A novel vaccine approach and the use of a synthetic standardized convalescent plasma until its development

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We have outlined the idea of creating a synthetic standardized convalescent plasma with assigned neutralization capacity and a vaccine strategy using a multi-peptide approach targeting the mucosal immune system.

Keywords: SARS-CoV-2, COVID-19, vaccine, convalescent plasma

Introduction

It is with concern that we observe the laudable efforts aimed at encouraging immunity towards the SARS-CoV-2 virus via vaccination. However, of concern are those efforts geared towards inducing immunity by targeting viral Spike (S) nucleotides or protein in any way, shape, or form.

This concern stems from the fact that S, while indeed an attractive antigen¹, is also unfortunately quite knowingly prone to mutation². While perhaps not nearly to the same extent as the S region of influenza³, a recent report² has already identified 14 mutations of circulating Novel coronavirus -- with all of the mutations occurring within the S encoding region. Our main concern is that any potential vaccine targeting S may result in a short-term gain followed by a long-term loss (i.e.: short-term immunity followed by a negating mutation). We address this concern by proposing what we believe to be the most efficient means to a vaccination that will provide immunity of sufficient durability and sufficient safety within the most reasonable timeframe.

Furthermore, with a vaccine in reach only in the near future (arguably overly optimistic by the first quarter of 2021), we believe that convalescent plasma represents the best treatment option so far for COVID-19 besides famotidine, dexamethasone, and heparin.

Even when a functional vaccine is produced, there will be such high demand that not enough doses will be available initially, and as such, convalescent plasma will still be an important substitute for this "treatment." Albeit there are and will be many people recovering from SARS-CoV-2 infection who are and/or will be willing to donate their antibody-rich plasma to be given to others with severe disease, this might also present a problem with availability and quality⁴⁻⁹. In the following we outline the idea of overcoming this shortcoming by producing synthetically engineered plasma with a defined neutralization capacity as a treatment option to cover the worldwide demand.

Antibody approach

Characterization of the epitopes of monoclonal antibodies (mAbs) from the plasma of recovered COVID-19 patients has been ongoing. Neutralising antibody (nAb) response appeared within 9-days along with specific immunoglobulin M (IgM) and immunoglobulin G (IgG) response which targeted particularly nucleocapsid and S proteins¹⁰. Nucleocapsid protein (NP)- and the RBD region of S protein ([S-RBD]-specific IgM and IgG antibodies) were both detected in the sera of newly discharged patients, and specific T-cell responses were detected as well¹¹. Approaches searching for a broader picture of antibody response in recovered COVID-19 patients found antibodies to 11 of the 15 SARS-CoV-2 antigens tested including NSP1, ORF3b, ORF7a and ORF8^{12,13}. With thousands of recovered patients and sera available, one could investigate a largescale study of the most prevalent epitope by high-throughput screening (HTS). Aim is to produce a drug of a mixture of 5 to 10 antibodies with different targets of most frequent appearing sequences. The aim would be to produce a drug consisting of a mixture of 5 to 10 antibodies with different targets of the most frequent appearing sequences. Taking into account that mAbs and T-cell and B-cell responses of SARS-CoV and other seasonal coronavirus infections were characterized and show cross reactivity similar as those characterized by higher homology to S glycoprotein of human endemic "common cold" coronaviruses^{9,14,15}, this may also want to be considered for addition.

At least Regeneron Pharmaceutical used a similar strategy for their Ebola drug REGN-EB3, where they mixed a cocktail of three monoclonal antibodies--as well as did Mapp Biopharmaceutical for Ebola cocktail ZMapp¹⁶. Also in the

meantime, Regeneron has developed a mixture of two potent neutralizing antibodies against S in preclinical testing called REGN-COV2 showing promising results¹⁷. In a large clinical trail, convalescent plasma (CP) proofed safe for use and also benefit for survival when admistered early¹⁸. The advantage is apparent of having a defined mixture and concentration allowing a distinct dosage over convalescent plasma. Also, shelf life and stability can be improved by the addition of substances like glucose and maltose (and if necessary) amino acids like glycine or proline for preventing clogging of IgG molecules. It should be noted that saccharose may produce kidney failure especially if the patient has pre-existing kidney problems¹⁹.

Even if one is administered a mAb with a known and approved safety profile and from an established cell line known for reliable production, we are dealing with a new biological entity (NBE) regarding the regulatory side--not to mention that the antibody sequences would need to be adjusted accordingly by cloning/editing. The product development timeline from lead mAb identification to phase 1 investigational new drug application (IND) is 10–12 months at many companies today (however, the emergency use application [EUA] function at FDA at least could negate this). Although alternative hosts, such as yeast, *Escherichia coli*, and plants have been proposed to have the benefits of rapid genetic engineering and production--major deficiencies which preclude them from enabling a rapid response to a pandemic outbreak.

A majority of therapeutic antibodies to date are produced from Chinese Hamster Ovary (CHO)-cells which provide for a number of advantages as host cells for production. CHO cells are

well characterized with established gene amplification methods and improved clonal selection strategies; thus, contributing to an overall increase in protein production yield. As such, CHO-derived proteins may lack or have differed glycostructures from human-derived glycoproteins²⁰.

Phage antibody display is rapidly maturing into a very effective tool for antibody generation and is one of the few methods for generating fully human antibodies²¹. The recent development of large primary antibody libraries enables selection of antibodies against most targets in under two weeks and many of these antibodies have relatively high affinities. As such, one possible method might be using phage display to generate human antibodies to these peptide sequences from those studies. Resulting in antibodies with affinities comparable to those obtained using traditional hybridoma technology can be selected from either large naïve antibody libraries or from immune phage antibody libraries. Automation of this technology is making it possible to identify hundreds of different antibody leads to a single therapeutic target. With the first antibody of this new generation, adalimumab (Humira, a human IgG1 specific for human tumor necrosis factor [TNF])²², already approved for therapy and with many more in clinical trials, these recombinant antibody technologies could provide a solid basis to produce the specific antibodies needed to substitute convalescent plasma.

Nonetheless, eukaryotic *in vitro* translation systems have supplemented the antibody production pipeline, as these systems are able to mimic the natural pathway of antibody synthesis in eukaryotic cells. We would suggest using such a microsome-containing cell-free expression system based on CHO cells. These systems have become a

well-proven and economically beneficial alternative to conventional cell-based protein expression systems²³. The protein synthesis is disconnected from cell viability and fate since the reactions are carried out using cell lysates. These lysates contain all the molecular components necessary for translation, such as ribosomes, translation factors, and enzymes. To initiate protein synthesis, the cell lysate is mixed with template DNA or mRNA and supplemented with amino acids and energy-regenerating components. Moreover, the system allows for rapid production in any country with minimal effort. A CHO-based cell-free system allows for antibody candidates to be synthesized in a very short time period in parallel reactions. In favor of cell-based expression, the cell-free system offers several major advantages such as the fact that antibody candidates can be synthesized rapidly within a few hours of incubation time compared to weeks when using cell-based expression without the need of cloning/gene editing, stability testing of the cell line, etc. Further, antibodies can be synthesized based on linear expression templates such as PCR fragments--an instance that circumvents time-consuming and labor-intensive cloning steps²⁴.

Vaccine approach

Only one Chinese and an Indian concept follows the old fashioned route of chemically inactivated virus. Most other common vaccine projects aim to target the S protein in one way or another although relying on different platforms and creating at least to this respect diversity. Even though it seems rather logical from a biochemist's point of view, it also harbors dangers.

Besides an overboarding immune response seen in some cases to S protein, it is also prone to strong

mutation already taking place and not predictable if evolutionary put to stress. Doubtless there are regions in S that cannot be mutated due to functional or stability reasons, but wouldn't it be better to take no chance and provoke an immune reaction and/or mutation against these regions? Also for SARS the S protein as a vaccine target showed to be a problematic solution²⁵. At least recently there have been reports about attempts that also use additional antigens to S like nucleocapsid (N) antigen²⁶. Furthermore, from sera of recovered patients there are also other targets obvious that should be included in a vaccination approach.

Even bioinformatics approaches are under way that predicted B- and T-cell epitopes and suggest potential concomitant sequences for vaccination targets^{27,28}. So a comparison with sequences yielded by bioinformatics and those occurring in recovered patients can help us in finding the right target. Whereas Enayatkhani et al. suggest to synthesize a polypeptide out of the yielded sequence, our suggestion would be a bit different²⁸. Our suggestion would rather be a mixture of several peptides picked from those attempts (modified i.e. by fusion to TAT sequence). TAT peptide is a protein transduction domain composed of an 11-amino acid peptide (TAT47–57: YGRKKRRQRRR)²⁹. The positive charge of this peptide allows protein antigen fused with it to improve cell penetration or polymers for endolysosomal or cytosolic delivery--like for instance polypropyl acrylic acid³⁰. While in the works, a paper from OSE Immunotherapeutics appeared on bioRxiv describing a similar approach. It is a very sophisticated attempt using data mining and AI to select specific epitopes with already positive data from preclinical and ex vivo studies³¹.

Antigen presentation leads to immunogenicity only when costimulatory molecules are presented with the MHC-class-I-associated or MHC-class-II-associated peptide antigen. To render the mixture immunogenic we would have to waive old adjuvants like alum and rather trigger pattern recognition receptors (PRR). PRR are abundantly expressed on dendritic cells and its activation via a danger signal gives instruction how to handle the antigen^{32,33}. Since it is a large group of receptors, we specifically think of Toll-like-receptors (TLRs) which are known to play an important role in pathogen recognition. Viruses like SARS-CoV2 are pleiotropic stimuli and likely trigger the intracellularly expressed Toll-like-receptors such as TLR3, 7, 8 and 9. Furthermore, since S protein is heavily glycosylated, it is also possible that it might interact with the TLRs expressed on the cell surface--namely TLR4 or TLR2 in concert with TLR1 or TLR6. To pretend a viral encounter and rendering the proteins immunogenic, we suggest triggering one or a combination of these TLRs. One such way is triggering of TLR3 since we are dealing with an RNA virus and it is also the natural mode of activation. One possible way to achieve this is to couple poly(I:C), a synthetic double-stranded RNA, to the other end of the peptide or formulating the mixture with poly(I:C)³⁴. Also adding the TLR2 agonist Pam₃CSK₄ to the formulation represents a possibility which has proven to be beneficial in dengue virus vaccine attempt³⁵. At least in a murine model of colorectal cancer a vaccine with a mixture of TAT-CEA fusion protein and poly(I:C) demonstrated that it can induce anti-tumor immunity²⁹. Lately, a new synthetic TLR1/2 agonist was described that was successfully used to vaccinate a healthy volunteer with a multi-peptide vaccine in a simple oil-in-water-emulsion³⁶.

Furthermore, a clinical phase I study was successfully carried out using a multiple peptide mixture to elicit an immune response against glioblastoma³⁷.

Since the route of infection of SARS-CoV2 is by droplets through the respiratory tract, it naturally seems that a good way to administer such a vaccine would be intranasally by some sort of nasal spray or the like. In some cases, it might also be beneficial to target the mucosal immune system instead intramuscularly or subcutaneously, since a B. pertussis vaccine applied through the nasal route released a breadth of antibodies, a T_H1-type cellular response and killing mechanisms are elicited³⁸. The idea of a nasal vaccine is also supported by a some scientists organised in the Radvac (Rapid Deployment Vaccine Collaborative) project associated with Harvard Medical School. They also don't prefer alum but suggest a different adjuvant³⁹.

Even though the OSE Immunotherapeutics platform favours subcutaneous injection for their multi peptide epitope vaccine we think a nasal distribution would be advantageous and probably also can be done using their adjuvant Montanide ISA-51³¹. Furthermore, it has some advantages such as easy immunization of large population groups which is especially in this pandemic situation of worth, better patient compliance and does not require needles and syringes⁴⁰. A very appealing system just came recently to our attention which uses a thermostable application of vaccine candidates and is produced by Stabilitech Ltd^{41,42}. The beauty lies within combining OSE Immunotherapeutics approach with that technology by Stabilitech Ltd of an oral-applied vaccine targeting the mucosal immune system similarly to a nasal application but instead with a

capsule and without the need of a cold-chain. Also no special trained medical personnel would be needed and a large area coverage can be reached in virtually no time.

We believe this to be the best path forward in overcoming the SARS-CoV-2 pandemic.

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