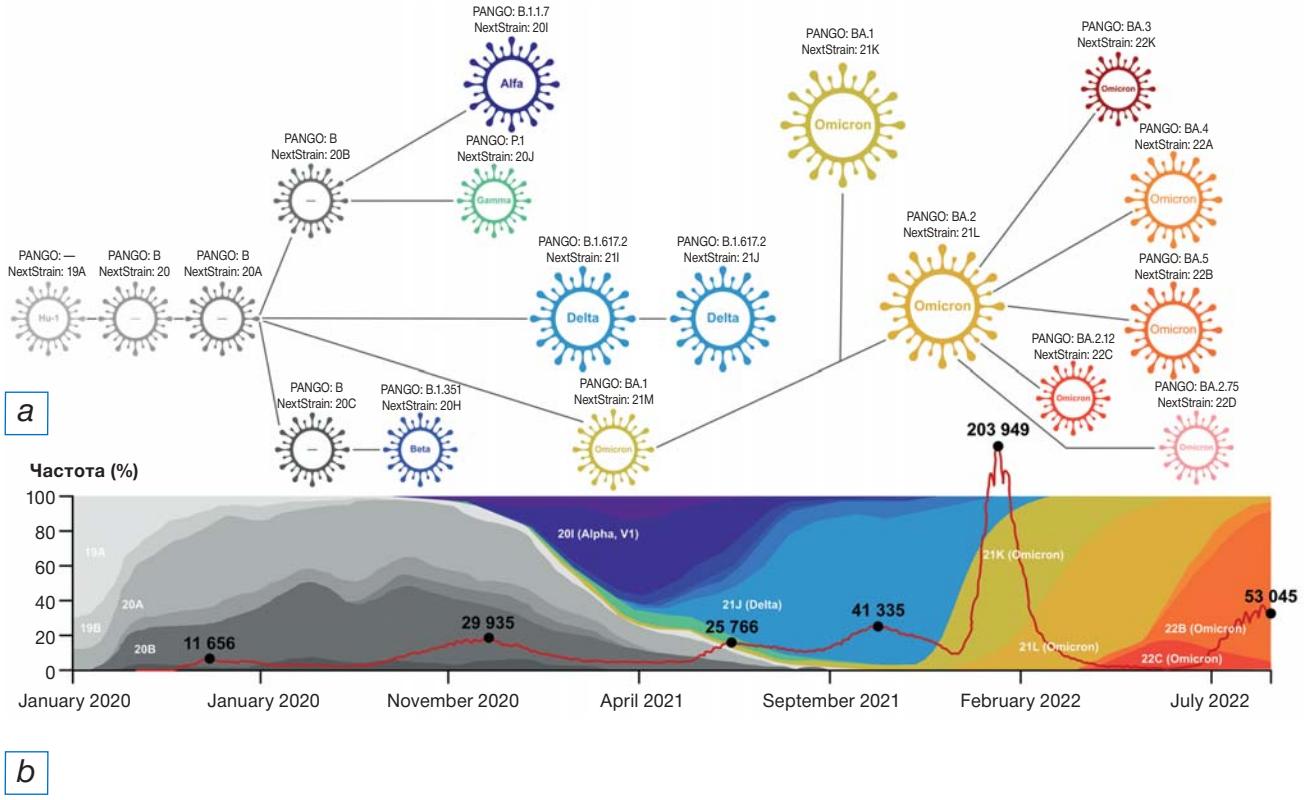

Fig. 1. Evolution of new variants of SARS-CoV-2 superimposed on the "waves" of the pandemic: *a* — phylogeny of "variants of concern"; *b* — histogram of the incidence rates of different variants of SARS-CoV-2 in Europe (according to Gissad, https://gisaid.org/) against the background of the morbidity curve in Russia (according to www.yandex.ru). The figures at peaks indicate the daily increase in cases according to the official data.

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(ACE2) on target cells and determine the dynamics of interaction with virus-neutralizing antibodies.

S-PROTEIN STRUCTURAL ASPECTS IN NEW SARS-CoV-2 VARIANTS

The phenotype and infectivity of new SARS-CoV-2 variants are determined by the structure and functional properties of the S-protein. Just like in other betacoronaviruses, SARS-CoV-2 S-protein consists of two main domains, namely, receptor-binding domain (RBD) and N-terminal domain (NTD) [21, 22]. RBD interacts directly with ACE2 and ensures that the virus penetrates the target cells; as a result, it is still considered the main target for virus-neutralizing antibodies that block virus entry into cells [23]. Moreover, NTD is the most immunogenic domain of the S-protein, and evidence shows that antibodies recognizing NTD can also be virus-neutralizing [24].

In contrast to the delta variant, which has 7–10 mutations in the S-protein, omicron BA.1 and BA.2 have 37 and 31 mutations in the S-protein, respectively [3] (Table 1). Both omicron stains (BA.1 and BA.2) bind the murine ACE2 receptor with high affinity. By contrast, the wild (Wuhan) variant of SARS-CoV-2 binds to human and cat ACE2, but not to murine ACE2. In this regard, a hypothesis arose of the origin of the omicron variant through evolution with a change of host, i.e., human–cat–mouse–human [2].

Although the S-trimer of the omicron variant, due to numerous mutations, acquired an increased affinity for ACE2 compared with delta, and protection from it requires a significantly higher concentration of virus-neutralizing antibodies (achieved by triple immunization with an mRNA vaccine or a combination of immunity convalescents with booster immunization [16]), the number of severe cases requiring hospitalization decreased by more than two times at the beginning of a new wave, and the risk of death decreased even more [25]. In the cell culture of the nasal epithelium, the replication of the omicron and delta variants was comparable; however, in alveolocytes and intestinal epithelium, omicron demonstrated a lower level of replication than delta, and it did not correlate with the expression level of transmembrane protease, serine 2 (TMPRSS2 protease) [16]. Thus, the alternative method of penetration into the cell acquired by omicron, which is not associated with the activity of TMPRSS2, is less effective and, possibly, underlies the lower pathogenicity and lethality of the new SARS-CoV-2 variant.

BA.2, which completely replaced BA.1 by the end of March 2022 (Fig. 1), exceeded the Wuhan variant by 11 times in the degree of affinity of the S-protein for ACE2 and exceeds the maternal BA.1 by almost two times [1]. Structural studies have shown that when the BA.2 S-trimer interacts with human ACE2, all three RBDs are predominantly in the open up-conformation, which greatly enhances the efficiency of equimolar (3:3) binding to ACE2 and, thus, increases significantly the transmissibility of this strain.

Some published sequences of the new omicron variant BA.2.75 (Centaur) carry the L452R mutation identified in BA.5, which is associated with the possibility of re-infection of patients, and this gives cause for concern. A study by Cao et al. [4] and several other studies published in August 2022 as preprints on the bioRxiv service investigated the possible mechanisms of increased virulence and avoidance of the immune response by BA.2.75 [4, 5].

Compared with BA.2, BA.2.75 S-trimer carries nine additional mutations, of which five (K147E, W152R, F157L, I210V, and G257S) are in NTD, and the remaining four (D339H, G446S, N460K, and R493Q) are in RBD [4–6] (Table 1).

Among the latest mutations in BA.1, G446S appeared, and the R493Q reversion is noted in the sequences of BA.4/BA.5. Mutations N460K and D339H have not previously been found in prevailing variants and their function are still unknown. An alarming factor is that India's BA.2.75 is characterized by a more efficient distribution than that of new omicron strains BA.2.38 (BA.2+N417T), BA.2.76 (BA.2+R346T+Y248N), and BA.5 [4]. The increased transmissibility of BA.2.75 suggests that this variant may become prevalent after the global wave driven by BA.4/BA.5.

Compared with BA.5, BA.2.75 has been shown to have a significantly higher affinity for ACE2. In addition, the BA.2.75 spike shows reduced thermal stability and a preferential RBD up-conformation under acidic conditions, which probably contributes to the increased endosomal entry of the virus into cells under acidotic conditions. Bioinformatic analysis of the S-protein of BA.2.75 showed that its RBD domain has a higher (more than 3000 times) affinity for ACE2 than B.1.1.7 (alpha) [26]. Such a high affinity of RBD BA.2.75 for ACE2 suggests the possibility of developing angiotensin intoxication when ACE2 is blocked by SARS-CoV-2 S-protein [27].

Omicron BA.2.75, to a lesser extent than BA.4/BA.5, avoids not only plasma neutralization of convalescents after omicron BA.1/BA.2, but also significantly higher plasma neutralization of convalescents after delta. The plasma of convalescents after infection with BA.5

also neutralizes BA.2.75 much weaker than BA.5 [4]. These data collectively indicate that BA.2.75 centaur may cause a significant increase in the incidence of COVID-19 in the near future. Conversely, on the collection of blood sera from Europeans, BA.2.75 did not demonstrate a more significant avoidance of the immune response than BA.5, which is currently predominant in Europe [5] and may indicate that the next wave will affect third-world countries to a greater extent.

IMMUNE RESPONSE TO NEW SARS-CoV-2 VARIANTS

All epidemically significant variants of omicron, such as BA.1 [28, 29], BA.2 [30, 31], BA.4/BA.5 [32–34], and BA.2.75 [4, 5], are characterized by a pronounced resistance to neutralizing antibodies obtained as a result of vaccination or infection with the previous version of SARS-CoV-2 and therapeutic monoclonal antibodies obtained during the delta wave. Just as omicron BA.1 acquired the ability to avoid the immune response resulting from infection with the delta variant, as a result of numerous amino acid substitutions in the S-protein, subsequent variants acquired the ability to evade virus-neutralizing antibodies developed against previous strains. Specifically, BA.2 is resistant to neutralizing antibodies induced against BA.1 [35–37], BA.5 avoids neutralization by antibodies from sera obtained from outbreaks of BA.1 [34] and BA.2 [38], and the new Indian variant BA.2.75 centaur, which emerged among the latest VOCs, appear to avoid successfully neutralization by antibodies against the BA.5 S-protein [4, 5].

In addition to the ability of new strains to avoid humoral and cellular immune responses, repeated COVID-19 infections are caused by the natural decline of immunity after an illness or after vaccination and a decrease in the titer of virus-neutralizing antibodies. The ability of new strains to avoid an antibody response in this aspect has a quantitative equivalent, and this is the minimum titer of virus-neutralizing antibodies that protects against infection. The more pronounced the ability to avoid the immune response, the higher this titer should be. Specifically, neutralization of the beta variant requires a 6-fold higher titer of virus-neutralizing antibodies than for the wild Wuhan variant, Alpha variant [39], etc., with subsequent variants.

Studies of humoral immunity in SARS-CoV-2 infection have shown that serum antibody levels usually decline significantly within 4–6 months, but remain detectable up to at least 11 months after illness [40]. The titer of antibodies to the S-protein correlates with the frequency of S-specific plasma cells in the bone marrow aspirate, which are at rest. Thus, the presence of long-lived plasma cells (LLPC) and S-specific memory B-cells after COVID-19 was detected. The duration of the humoral immune response is determined by the count and lifespan of memory B-cells and LLPCs in the bone marrow [41].

RATIONAL STRATEGY TO MAINTAIN PROTECTIVE IMMUNITY AGAINST SARS-CoV-2: ENDONASAL IMMUNIZATION

All currently registered SARS-CoV-2 vaccines are administered intramuscularly. Moreover, the mucous membranes of the upper and lower respiratory tracts are the site of entry of SARS-CoV-2; therefore, the local mucosal immune response is very important for protective immunity [42–45]. An alternative non-invasive method of immunization is intranasal vaccination, which is currently actively investigated for the possibility of generating a sterilizing mucosal immune response in COVID-19 [44, 46]. At the moment, several new intranasal vaccine agents are undergoing preclinical and clinical phase I–III trials (Table 2).

The most promising results are demonstrated by intranasal adenovirus-based vaccines, probably because the mucous membranes are the entry sites of adenoviruses, and viral particles can serve as natural adjuvants for intranasal immunization. Two adenoviral vectors are used as carriers in currently ongoing clinical trials of vaccine agents, namely, Ad5 (AdCOVID [51], Ad5-nCoV [49, 50], and Sputnik V [53]) based on adenovirus serotype 5 and ChAd (AZD1222 [47], ChAd-SARS-CoV-2-S [48], and BBV154 [52]) based on chimpanzee adenovirus.

Preclinical studies of adenovirus vaccines based on Ad5 and ChAd when administered intranasally have shown their ability to induce persistent systemic and local mucosal immunity, characterized by high titers of secretory anti-RBD IgA and serum virus-neutralizing antibodies, increased levels of specific CD4+ T-cells and CD8+ cytotoxic cells, and increased level of Th1 cytokines [48, 51, 52, 61]. Intranasal booster vaccination after intramuscular vaccination induces the development of durable immunity against new strains of SARS-CoV-2, an increase in specific T- and B-cells, including in the secretion of mucous membranes [44, 45].

Phase I/II clinical trials for the safety and efficacy of adenovirus vaccines are currently performed in the US, UK, India, and Russia (Table 2). The Russian

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Table 2

Intranasal vaccines against SARS-CoV-2 undergoing preclinical and clinical trials

Vaccine candidate name	Vaccine product basis	Developer	CT phase	CT identifier	Source
Vaccines based on recombinant adenoviruses					
AZD1222 (ChAdOx1 nCoV-19)	Recombinant viral vector ChAd expressing S-protein	Imperial College London, University of Oxford AstraZeneca (UK)	I	NCT05007275 NCT04816019	[47]
ChAd-SARS-CoV-2-S	Recombinant viral vector ChAd expressing stabilized S-protein	Washington University School of Medicine (USA)	I	NCT04751682	[48]
Ad5-nCoV	Recombinant viral vector Ad5 expressing the RBD domain of the S-protein	CanSino Biologics Inc. jointly with Beijing Institute of Biotechnology (China)	I/II	NCT04840992	[49, 50]
AdCOVID	Recombinant viral vector Ad5 expressing the RBD domain of the S-protein	Altimmune, Inc. (USA)	I	NCT04679909	[51]
BBV154	Recombinant viral vector ChAd expressing stabilized S-protein	Bharat Biotech International Limited (India)	III	NCT05522335	[52]
Gam-COVID-Vac (Sputnik)	Recombinant viral vector Ad5 expressing S-protein	The Gamaleya National Center of Epidemiology and Microbiology (Russia)	I/II	NCT05248373	[53]
Vaccines based on attenuated viruses					
COVI-VAC	Live-attenuated SARS-CoV-2	CODAGENIX Inc (USA)	I	NCT04619628	[54]
DelNS1-NCoV-RBD LAIV	Live-attenuated SARS-CoV-2	Beijing Wantai Biological Pharmacy Enterprise jointly with Hong Kong University (China)	I	NCT04809389	[55]
MV-014-212	Live-attenuated respiratory syncytial virus vaccine expressing SARS-CoV-2 S-protein	Meissa Vaccines, Inc. (USA)	I	NCT04798001	[56]
ACM-001	Protein subunit vaccine (ACM-CpG) based on S-protein from strain B.1.351 and adjuvant CpG7909, packaged in an artificial cell membrane	ACM Biolabs (Singapore)	I	NCT05385991	[57]
CROWNase	SARS-CoV-2 S-protein envelope degrading enzyme	Illinois Institute of Technology (USA)	-	Preclinical study	[58]
CovOMV	*Neisseria meningitidis* outer membrane vesicles mixed with recombinant S-protein	Intravacc (Netherlands)	-	Preclinical study	[59]
STINGa-	S-trimer in PEGylated liposomes	AuraVax Therapeutics (USA)	-	Preclinical study	[60]

Note: Revised and supplemented from [44]. ChAd — chimpanzee adenovirus; Ad5 — adenovirus 5 serotype.

intranasal vaccine is being developed based on component 2 (Ad5) of the registered Sputnik V vaccine at the Gamaleya National Center of Epidemiology and Microbiology [53].

Various variants of attenuated viral vaccines can serve as an alternative to adenovirus vaccines (Table 2). An attenuated live vaccine was obtained by passaging SARS-CoV-2 in Vero cells at a temperature reduced from 37°C to 22°C. A single administration of such a vaccine to humanized K18-hACE2 mice, in which SARS-CoV-2 causes a lethal infection, made them insensitive to infection due to a pronounced B- and T-cell immune response and a high titer of secreted IgA [62]. The advantage of a live-attenuated endonasal vaccine based on SARS-CoV-2 is a polyclonal immune response to all antigens of the virus, which can more effectively activate T-cell immunity.

The development of a T-cell immune response is another promising strategy for acquiring long-term immunity covering different SARS-CoV-2 variants. Conserved peptide epitopes of the nucleocapsid (N-protein) may be no less important than the peptide epitopes of the S-protein, and probably more important, for the implementation of the T-cell response, because the latter is the most immunogenic antigen of SARS-CoV-2. In BALB/c mice, intranasal immunization with recombinant adenovirus serotype 5 expressing the SARS-CoV-2 N-protein is accompanied by a significant activation of the T-cell response in the bronchoalveolar tree. Moreover, after such intranasal immunization, specific CD4+ T-cells were detected in the spleen, which, along with an increase in the titer of specific antibodies, indicated the triggering of a systemic humoral immune response [63].

Along with adenoviral vectors and attenuated vaccine agents, new nanotechnology-based vaccine platforms are being actively developed. Liposomal nanoconjugates [57, 60] are tested in preclinical studies, including those with shRNA [64], various organic nanoparticles, for example, nanoparticles based on inulin acetate, a plant polymer that can activate the TLR4 receptor [65], or nanoparticles of chitosan conjugated with RBD, which increases significantly its immunogenicity compared with soluble RBD [66], vesicles based on bacterial membranes [59], and other approaches that activate the immune response.

A key role in the local humoral and cellular immune response on the mucous membranes of the bronchopulmonary tree belongs to cytokines from the pro-inflammatory superfamily tumor necrosis factor, namely, B-cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL), as well as chemokines CXCL13, CCL19, and CCL21, which induce a local response of T- and B-cells in bronchial lymphoid tissue [43]. The addition of BAFF/APRIL sequences and/or the listed chemokines to the composition of new polyvalent nasal vaccines was assumed to significantly increase the efficiency of the mucosal immune response.

A separate area is the development of polyvalent nasal vaccines of a new-generation. For example, a trivalent vaccine containing the sequences of the S1 domain (RBD+NTD) of the S-protein, full-length nucleocapsid protein, and nsp12 fragment (RNA-dependent RNA polymerase, RdRp) was created based on adenovirus vectors Ad5 and ChAd68 [67]. The S1 domain in the construct was fused to the transmembrane domain of the vesicular stomatitis virus G protein, which provides trimerization and exosomal targeting [68] for a better immune response. The full-length N-protein richest in T-cell peptide epitopes and the selected RdRp fragment, which, according to bioinformatic analysis, exhibits the highest affinity for T-cell receptors, were included in the vaccine to activate cellular immunity. Intranasal (but not intramuscular) immunization with a single dose of such a trivalent vaccine leads to the formation of protective mucosal immunity against both B.1.1.7 and B.1.351 VOCs [67]. Thus, intranasal immunization with new-generation multivalent vaccines may be an effective future vaccination strategy against COVID-19.

IS THE CREATION OF STERILIZING IMMUNITY AGAINST NEW STRAINS REAL?

To analyze mucosal immunity to new SARS-CoV-2 strains in humans, Tang et al. [69] evaluated S-specific total and virus-neutralizing antibodies, as well as B- and T-cell immune responses in bronchoalveolar lavages and in the blood of patients vaccinated with mRNA vaccines and patients who recovered from COVID-19. In vaccinated patients, the levels of neutralizing antibodies against strain B (D614G), strain delta (B.1.617.2), and omicron BA.1.1 in the bronchoalveolar lavage were significantly lower than those in patients who had recovered from COVID-19, despite a comparable virus-neutralizing activity of blood [69]. Notably, vaccination with mRNA vaccines induced circulating S-specific B- and T-cell immunity; however, unlike COVID-19 convalescents, these responses were absent in the bronchoalveolar lavage of vaccinated individuals. By using a mouse immunization model, systemic mRNA vaccination induces weak mucosal

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immunity, especially against omicron BA.1.1. The combination of systemic mRNA vaccination with endonasal immunization with pseudotyped S-adenovirus induced the production of mucosal virus-neutralizing antibodies not only against delta but also against omicron BA.1.1 and reduced significantly the viral load in experimental animals. Thus, in a rational strategy for developing antiviral immunity to new SARS-CoV-2 strains, endonasal vaccines that create sterilizing immunity in the respiratory tract against SARS-CoV-2, including the latest versions of omicron and new potentially dangerous strains, can come into prominence [69].

CONCLUSION

New SARS-CoV-2 variants, characterized by high contagiousness and the ability to evade virus-neutralizing antibodies, require a new antiviral defense strategy. Such a strategy could be the activation of the mucosal immune response of the bronchoalveolar tree by intranasal and/or inhalation immunization with vector vaccines along with the development of new-generation multivalent vaccines that activate specific B- and T-cells and promote the production of broadly neutralizing secretory antibodies that provide sterile immunity.

ADDITIONAL INFORMATION

Author contribution. *Baklaushev V.P.* — the concept of the review, literature search, manuscript writing and editing. *Yusubalieva G.M., Bychinin M.V.* — literature search, manuscript writing and editing. *Yusubalieva S.M.* — literature search, manuscript editing, preparation of tables. *Kalsin V.A.* — literature search, manuscript editing. *Troitsky A.V.* — general guidance, manuscript editing. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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AUTHORS' INFO

The author responsible for the correspondence:
Vladimir P. Baklaushev, MD, PhD;
address: 28, Orekhovy blvd, Moscow, 115682, Russia;
e-mail: baklaushev.vp@fnkc-fmba.ru;
eLibrary SPIN: 3968-2971;
ORCID: https://orcid.org/0000-0003-1039-4245

Co-authors:
Gaukhar M. Yusubalieva, MD, PhD;
e-mail: gaukhar@gaukhar.org; eLibrary SPIN: 1559-5866;
ORCID: https://orcid.org/0000-0003-3056-4889

Mikhail V. Bychinin, MD, PhD;
e-mail: drbychinin@gmail.com; eLibrary SPIN: 6524-9947;
ORCID: https://orcid.org/0000-0001-8461-4867

Saule M. Yusubalieva;
e-mail: sm_yusubalieva@mail.ru;
ORCID: https://orcid.org/0000-0002-8258-8148

Vladimir A. Kalsin;
e-mail: vkalsin@mail.ru; eLibrary SPIN: 1046-8801;
ORCID: https://orcid.org/0000-0003-2705-3578

Aleksandr V. Troitskiy, MD, PhD;
e-mail: dr.troitskiy@gmail.com; eLibrary SPIN: 2670-6662;
ORCID: https://orcid.org/0000-0003-2143-8696