



B-cell engineering: A promising approach towards vaccine development for COVID-19

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ARTICLE INFO

Keywords:

Vaccine
CRISPR
Genome editing
COVID-19
Coronavirus
B-cell
Antibody

ABSTRACT

With the number of cases crossing six million (and more than three hundred and seventy thousand deaths) worldwide, there is a dire need of a vaccine (and repurposing of drugs) for SARS-CoV-2 disease (COVID-19). It can be argued that a vaccine may be the most efficient way to contain the spread of this disease and prevent its future onset. While many attempts are being made to design and develop a vaccine for SARS-CoV-2, pertinent technological hitches do exist. That is perhaps one of the reasons that we don't have vaccine for coronaviruses (including SARS-CoV-1 and MERS). Recently developed CRISPR-mediated genome editing approach can be repurposed into a cell-modification endeavor in addition to (and rather than) correcting defective parts of genome. With this premise, B-cells can be engineered into universal donor, antigen specific, perpetually viable, long lasting, non-oncogenic, relatively benign, antibody producing cells which may serve as an effective vaccine for SARS-CoV-2 and, by the same rationale, other viruses and pathogens.

Introduction

COVID-19, caused by a positive sense single strand RNA virus (a member of the coronavirus family) called SARS-CoV-2 [1,2] does not, as of now, have any treatment and a majority of its aspects are yet unknown [3]. Initial attempts with repurposing of certain drugs have seen little success. Though previous coronavirus outbreaks can be used to model or understand SARS-CoV-2 and the disease it causes, but it is to be understood that no vaccine has yet been developed for any of the coronaviruses (including SARS-CoV-1 and MERS). As is the case with many viral diseases there is no vaccine for COVID-19. This is despite the fact that arduous efforts are being effectuated globally in this direction [4]. None of these efforts have yet been successful. This paper proposes B-cell genome engineering as a coherent technique to foster the development of an effective vaccine against SARS-CoV-2 and many other viruses that have evaded the possibility of vaccine development through conventional methods.

Since vaccines are presumably the most sought-after treatment for any disease. To this effect, a vaccine must elicit a controlled immune response in the recipient without complications and prompt the immune potency to persist. Despite decades of dedicated attempts, such vaccines intended to provide lifelong protection against many viral agents like respiratory syncytial virus (RSV), human immunodeficiency virus (HIV), influenza and Epstein-Barr virus (EBV) have not yet been a

reality. While many reasons can be attributed to this verity, a genome editing based approach to substitute/replace the endogenously-encoded antibodies with antibodies targeted at specific antigens (various parts of the SARS-CoV-2 in this case) in human B-cells may prove to be an efficient strategy to develop a safe, effective, and long-lasting vaccine. This paper proposes/hypothesizes B-cell genome engineering as a cogent rationale to develop a viable vaccine for SARS-CoV-2. This paper also explicates the stepwise methodology for translating this idea into reality. This paper also discusses the potential technological constraints and deliberates upon the coherent modus operandi to overcome such impediments.

Theory

In principle, CRISPR/Cas9 mediated genome editing approaches have a potential to edit mammalian cell genomes with extreme precision and this approach is not restricted to correcting the defective parts of the genome. Genomes can be modified and specifically repurposed towards important goals of improved and refined functions. With this premise, it can be hypothesized that a similar approach would be plausible to engineer human B-cells. To this effect, well-orchestrated expression of specific antibodies can be achieved under the control of endogenous regulatory elements responsible for antibody production (expression and secretion of normal antibodies) in these cells.

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<https://doi.org/10.1016/j.mehy.2020.109948>

Received 14 April 2020; Received in revised form 4 May 2020; Accepted 1 June 2020

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The fundamental mechanism through which many vaccines work is the production of antibodies by activated B-cells. This approach appears articulate at the outset but does have its own handicaps particularly pertinent to RNA viruses. Refashioning B-cells through genome-editing technology (like CRISPR/Cas9 mediated gene editing) to acquire certain imperative properties may resolve this difficulty. In this case the B-cells may be aimed at acquiring certain properties like (1) sufficient expression of the specific antibody, (2) negligible or no expression of the unintended antibody, (3) higher temporal viability of the so engineered B-cell clones inside the body and (4) the salience of being relatively benign and non-oncogenic. A repertoire of such cellular clones is likely to solve the problem not only for the SARS-CoV-2 but also of other viral pathogens.

Vaccines prompt B-cells to produce antibodies against specific antigens (epitopes) of the pathogen (e.g. S-spike protein in case of SARS-CoV-2). B-cells achieve this fate by rearrangement of the three imperative components of the antibodies in their genomes, the V, D and J regions. Some reasons for failure of vaccines are that such a gene rearrangement (1) may not effectively take place, (2) may be delayed, (3) may not be long-lasting and (4) may not be able to mount a sufficient and sufficiently specific response. Another critical issue with antibody-based vaccines is that the antibodies may get depleted within a short span of time and hence need to be administered repeatedly at definite intervals of time. This is an additional reason to engineer B-cells in a way so that they keep on producing the required antibodies perpetually as and when they are required.

Practical considerations

B-cells can be harvested, cultured and engineered using the CRISPR/Cas9 genome editing technology. Once the guide RNA and the required vector enters the cells, it can precisely rescript the DNA at the required position to modify the B-cell genome in order to express the antibody that is specific for a particular antigen/epitope. A combination of multiple B-cells can be used to generate a swarm of specific antibodies against a multitude of epitopes (generated as a result of antigenic drift). A nucleic acid segment can be delivered to the primary B-cells in culture through a viral delivery system like adeno associated viruses (AAV). In this case a precisely engineered nucleic acid stretch for antibodies against SARS-CoV-2 can be inserted in the host cell genome (though this approach can work for other pathogens also like HIV, flu, or Epstein-Barr virus). It can be safely assumed that such an approach will generate the required B-cells with a significant percentage of cells successfully getting their DNA edited. Once these engineered B-cells are ready, they can be injected into the patient (or a healthy person depending on the scenario) to elicit an immune response against the pathogen.

It should not escape our notice that an imperative facet to this approach is immune rejection of transplanted B-cells by the host immune system. For that reason, the activated B-cells would have to be engineered on individual basis as an allogenic B-cell might evoke an immune response. Though such a scenario appears to be a huge hurdle at the outset but might not be that a severe obstacle. This problem can be tackled by many approaches. One is to harvest the B-cells from the individual to be vaccinated, culture and genome edit them and then transplant the modified cells. The individual specific B-cells are likely to be expensive. Another approach to evade this difficulty is generating what can be termed as the universal donor B-cells. To augment this notion, recently universal donor cells have been shown to be an immunologically coherent possibility [5]. In this case a universal donor B-cell can be generated and engineered to produce antibodies. Such cells will not be rejected by the host immune system and at the same time will be viable enough to produce the specific requisite antibody.

Touching upon a slightly different note, viruses often mutate and render the antibodies ineffective. The proposed vaccine system can outmaneuver this snag also. A library of universal donor CRISPR

engineered B-cells can be generated that would outsmart this antigen drift and hence overcome this obstacle. As of now, three different strains of SARS-CoV-2 have been isolated and such a variety of strains for a virus can be tackled by generating a library of B-cells [6]. Molecular docking and simulation studies can be employed for designing such libraries. Through this process, a swarm of different B-cells- each aimed at different (or slightly different) antigen of the virus- can be produced using a repertoire of CRISPR mediated nucleic acid delivery.

The next question is to make sure that the engineered B-cell is not oncogenic. To this effect, repeated culture of B-cells can be done and various assays performed to know if the strain is safe for the recipient. A set of assays and tests can be developed to create a worksheet and protocol to ensure the selection of viable and safe engineered B-cells.

How to test the hypothesis

The proposed hypothesis can be tested in both mice and human B-cells in a two-facetted outlook. B-cells from mice can be harvested and engineered. The successfully engineered B-cells can then be injected into mice which were not exposed to the antigen previously. After exposing the mice to the antigen, the antibody titer can be evaluated against the control group (which did not receive the B-cell therapy). Presence of specific antibodies and the quantification of these antibodies against the antigen would confirm the success of development of B-cells. Alternatively, B-cells can be harvested from humans and engineered against the viral antigen. Then the successfully engineered cells can be injected into nude mice. The detection of antibodies in these mice after antigen exposure can account for the evidence that the B-cells have been correctly engineered. Also, the absence of any cancerous growth in these mice will be indicative of safety of these so engineered cells.

Technical difficulties

One of the technical hurdles in this sort of approach is that functionally viable B-cells require alternative splicing and polyadenylation to breed membrane bound as well as secreted antibodies. This appears to be a relatively tough venture in the context of a viral transgene [7,8]. This is further complicated by the fact that functional antibodies are a result of a synergistic expression of two genes viz. the heavy chain (IgH) and a kappa (Igκ)/ lambda (Igλ) light chains. Since the heavy chain locus requires a large size of the construct to which another level of intricacy is added by the heterogeneity of the heavy chain. B-cells generally undergo recombination events in V, D, and J segments which is approximately a megabase of DNA stretch within the heavy chain VDJ locus. Such a process gives rise to a kind of heterogeneity that is unique to each B-cell [9]. As a result, it becomes challenging to directly target the antibody region in the B-cells. Such a barrier can be overcome by replacing the complete stretch of the heavy chain locus with the heavy chain VDJ stretch of choice. This has recently been shown to be possible [10]. However, this approach may render the process limited only to those antibodies that act without the involvement of the light chain. One more similar approach is to focus on the light chain [11]. This approach however, has its own limitations as we may not be sure about the background expression of the endogenous antibodies. This is in addition to the fact that such an approach has been found to work only for secreted antibodies [11]. However, all these shortcomings can be circumvented by engineering a single insertion stretch with a full-length light chain and a part of heavy chain VDJ region that could be inserted in an intronic region of the heavy chain locus. Such an approach may work and generate antibodies of choice. This possibility also has some experimental evidence [12].

The “off-target” cutting by Cas9 as it happens in certain instances with the CRISPR/Cas9 mediated genome editing may be another difficulty. This problem can be circumvented by utilizing a single gRNA for the entire string of both heavy and light chain. This can be done by

employing pre-complexed gRNA and Cas9 [13]. In addition to this, it would always be advisable to perform a comprehensive analysis of the off-target genomic changes in the B-cells to be employed for further downstream processing [14].

Additional critical feature to take a note of is allelic exclusion which means that though there are two alleles (as is the case with diploid genomes) for each antibody chain within the B-cells, but they only express one heavy and one light chain gene [15–17]. Insertion of extra genes for a non-native antibody would predispose the cell to numerous permutations of light and heavy chain combinations. Such, so produced antibodies, may be incompetent. In that case, the deletion of the native/endogenous (light and heavy chains) would be desirable. This, in turn, will thwart the expression of chimeric B-cell receptors (which is a combination of one fragment from the transgene and from the endogenous gene for the antibody). Some experimenters have produced evidence for such a possibility [18].

Conclusion

Given the advances in the precision genome editing and our understanding of difficulties in devising effective vaccines against SARS-CoV-2 (and other related viruses), it can be concluded that precisely engineering the genomes of human B-cells might be an effective and promising approach to develop long lasting and effective vaccines. Though there are some logistic, economic and technical difficulties, B-cell engineering is highly likely to yield promising results and an effective vaccine not only for SARS-CoV-2 but also for other pathogens like HIV, EBV, Ebola and dengue.

Conflict Of Interest

The author declares “No conflict of interest”.

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