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COMMENT

Post-genomic platform for development of oligonucleotide vaccines against RNA viruses: diamond cuts diamond

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Abstract

The coronavirus pandemic has starkly demonstrated the need to create highly effective vaccines against various viral diseases. The emerging new platforms for vaccine creation (adenovirus vectors and mRNA vaccines) have shown their worth in the fight against the prevention of coronavirus infection. However, adenovirus vectors and mRNA vaccines have a serious disadvantage: as a rule, only the S protein of the coronavirus is presented as an antigen. This tactic for preventing infection allows the ever-mutating virus to escape quickly from the immunity protection provided by such vaccines. Today, viral genomic databases are well-developed, which makes it possible to create new vaccines on a fundamentally new post-genomic platform. In addition, the technology for the synthesis of nucleic acids is currently experiencing an upsurge in demand in various fields of molecular biology. The accumulated experience suggests that the unique genomic sequences of viruses can act as antigens that trigger powerful humoral and cellular immunity. To achieve this effect, the following conditions must be created: the structure of the nucleic acid must be single-stranded, have a permanent 3D nanostructure, and have a unique sequence absent in the vaccinated organism. Oligonucleotide vaccines are able to resist the rapidly changing genomic sequences of RNA viruses by using conserved regions of their genomes to generate a long-term immune response, acting according to the adage that a diamond cuts a diamond. In addition, oligonucleotide vaccines will not contribute to antibody-dependent enhanced infection, since the nucleic acid of the coronavirus is inside the viral particle. It is obvious that new epidemics and pandemics caused by RNA viruses will continue to arise periodically in the human population. The creation of new, safe, and effective platforms for the production of vaccines that can flexibly change and adapt to new subtypes of viruses is very urgent and at this moment should be considered as a strategically necessary task.

Keywords Oligonucleotide vaccine · SARS-CoV-2 · RNA viruses · Immunity · Pandemic · Post-genomic platform · La-S-so

Introduction

The current SARS-CoV-2 pandemic is creating favorable conditions for the rapid development and introduction of new vaccine platforms because traditional approaches have not proved sufficient. The development of genomic databases of viruses has provided vital information to allow the necessary viral genetic information to be embedded into adenoviral vectors and mRNA vaccines. Humans are striving to end the pandemic as soon as possible, using the entire arsenal of existing weapons, old and new. We wish we could create just one vaccine that would rid us of the danger of dying from the coronavirus forever. In this regard, history and microevolution, both human and viral, shows us that this is rarely possible. Therefore, it is urgent to search for new platforms for use in the creation of universal vaccines with

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long operating life, despite the existing subtypes of SARS-CoV-2 (starting with the alpha subtype and stretching currently to omicron and deltacron) and those that will appear in the future. History has certainly taught us that each person either gets sick with coronavirus ('fast and tough' vector), or gets vaccinated, but then still gets sick ('slow and mild' vector), before humanity reaches herd immunity.

Vaccines with a long operating life will help reduce the number of victims by helping us move towards herd immunity. While it is quite difficult to track the emerging subtypes of coronavirus and respond to them quickly enough to maintain the effectiveness of vaccines at the proper level, so far this has been our only option.

Innovative platforms for creating vaccines against RNA viruses: is the glass half empty or half full?

As of March 25, 2022, coronavirus pandemic has already claimed more than 6.1 million lives worldwide in 2 years, while in several of the worst-affected countries, the excess mortality was 50% greater than the expected annual mortality (Peru, Ecuador, Bolivia, Mexico) [1]. Against the backdrop of the COVID-19 pandemic, vaccines created using innovative platforms have made a big leap forward, and their use around the world has already reached billions of doses. Nevertheless, vaccines based on innovative platforms (mainly adenoviral vectors and mRNA vaccines) are constantly having to play catch up with the variability of the coronavirus. Before the coronavirus pandemic, the same situation existed with the human influenza virus [2]. The presence of RNA-dependent RNA polymerase, which makes many mistakes during the replication of RNA virus genomes [3], makes this race endless. However, in some cases, when mortality from the disease is high, it is necessary first to rely on such biotechnology, which allows the fastest possible response and takes into account the characteristics and dynamics of the changing system of antigens of the target virus.

The sequence of the S protein of SARS-CoV-2 represents ~ 24% of the genome of the virus, which means that the newly created vaccines currently in use provide the immune system with only partial information. In a very real sense, these innovative vaccines employ an equally narrow approach to creating an immune response, which is bound to fall short with time [4]. The S protein has been identified as perhaps the key glycoprotein required for viral entry of the host cell. However, as has been amply demonstrated by the omicron variant, mutations in the S protein gene sequence allow the virus to substantially circumvent the immunity provided by vaccines. As new subtypes of the SARS-CoV-2 coronavirus have emerged, mounting evidence has shown the effectiveness of these vaccines decreases [5, 6].

General side effect accompanying use of adenoviral vectors and mRNA vaccines is an unwanted increase in protein levels in human cells caused by their active expression of the SARS-CoV-2 S protein. This protein has been shown to accumulate in the blood (plasma), in human renal tissue, and in the brain, in glial cells [7] and neurons [8]. Studies in male mice have shown that radio-iodinated spike S protein (I-S1) injected intravenously readily crossed the blood–brain barrier, was taken up by brain regions, and entered the parenchymal brain space. Several other alarming side effects have occurred with reportable frequency following vaccine administration. People who received mRNA vaccines reported hearing impairment, exacerbation of asthma, bloating, rosacea, and skin sensitivity disorders [9, 10], while those who received adenovirus vaccines reported laryngeal and facial edema, thrombosis, anemia, thrombocytopenia [11], nausea, and swollen lymph nodes [12].

In addition, there is a possibility of the development of antibody-dependent enhancement (ADE) of infection in response to vaccines. Previous potential vaccine trials with inactivated and live attenuated vaccines for two respiratory viruses, syncytial virus and dengue virus, failed due to human clinical safety risks related to ADE [13]. This has been a problem with all attempts to develop vaccines for coronaviruses; research into vaccines for severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus encountered SDE responses in animal models. In humans, there are two different mechanisms that may account for ADE: SARS-CoV-2 antibodies bound to Fc receptors on macrophages or on mast cells. This complicates the current widespread use of SARS-CoV-2 *B* cell activated vaccines (inactivated vaccines, mRNA vaccines, and adenovirus vaccines) for different groups because of age, cross-reactive antibodies, variabilities in antibody levels over time, and pregnancy [14]. Clearly, the need to develop safe SARS-CoV-2 *T* cell vaccines not dependent upon antibodies is desirable [15, 16].

Overall, mRNA vaccines and adenoviral vector vaccines for SARS-CoV-2 have been successful, with an efficacy of 76.7–94.5% [17]. These vaccines act by forming memory *B* and *T* cells [18] as well as specific antibodies to the viral spike S protein of the virus [17]. Their use has reduced mortality [19] and mitigated the course of the disease, giving researchers a chance to learn more about this virus and how to handle it. Of course, at their best, vaccines are a force that can quickly and effectively reduce the number of victims during a pandemic [20]; however, the flip side is the unavoidable negative aspects associated with side effects [9–12].

When using innovative vaccines in a pandemic, given that their use comes with certain caveats, it is obvious that

humanity will have to choose the lesser of two evils. Thus, if the use of a particular vaccine results in mild side effects, but reduces mortality and leads to an milder course of the disease, then that vaccine should be used, especially for people at risk.

Today, to a certain extent, we can say that global vaccination for SARS-CoV-2, which primarily involves use of vaccines developed using the aforementioned innovative approaches, is a global experiment. Unfortunately, while humanity does not yet have sufficient experience to assess the long-term effects of such vaccination, some individuals have already received 3 or more doses of vaccines and boosters. It is assumed that in the near future a rational algorithm for reducing the number of doses of vaccines used per person will be considered and adopted while maintaining the maximum effectiveness of the preparation.

Thus, the innovative approach to the creation of vaccines in the form of adenoviral vectors and mRNA vaccines has been relatively successful at preventing severe forms of the disease and reducing mortality. However, the effectiveness of these vaccines is highly dependent on the frequency of occurrence of various subtypes of the viruses and emergence of the new ones.

The creation of new, safe, and effective platforms for the production of vaccines that can flexibly change and adapt to new subtypes of RNA viruses (coronaviruses, influenza viruses, HIV, etc.) is urgent and should be considered strategically necessary. The emergence of new effective platforms for the creation of antiviral vaccines is largely facilitated by the development of viral genomic databases and the improvement of methods for the synthesis of nucleic acids.

Genomic virus databases and nucleic acid synthesis: riding the wave

To date, the most actively sequenced genomes are those of viruses, since they are the shortest, which means that there are thousands of known viral genome sequences. The NCBI Viral Genomes Resource contains over 5600 complete reference viral genomes. In total, more than 2 million sequences of viral origin have been deposited in INSDC (International Nucleotide Sequence Database Collaboration) databases. The rapid increase in sequenced genomes made possible by next-generation sequencing technology has resulted in new challenges associated with the annotation and maintenance of viral genomes [21]. Analysis of these genomes is a powerful tool with which to explore and gain a better understanding of the human microbiome and how its constituent microbiota affects human health. The symbiotic microbial cells of each person's microbiota number in the trillions; the genes in these cells comprise the microbiome. Therefore, it is absolutely critical that we identify the major characteristics of the human virome as a step towards understanding the role of the microbiome. The reliance of traditional characterization of virome datasets on homology-based approaches is one of the challenges facing researchers when attempting to annotate viral sequences. While these approaches can accurately identify sequences from virus families that have already been characterized, as well as the sequences of distant relatives, they fail when presented with sequences that share little similarity or none at all, with known sequences [22].

There are various public databases that store and distribute information regarding viral genome sequences, annotation of genes and proteins, and information on viral structure, biology, pathology, and taxonomy. Primary sequence databases, INSDC (International Nucleotide Sequence Database) databases (GenBank/ENA/DDBJ), and Uniprot constitute the main repositories of sequence data concerning nucleotide and protein levels. Reports published by the International Committee on the Taxonomy of Viruses (ICTV) are considered both the standard and the definitive reference for the taxonomy of viruses. These taxonomy releases are also available online (www.ictvonline.org). In addition, there are databases focusing on specific groups of viruses, e.g., OpenFluDB, Ebola Virus Knowledgebase, HBVdband, and many others [21].

Basically, the primary use for the information found in the genomic databases of viruses is genetic engineering. Very rarely, the nucleic acid sequence of the virus is used as an active tool, which functionally affects the cell. These examples include antisense oligonucleotides used as drugs [23, 24]. So far, there are no accounts in the literature of the use of a unique oligonucleotide which would play the role of antigen. This is quite feasible since such oligonucleotide antigens can be synthesized quite simply and quickly.

Chemical synthesis of oligonucleotides [25] is currently widely used in molecular biology and medicine to create primers and probes for the diagnosis of diseases [26, 27], gene assembly [28], vector inserts [29, 30], genome sequencing [31, 32], genomic editing [33–35], and gene modification [36, 37], as well as the development of drugs using antisense therapy [38–41]. The method most widely used in the synthesis of oligonucleotides is the automatic solid-phase phosphoramidite synthesis method, which makes it possible to obtain the specified sequences of oligonucleotides relatively quickly, with high yield and purity [42, 43], for use in solving a wide range of problems.

However, solid-phase synthesis is inefficient, consuming on average several times more than the required amount of reagents to create oligonucleotide chains. In addition, solid-phase synthesis uses large amounts of silica-controlled pore glass (CPG) [44], which is the most expensive component of solid-phase synthesis, as well as expensive automatic nucleic acid synthesizers. A rational alternative to this technology is liquid-phase synthesis, which can significantly reduce the

cost of the process by removing the need for CPG, reducing the amount of phosphoramidites required, and removing the automatic synthesizers by transferring the synthesis to chemical reactors [45–48]. According to approximate calculations, liquid-phase synthesis can reduce the cost of oligonucleotides by 100-fold.

The Achilles heel of liquid-phase synthesis so far is that the process lacks automation and the creation of effective anchors [49, 50]. It is obvious that liquid-phase synthesis will be more competitive compared to solid-phase synthesis for the production of oligonucleotides of short length, such as DNA insecticides [51], DNA drugs [52], and DNA regulators of plant secondary metabolism [53]. This will make it possible to obtain large volumes of targeted short deoxyribonucleic acid sequences at low cost.

In the case of relatively long oligonucleotide chains (50–60 nucleotides) for the creation of phosphorothioate oligonucleotide vaccines of the 'lasso' type [54], solid-phase synthesis will be suitable, since in this case the cost of one dose of such a vaccine (5 mg of active ingredient) will be between 3 and 4 USD. Indeed, if highly conserved viral sequences are used to create oligonucleotide vaccines, using genomic databases to search for targets, then such vaccines will have a long operating life. Despite the apparent simplicity and availability of the approach, it is necessary to answer the question: is it possible to use an oligonucleotide sequence as a stable and effective immunogen?

Oligonucleotide vaccines: the joker in the vaccine deck

Studies carried out over the past few decades have accumulated sporadic but convincing data on the possibility of using nucleic acids as an active immunogen, but for this to succeed, it is necessary to set certain parameters:

1. The nucleic acids must be single-stranded rather than double-stranded [55, 56];
2. The nucleic acids must have a permanent multidimensional nanostructure [57, 58]; and
3. the nucleic acids must have a unique sequence that is not present in the host genome [59, 60].

The characteristic sequence motif of bacterial DNA, which contains the unmethylated cytosine-guanine dinucleotide CG at its core (CpG), activates the mammalian immune system to produce antibodies to the sequence rather than backbone of DNA [55]. When foreign DNA enters a host, like any other foreign, unknown protein, it causes an antigenic reaction, since its epitope structure carries sequences not shared with the host. This reaction to foreign DNA is universal and independent of immunity status, as numerous data have demonstrated that hosts with both normal and aberrant immunity produce anti-DNA. We agree with the prophetic words of David Pisetsky, who said in 1998, with the recognition of the epitope structure and immunostimulatory properties of bacterial DNA, DNA has been transformed from a uniform and inert molecule into a powerful presence whose activities are extensive and pervasive. The coming years should be exciting as investigators elucidate these immune activities and develop techniques for their manipulation in the treatment and prevention of human disease [55]. Nevertheless, until 2020 [54], not a single attempt was made to create a working vaccine based entirely on oligonucleotide constructs, although oligonucleotides themselves have been used in vaccine formulas as adjuvants for several decades [61–63].

All the coronavirus vaccines in use today employ whole proteins or protein fragments to induce an immune response. The idea of oligonucleotide vaccines was first proposed in the form of a lasso-like oligonucleotide phosphorothioate (PS) construct containing an antigen-presenting 'head' with a unique sequence for activating acquired immunity, a tail with CpG islands to activate innate immunity, and a 'neck' connecting the 'head' and 'tail' [54]. In the proposed 'lasso' (La-S-so, lamellar anti-SARS-CoV-2 sulfur-containing oligonucleotide) construction, we placed the CpG motifs in the 'tail, region in the most efficient 5'-purine-purine-unmethylated deoxycytosine-deoxyguanosine-pyrimidine-pyrimidine -3' position [54].

While the inhibitory effect observed for polyG motifs of CpG oligodeoxynucleotides (ODN) reduces the level of *B* cell activation in single-stranded polyG motifs [64, 65], in our first construct [54], this motif is found in the double-stranded 'neck' and may have a positive inhibitory effect during the acute inflammation found to occur after vaccination in some individuals. Also, the presence of such a loop (formed by the 'neck') allows us to take advantage of the formation of the stabilized 3D structure in the antigen-presenting 'head '. Only adenine, cytosine, and guanine residues are present in the construct, which makes synthesis of a La-S-so vaccine more affordable since the cost for deoxyuridine phosphoramidite is 6- to sevenfold higher than for other phosphoramidites. Of course, uracyl-containing constructs can be created if required.

Since the PS backbone is the basis of the 'lasso ' construct, the presence of deoxyribose is of little importance as everything will depend on the nucleotide sequence of the construct [66]. CpG motifs in nuclease-resistant PS backbones have been found to dramatically enhance *B* cell stimulatory properties. In almost all vertebrates, the optimal PS CpG ODN are extraordinarily strong mitogens for *B* cells [67–70].

When ODN containing unmethylated CpG stimulate cells, the subsequent expression of Toll-like receptor 9

(TLR9) induces inflammatory cytokines, which activate humoral or cellular immunity [71]. TLR9 agonists, often referred to as CpG ODN, also induce secretion of IFN-immature plasmacytoid dendritic cells, causing *B* cells to differentiate into antibody-secreting plasma cells. Preclinical and early clinical data provide support for the use of TLR9 agonists as vaccine adjuvants; in addition to improving the function of the professional antigen-presenting cells, they generate both humoral and cellular responses to a variety of antigens [72]. These effects work best when the ODN and the vaccine remain in close proximity, as in the 'La-S-so' construct. Several independent studies have now demonstrated that the dependence on CpG motifs for TLR9 activation is restricted to synthetic phosphorothioate oligodeoxynucleotides (PS-ODN), and that natural phosphodiester oligodeoxynucleotides (PD-ODN) bind and activate TLR9 via the 2' deoxyribose backbone in a sequence-independent manner [73–75]. In humans, TLR9 is expressed in plasmacytoid dendritic cells and *B* cells. In addition, recent studies have revealed the presence of TLR9 receptors in normal gastrointestinal organs [76] and liver [77].

Suppression of CpG motifs is a well-known mechanism used by many mammalian RNA viruses, including the influenza virus, to adapt to the human host. For example, viruses that have evolved CpG suppression are able to elude the zinc finger antiviral protein (ZAP). This antiviral factor degrades invading viral DNA by selectively binding to CG-dinucleotide-enriched RNA sequences. This is important because ZAP, which is expressed in human lung cells, has been identified as an important antiviral effector of the IFN response needed to combat SARS-CoV-2. The researchers who studied this knocked down ZAP and observed a significant increase in the production of SARS-CoV-2 by lung cells [78]. In some unfortunate people, the human body has responded to coronavirus infection by overexpressing cytokines and letting loose a dangerous and sometimes lethal 'cytokine storm'. We propose development of a vaccine construct with the CpG motifs that will help human immune systems train for this inflammation reaction in the absence of a real pathogen and real danger [54], and help immunocompetent cells form an immunological memory to a unique antigen-presenting sequence of the virus.

Phosphorothioate oligonucleotides, which contain a non-bridging sulfur, are the most widely studied oligonucleotides; compared to unmodified oligonucleotides, they possess higher solubility and nuclease stability [79], with improved membrane penetration [80].

Phosphorothioate oligonucleotide transient activation of the complement cascade represents the most evident toxicological response, as demonstrated by in vivo studies. These compounds have been shown to prolong the activated partial thromboplastin time, a reaction which is often highly transient and proportional to the oligonucleotide plasma concentrations, making that effect clinically insignificant for the current treatment regimens. In summary, current evidence shows limited untoward effects and reversibility of the damage induced, at least for some of those compounds, with promising effectiveness for treatment of various pathologies [81–83]. Following intravenous administration of oligonucleotides, the highest concentrations are found in the kidneys. These high concentrations are associated with the infiltration of macrophages and monocytes, along with intracytoplasmic eosinophilic granules and vacuolation in renal tissue [24, 50]. Oligonucleotides can be used to selectively target the liver. One of the resulting morphopathological features following administration is hypertrophic change to the Kupffer cells, due to basophilic inclusions, which may be dose related [82, 84, 85].

In all phosphorothioate oligonucleotides tested in mice, the resulting acute LD50 was in excess of 500 mg/kg. Several studies in rodents have evaluated the acute and chronic toxicities of multiple phosphorothioate oligonucleotides administered by multiple routes [86, 87]. The consistent dose-limiting toxicity was immune stimulation, manifested by lymphoid hyperplasia, splenomegaly, and a multiorgan monocellular infiltrate. These dose-dependent effects occurred only with chronic doses around 20 mg/kg. Monocellular infiltrates had their greatest effect on the liver and kidneys. While all of these effects appeared to be reversible, chronic intradermal administration was the most toxic, probably because higher local concentrations of the drugs resulted in local cytokine release and initiation of a cytokine cascade. The sequence of administration had no obvious effect. In the treatment group, there was a significant increase in the area of the parenchyma due to fibrosis and proliferation of alveolocytes in the later stages of the disease. At the same time, in the untreated group, similar values had already been observed on the 5th day from the onset of the disease. At dose of 100 mg/kg and greater, minor increases in liver enzyme levels and mild thrombocytopenia were also observed [87]. Since for the 'La-S-so' type vaccines in our studies, the mass of the active substance is 30 μg per mouse (average weight 20–25 g), this dose is far from dangerous. We have not noticed any side effects other than the apparent protective characteristics of the 'La-S-so' vaccine (under publication).

The key to the effectiveness of 'La-S-so' oligonucleotide vaccines is their ability to activate acquired immunity and act as effective immunogens capable of generating a strong immune response. This leads to several key points worth considering: (a) how antibodies formed on a nucleic acid neutralize the virus particle with this nucleic acid within a virion; (b) whether the dendritic cells are capable of antigen presentation of the vaccine fragment containing the unique nucleic acid sequence of the virus; and (c) what is the likelihood that ADE will occur in response to the use of an

oligonucleotide vaccine? Formed in response to the 'La-S-so ' vaccine, antibodies will not be able to attack the mature viral particle because the nucleic acid is inside the virion. Thus, the emphasis should be placed on antibodies that are capable of 'seeing' the viral nucleic acid within the cell during virus replication. Antibodies have been used therapeutically for extracellular pathogens and for targeting cell-surface antigens. While in general antibodies do not pass easily through intact cellular or subcellular membranes in living cells [88], we assume that the human body can harbor antibodies capable of penetrating human cells during viral infection and targeting specific fragments of nucleic acids in RNA viruses. Over the years, multiple studies carried out primarily in cultured cells have shown that facilitating the cellular internalization of antibodies is achievable [89–91]. Furthermore, a number of studies evaluating a nuclear-penetrating lupus anti-DNA autoantibody have demonstrated its potential in vivo therapeutic benefits [92–95]. This anti-DNA autoantibody was able to enter the nucleus of the cell. Once inside, in addition to inhibiting DNA repair, it located and selectively killed cancer cells highly vulnerable to DNA damage [94, 96]. Some studies have shown that PS DNA oligos linked covalently to antibodies are better able to penetrate cells [96]. Antibodies are extremely specific, with a high affinity for their targets. In addition to their utility as experimental tools, they have become the standard of care for rheumatoid arthritis [97], psoriasis, solid tumors [98], and blood cancers [99]. The clinical success of antibody therapy in the clinic has heightened interest in the field itself and in the effects of therapy. This has led to the development of specifically engineered humanized molecules. The improvements made to the physical and chemical properties of these molecules have made them safer for patient use [100, 101]. Generally, the antibody must be delivered inside the living cell to become effective. While difficult, the search for cells to facilitate antibody penetration is not over yet (Fig. 1).

Another key to strong immune response are dendritic cells, leukocytes derived from bone marrow. Able to migrate within the body, throughout which they are distributed sparsely, these cells specialize in the uptake, transport, processing, and presentation of antigens to *T* cells [102]. After encountering and taking up antigens in the peripheral tissues, dendritic cells process the antigens into proteolytic peptides, which are then loaded onto major histocompatibility complex class I and II molecules. Before they can present these antigens to T lymphocytes, dendritic cells must migrate to the secondary lymphoid organs, where they become competent. Subsequent presentation of the antigens to T lymphocytes initiates antigen-specific immune responses. While there are other antigen-presenting cells, the processes that make dendritic cells particularly effective at stimulating the immune response are different, with

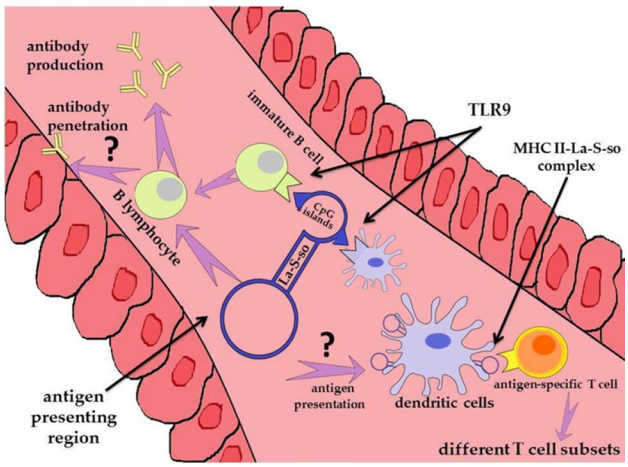

Fig. 1 Presumptive scheme of action of oligonucleotide vaccines: pathways supporting *B* cell and *T* cell activation are discussed

meticulous regulation of each step (antigen uptake, intracellular transport and degradation, loading of MHC molecules). These specializations allow dendritic cells to play a unique and necessary role in the initiation of immune responses and the induction of tolerance [103]. It remains unknown whether dendritic cells are capable of antigen presentation of nucleic acids. Perhaps no one has asked quite the right question yet.

When an unknown protein presents itself and its ligands to these cells, they respond by stimulating the innate immune system and causing it to produce cytokines and chemokines and initiate *T* cell differentiation [104]. Among the receptors found on these dendritic cell subpopulations are those in the Toll-like receptor family (TLR3, 7, 8, 9) and the cytosolic RNA helicase family (RIG-I, MDA5, LGP2). When viral DNA and RNA bind with these receptors, causing dendritic cells to activate, they trigger the specifically antiviral innate immune response that creates antiviral immunity [105]. Based on how they carry out their usual functions, it makes sense that dendritic cells, among other things, are capable of antigen presentation of unique fragments of viral RNA nucleic acids. This idea is prompted by the fact that specific antibodies can form in response to nucleic acids, which means that a connection exists between the *B* and *T* cells.

So far, not a single antigen has been found that activates only B cells without affecting *T* cells, the versatile activation of which depends on dendritic cells. *B* cells and *T* cells sample antigens in secondary lymphoid tissues, lymph nodes, and the spleen, all of which provide microenvironments ideal for facilitating physical interactions among *B* cells, *T* cells, and dendritic cells [106]. In the secondary lymphoid organs, *B* and *T* cells act in concert to generate the optimal immune response against invading pathogens. For example, within follicles, *B* cells rely on the highly specific *B* cell

receptor to recognize antigens. The *B* cells then internalize, process, and present the antigens to the *T* cells. This unique, precise process comprises five consecutive and interdependent steps by which the *B* cells prepare antigens for the *T* cells. Pathogen recognition by the *B* cell receptor results in a signal that initiates the following: actin remodeling; endosomal formation and transport; synthesis of HLA class II molecules, which are trafficked to specialized late endosomes; and finally, processing of the antigen, which is loaded on the HLA class II molecules and presented to the CD4+ Th cells [107]. In other words, they always work together to get the immune response rolling.

Data from the study of SARS-CoV and other respiratory viruses suggest that anti-SARS-CoV-2 antibodies could exacerbate COVID-19 through antibody-dependent enhancement (ADE) [108]. It should be noted that the likelihood that ADE will occur with the use of oligonucleotide vaccines is extremely low, since the nucleic acid is inside the viral particle and the specific antibodies formed are in suboptimal concentrations that cannot facilitate the penetration of the virus into the host cells.

In March 2020, our research group, which has been working with oligonucleotide constructs for about 15 years [51], thought about the possibility of using phosphorothioate oligonucleotide constructs to create vaccines against the coronavirus. We did a preliminary review of the concept of oligonucleotide vaccines in the journal of Inflammation Research [54]. To date, we continue to research and test two main types of oligonucleotide constructs, semi-natural and natural (Fig. 2).

In a number of our experiments with SARS-CoV-2, a semi-natural vaccine of the La-S-so type was found to have a moderate immune response, expressed by formation of specific antibodies. In addition, studies of the La-S-so vaccine have been conducted in humanized mice with the human ACE2 receptor. By day 30 of the experiment, it was shown that, while all the vaccinated animals were alive and beginning to recover, all the control mice had died. The morphological parameters of the lung parenchyma in vaccinated animals were comparable to those of intact animals (under publication). We assume that the effects observed cannot be explained solely by the adjuvant role of oligonucleotides. Research continues on the selection of doses (fine tuning to discover the most effective amount) and the refinement of constructs like 'La-S-so', to find those with the best immune response.

Conclusion

As SARS-CoV-2 has shown us, it is impossible to predict the emergence of new mutations of RNA viruses. Modern vaccines are vulnerable to these unpredictable shifts in

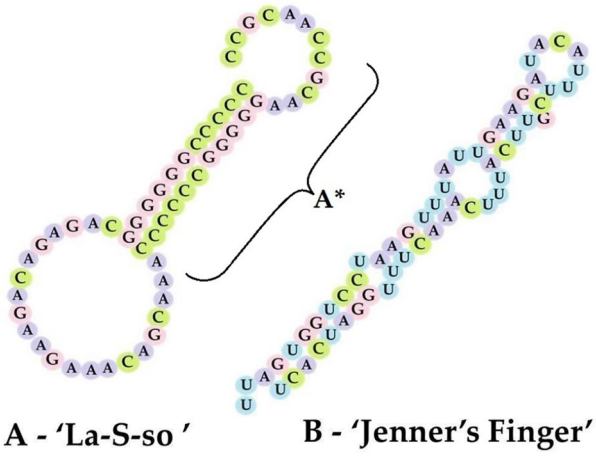

Fig. 2 Variants of oligonucleotide vaccines **A** semi-natural (has inserts (A*) of oligonucleotides that do not belong to the RNA virus [54]), **B** natural (consists of the full RNA virus genome fragment). The constructs are performed by RNAfold WebServer; https://rna.tbi.univie.ac.at)

nucleotide sequences, since the principle of their action and effectiveness depends on the presence of the certain specific extended amino acid sequences in the peptide chains of the pathogen that play the role of antigen.

For this reason, the use of conserved amino acid sequences of surface proteins of RNA viruses, such as S proteins, has little chance of long-term success.

In fact, they are constantly changing, undergoing microevolution to ensure maximal adaptation to the host cells, whose genetics are also constantly changing. However, the nucleic acids of RNA viruses contain extended, highly conserved regions that hardly change over time, which represent an ideal target for therapeutic development using principle 'diamond cuts diamond'. It has been impossible to hit the target thus far is because the concept has been poorly studied. However, these vaccines can be versatile and very effective. Oligonucleotides have been acting as excellent adjuvants for decades, and more and more data are accumulating that they can be antigens as well. We may be running out of options with modern vaccines, which depend on the amino acid sequences of RNA viruses' proteins, but we are well on the way to creating new, less limited options with universal oligonucleotide vaccines.

Two obstacles stand in the way of the development of oligonucleotide vaccines: the absence of knowledge about antibodies capable of penetrating cells and attacking the unique nucleic acid sequences of RNA viruses, and the unknown antigen presentation ability of dendritic cells with respect to nucleic acids. At the same time, there is no doubt that oligonucleotide vaccines based on highly conserved regions of the RNA virus genomes have great potential in

the prevention of viral diseases, despite the fact that they do not yet fit within the framework of a modern textbook on immunology.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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