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Commentary

Multicomponent vaccines to fight SARS-CoV-2 variants of concern

Antonio Cassone [a,*], Roberto Cauda [b,1]

[a] Polo d'innovazione della genomica, genetica e biologia, c/o Toscana Life Sciences, Strada del Petriccio e Belriguardo, 53100 Siena, Italy
[b] Dipartimento di Sicurezza e Bioetica, Università Cattolica del Sacro Cuore, Rome, Italy

1. Effective vaccines against COVID-19 pandemia

COVID-19, the pandemia caused by the new coronavirus SARS-CoV-2, continues to ramp up worldwide, with more than 215 million cases and nearly 4.5 million deaths as of August, 31st, 2021 (www.who.int). To fight this dreadful health threat, a number of SARS-CoV-2 vaccines have been generated and authorized for emergency use by regulatory authorities. Most of them have been made using the viral gene coding for SARS-CoV-2 Spike (S) protein, either as RNA or DNA, vectored by a safe, non-replicating human or chimpanzee adenovirus, as immunogens [1].

The S protein plays a critical role in SARS-CoV-2 infection in that its S1 subunit exposes a domain of around 25 amino acids (RBD) which bind to ACE2 receptor of human cells while its S2 subunit interacts with the host cell membrane and allows the viral fusion with, and entry, the cell so initiating infection [1]. The choice of nucleic acid technology and S protein as antigen for vaccine formulation was pathogenically logical and allowed generation of safe and highly efficacious vaccines at an unprecedented speed (around 1 year). These vaccines elicit the production of specific antibodies that inhibit S protein binding to ACE2 receptor and neutralize virus infection. They also activate cell-mediated, B and T memory immunity, and are highly effective in protecting against severe disease, hence abating COVID-19 mortality [2].

2. Vaccines challenged by SARS-CoV-2 variants

Unfortunately, though expected, viral mutations have soon appeared among spreading SARS-CoV-2 lineages, the so-called Variants of Interest (VOI) or Concern (VOC), which seriously threaten vaccine effectiveness. The most threatening mutations are those leading to amino acid substitutions or deletions in the S protein, particularly in its RBD sequence. Some of them make SARS-CoV-2 more contagious because they confer to the S protein increased affinity for ACE2 receptor but do not appear to markedly undermine vaccine effectiveness. Some others, however, appear to substantially lower antibody protection as shown by in vitro experiments with hyperimmune sera from convalescent or vaccine-recipients. One of them is the VOC B.1.351, first identified in South Africa, bearing the E484K mutation [3], A breakthrough infection by a SARS-CoV-2 variant with the E484K and other mutations has been reported in two fully vaccinated women with high titers of neutralizing antibodies [4]. Additional evidence of virus immuno-escape has recently been provided with the VOC B.1.617, first isolated in India and, more recently, with one isolated in Peru [5]. Increased contagiousness and immuno-escape ability, in a context of a substantial lack of vaccine availability in low-income countries, and vaccine geopolitical usage, make the prospect of an uncontrollable pandemic not so unrealistic.

3. Proposal

Scientists, public health administrators and stakeholders appear to be well aware of the above threatening situation, and some vaccine manufacturers have started production and validation of a second generation vaccines for VOC better fighting. To do this, the high efficiency and flexibility of nucleic acid-based technologies, as witnessed by the success of current vaccines, is being rightly advocated, and a logical option would seem to be just replacing, or adding to, the present nucleic acid sequences with those of coronavirus variants [1,2].

We believe there is time to consider an integrative option. We posit the necessity of broadening antigen composition of the second generation, SARS-CoV-2 vaccines by including sequences of genes encoding non RBD, non S constituents of the coronavirus genome. We invite vaccine companies to seriously consider the potential of a multicomponent vaccine to generate protective antibodies and cell-mediated immunity to which SARS-CoV-2 could much less likely escape by mutations than with the current, single component vaccines. A number of structural and non-structural viral proteins of critical importance for the reproduction cycle and architecture of the beta-coronaviruses, as well as for their interaction with human cells, could in principle be considered. Past studies have indeed documented that SARS-CoV-2 -infected subjects raise strong immune responses to antigenic components other than S protein, some of which are likely to contribute to the immune protection against the virus, as discussed below. Most of these immune responses are targeted to the nucleoprotein (N) constituent of the viral particle.

* Corresponding author.
E-mail addresses: a.cassone@pologgb.com (A. Cassone), roberto.cauda@unicatt.it (R. Cauda).
[1] Istituto di Clinica della Malattie Infettive, Università Cattolica del Sacro Cuore, Largo Francesco Vito, 1, 00168-Roma, Italy.

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We here propose the formulation of a nucleic acid vaccine, either RNA or vectored DNA, which contains both S and N gene sequences.

The N protein, which is the only protein present in the coronavirus nucleocapsid, plays a critical role in ensuring coronavirus replication and successful intracellular lifecycle [6]. As other structural constituents of the viral particle i.e. the M and E proteins, the N protein is highly conserved but differently from the other two proteins, it is at least as immunogenic as the S protein, as largely documented in SARS-CoV-2-infected subjects and convalescent COVID-19 patients. While there is evidence of a protective role of the N protein in vaccines against animal infections by coronavirus and partial protection against MERS infection [6], other studies with SARS-CoV-1 virus have suggested that the N protein would be unsuitable as a constituent of coronavirus vaccines in humans [7]. However, the animal model used in those studies (aged Balb/c mice not engineered to express human ACE2) is not representative of SARS-CoV-2 human infection, which progresses to lung and other internal organs via binding to the ACE2 receptor. Moreover, the animals were immunized with the full protein rather than some of its gene sequences as we are here proposing (see below).

4. Data supporting the proposal

There are several recent indications that the addition of N protein sequences to S protein ones has the potential to contribute to the generation of an efficacious and effective COVID-19 vaccine. It has been shown that convalescent plasma samples had a complex set of diverse, antibody-mediated antiviral activities, with both neutralizing and other, Fc-mediated, effector functions [8]. Although the epitope -specificity of these antibodies have not been reported, the Authors suggest that convalescent plasma could retain activity against virus variants because of their extensive poly-functionality, implying that other SARS-CoV-2 antigens could also be involved in addition to the S protein. More specifically, it has been shown that patients infected by SARS-CoV-2 generate approximately similar levels of both anti-S and anti-N antibodies, but these latter were of longer duration and switched from IgM to IgG class more rapidly than the former [9]. This early report has been confirmed and expanded in a much larger COVID-19 patient cohort by Secchi and collaborators who identified a strong antibody response against a monomeric formulation of the N protein [10]. Of particular relevance to our proposal, Morgenlander and collaborators, using a phage display technology (VirScan library of 3466 peptides across the whole SARS-CoV-2 protein composition), were able to identify four regions of the N protein recognized by 59–100% of convalescent sera with high neutralizing titers and other antibody functionalities of potential antiviral impact such as antibody-dependent cellular cytotoxicity [11]. Similar four regions of N protein were identified in another study using Vir Scan library targeted by antibodies from most COVID-19 patients [12]. An antibody-binding epitope from human convalescent sera located in a C terminus region of the N protein has recently been reported by Casasanta and collaborators using a recombinant N protein fixed in engineered functionalized microchips [13].

It remains unclear as to how anti-nucleoprotein antibodies express their antiviral activity. Working with LCMV as a model system, Caddy and collaborators have suggested that anti-N antibodies activate the cytosolic Fc receptor and E3 ubiquitin ligase TRIM21 thus inducing cytotoxic T cells able to kill viral N-peptides expressing cells [14]. While it is unknown whether this mechanism operates in coronavirus infections, it is known that T cells are critical components of protective antiviral immunity. In fact, a remarkable set of data have shown that the N protein is a dominant target of CD4 and CD8 T cells of COVID-19 patients [15]. Importantly, Grifoni and collaborators do also make an explicit statement about the possible advantage of including N (and M) protein in the formulation of new vaccines to better mimic the natural T cell responses in COVID-19 patients.

The overall set of the above data and their interpretation we have here discussed, appears to support the potential added value of inclusion of N protein in novel COVID-19 vaccine formulations. In particular, the identification of N protein sequences as targets of antibodies and T cells could allow the selection of the corresponding nucleic acid sequences for an easy insertion in a multicomponents, second generation nucleic acid vaccines capable of conferring enhanced protection against SARS-CoV-2 VOC.

Vaccines which combine different protective antigens against a single pathogen are widely used and effective either to protect against disease caused by microbes with a vast array of virulence factors, such as the acellular pertussis vaccines, or to account for the variety of circulating bacterial and virus types, and decrease the likelihood of immuno-escape by antigenic shift, as for pneumococcal and papillomavirus vaccines. Future, second generation of COVID-19 vaccines would belong to this highly meritorious category of human vaccines.

5. Note added at the revision stage

After paper submission, we came across a posted publication by Jim Young Ahn and collaborators: (doi: org/10.110/2021.05.26.21257700(doi) reporting promising preliminary results of a Phase 1 study of safety and immunogenicity of a DNA vaccine comprising both S and N sequences.

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Declaration of Competing Interest

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