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Commentary

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Inactivated vaccines: a promising old tool against Covid-19

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Abstract

Global dissemination of the novel coronavirus SARS-CoV-2 associated to the acute severe respiratory syndrome (COVID-19) has already reached more than 30 million of persons. Fortunately, lockdown and social distance measures have proven to be effective at limiting viral dissemination during early stages of epidemic at regional or country level. However, relaxing of those measures resulted in a rapid growth of new infected cases. Because of that most researchers believe that only an effective vaccine would allow controlling the disease and later on eradication. Thus, a race has been launched and several approaches are evaluated. Among the platforms, the use of killed virus, recombinant viral vectors and encapsulated mRNA are leading the pipeline. Considering advantages and drawbacks of the leading approaches, it seems possible that an inactivated vaccine versus COVID-19 will be available in the near future.

Inactivated vaccines for Covid-19 in the pipeline

There is an urgent demand of a vaccine to control COVID-19 disease and SARS-CoV-2 dissemination worldwide. The re-emergence of new cases of COVID-19 in countries and territories where lockdown measures were relaxed suggests that only the immunity provided by well implemented vaccination programs would control further dissemination of the virus or the fatal rate of the disease.

Several pharmaceutical companies, universities, institutes of research and governmental organizations are working in accelerated programs to achieve this goal. Government-funded projects have achieved an unprecedented increase and for the moment financial support is not limited. This scenario allows that many approaches and vaccine platforms are currently tested to elicit specific immunity against SARS-CoV-2.

Among the methods to produce vaccines, the inactivation of pathogens is regarded as an "old fashion" of making vaccines. However, nowadays this methodology is leading the race against Covid-19. A look at the pipeline of vaccines currently in clinical trials shows that two candidates based on viral inactivation were developed by Chinese companies, Sinopharm and Sinovac [1]. According to press releases of results of ongoing phase I and II clinical trials, these two inactivated vaccines are very promising. It was reported that around 90% of volunteers immunized with PicoVacc (the candidate developed by Sinovac) developed neutralizing antibodies (NAb) [2] and Sinopharm's vaccine candidate, BBIBP-CorV, achieved 100% NAb sero-conversion in the groups of two doses at 28 days interval and more than 97% of people who received shots at 14 and 21 interval with the medium level of dose tested [3]. Although the correlates of protection are still unknown all competitors bet on this effector arm of the adaptive immune system to develop a prophylactic vaccine.

If it is confirmed that NAb may provide immunity for SARS-CoV-2 then previous results must be considered remarkable achievements from several points of view. First, it has been calculated that a combined effect of vaccination and herd immunity due to natural infection of 55-82% would be necessary to achieve COVID-19 extinction [4]. Second, the FDA Guidance for Development and Licensure of Vaccines to Prevent COVID-19 [5] only demands 50% efficacy to prevent the disease or decrease its severity to grant a license. Third, according to the same guideline ¨ For non-inferiority comparison to a COVID-19 vaccine already proven to be effective, the statistical success criterion should be that the lower bound of the appropriately alpha-adjusted confidence interval around the primary relative efficacy point estimate is >-10%.¨ Taking together points 2 and 3, it means that it will be more easy to obtain a license for those companies that go faster to the efficacy trial because the threshold to obtain it might be very high for those that arrive later to the market. Obviously, if such very good results in terms of NAb obtained in phase II trials with inactivated vaccines are translated in similar results in efficacy trials then it might be more difficult to other developers to get a license. Nevertheless, in the scientific ground registration of some vaccine for COVID-19 might contribute to a better understanding of the correlate of protection if it is widely distributed and administered to a significant number of people in a high incidence geographic area or country.

Which previous research is relevant to develop an inactivated vaccine candidate against SARS-CoV-2 infection or Covid-19 disease?

There are still important un-answered questions that would help to develop a vaccine against SARS-CoV-2 based on a rational design. That is why an accelerated development of a vaccine must be based on previous relevant research.

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SARS-CoV-2 is a new member of the Betacoronavirus genus where SARS-CoV and MERS-CoV viruses are also included. The homology of a consensus SARS-CoV-2 protein sequence to other Betacoronaviruses showed a higher degree of identity with SARS-CoV. In particular, the coding region for the S antigen has a percent of homology higher than 75% [6]. In this regard, although there is not any registered vaccine against the SARS-CoV the accumulated experience about this virus might guide the way forward for developing a vaccine against SARS-CoV-2.

Several important questions need to be addressed to develop an effective inactivated vaccine. For instance, the impact of different viral strains on the development of NAb and of the method of inactivation. Fortunately, several studies showed that independently of the SARS-CoV strain, the inactivation method (e.g., β-Propiolactone, Formalin, UV) and even the adjuvant (e.g., Alum, CFA/IFA, MLP+TDM, CpG ODN) all inactivated vaccine candidates tested in mice via parenteral routes of immunization developed NAb [7-11].

Unfortunately, although it is supposed that NAb or sIgA at the mucosal surface of the throat are relevant to stop dissemination of coronavirus, experiment in mice suggested that only when adjuvated, inactivated vaccines can elicit sIgA [12]. Furthermore, these antibodies lacks neutralizing activity or it is very low [10] unless using strong experimental adjuvants [13]. In this regard, previous results with inactivated vaccine candidates for SARS-CoV suggest that prevention of upper respiratory tract infection by SARS-CoV-2 mediated by mucosal Abs could be difficult to attain. But, to our knowledge, no one has evaluated the coadministration of inactivated vaccine candidates through a parenteral (e.g., intramuscular) and intranasal route in the same schedule of immunization. Previous studies have shown that it produced an enhancement of the immune response in the mucosal and systemic compartment compared with single route schedules of immunization [14-17]. But, after all, it is maybe not necessary because in a rhesus macaque model of infection after parenteral immunization with PiCoVacc (inactivated vaccine candidate for SARS-CoV-2 developed by Sinovac) and BBIBP-CorV (developed by Sinopharm) the viral replication in throat, the gut and lung tissue was aborted [18,19].

There is still a debate about the impact of NAb titers on protection against SARS-CoV considering that previous research in animal models and human converged to show that they are not long lasting [20,21]. However, challenge experiments also evidenced a huge booster effect that amplified the neutralizing titers even against heterologous isolates to achieve protection [21]. It is also something relevant for the SARS-CoV-2 because preclinical experiments with inactivated vaccines showed a similar trend [18]. Analysis of results of ongoing efficacy trials might provide an answer to this question.

It is important to notice that it was demonstrated using an inactivated vaccine candidate for SARS-CoV that up to 5 mg of the vaccine was very safe and tolerated in macaques [22]. This seems to indicate that a similar vaccine for SARS-CoV-2 would benefit of a huge range of dosing for testing the appropriated dose in humans to warrant the best response possible for eliciting neutralizing antibodies.

There is still a "black spot" that raise a lot of concern for the development of a vaccine against coronavirus in general which is the possibility to promote antibody-dependent enhancement (ADE) or enhancement of the respiratory disease (ERD). This has been evidenced in vitro for SARS-CoV infection [23]. Accordingly, it has been hypothesized that ADE or ERD might be induce if a vaccinated person is later on infected by a strain with a genetic variant of the S antigen of the same virus or any related coronavirus at the time when anti-S titers due to vaccination have waned. In this regard, the evidence for SARS-CoV-2 is still lacking [24]. In any case, limited evidence in macaques suggests that leading inactivated vaccine candidates PiCoVacc and BBIBP-CorV does not induce such phenomenon [18,19]. Additionally, it is possible to speculate that selection of a well representative strain of field isolates would provide less change to the occurrence of ADE or ERD in vaccinees.

Which are the Pros and Cons for the production of an inactivated vaccine?

It is impossible that a vaccine platform will fix every situation. But, considering that two of eight vaccine candidates which are entering into efficacy trials are killed vaccines [1], the inactivation approach for SARS-CoV-2 seems very promising. In general terms, it might be extended to other coronaviruses.

One important point in favor of the approach is the fact that there are several inactivated vaccines in use today against other viruses [25] and this will speed up the licensure process [5] and it is a clear advantage over novel approaches for which not licensed vaccine exist. Furthermore, if, as expected, good results are obtained in efficacy trials for inactivated vaccines then according to new regulations there will be a clear advantage to get licensure in many territories because of the emergency status. It would be a major drawback for other vaccine candidates that arrive later to efficacy trials because for non-inferiority comparisons the threshold criterion for statistical success will be set by a previous licensure process [5]. In fact, when considering the high levels of NAb elicited in phase I/II trials for inactivated vaccines and the fact that they are leading the vaccine pipeline, it is possible to speculate that such threshold value might be very difficult to reach.

Additionally, the platform allows easy scalability of a viral stock that replicates very fast reaching a peak titer of more than 6.0 log10 $TCID_{50}$/mL by 48-72 hours post-infection at multiplicities of infection (MOI) of 0.0001-0.01 [18]. Nevertheless, it is also its main drawback in addition to the high level of transmission reported for SARS-CoV-2. Consequently, it is necessary Biosafety Level 3 (BL-3) facilities for virus culturing and inactivation. This is a major limitation for some vaccine manufacturer countries. However, if a novel bioreactor is developed that allows culture of the Vero cells, 48-72 h of viral replication, and inactivation without harvesting of the viral solution and cell debris out of the bioreactor to avoid the airborne route of transmission, perhaps it would be a feasible solution for obtaining the inactivated product under BL-2 safety conditions. In addition, to ensure safety, two inactivation steps can be programmed in tandem to guarantee viral inactivation before the final formulation process with the adjuvant takes place.

It is also important to warrant the genetic stability of the viral strain for the industrial production process of the vaccine. In this regard, it seems that genetic stability of SARS-CoV strains when cultured in Vero cell line are pretty stable, perhaps even more than natural viral transmission without significant changes after at least 10 passages in the amino acid sequences of the S protein target of neutralizing antibodies [26]. There are evidences that SARS-CoV-2 strains also have a high degree of genetic stability [18,19].

Another important issue for an approach to deal with pandemic coronaviruses is the possibility of fast implementation and versatility. In this regard, among the traditional ways of vaccine development the inactivation approach is the one that needs less information about the virus compared to, for example, the subunit approach. The last approach

requires some knowledge of the nucleic acid sequence and also the 3D structure of the surface antigen beforehand to ensure proper folding, etcetera. In the case of killed vaccines, if necessary a similar production process might be implemented with only few changes for a novel field isolate to deal with some genetic variant to which cross-protection is not observed. It might take much time for other approaches to implement a new production process, except for those based on mRNA or DNA for which there is not any registered vaccine yet. Finally, because the production process is not very expensive the price of the vaccine could be reduced to reach worldwide distribution.

Conclusion

Broadly speaking, the killed vaccine platform is "an open road" to everybody for developing vaccines because it does require neither too much science nor technological level compared to other approaches. This holds true when the growth of the microorganism does not pose a threat of dissemination by the airborne route as it is the case of SARS-CoV-2. In such circumstance a BL3 facility is required and currently this is the main limitation. Nevertheless, on other grounds it seems very feasible for SARS-CoV-2 because it is not limited neither by the selection of the viral strain nor the adjuvant or method of inactivation among other important variables of the production process.

When looking to the pipeline of vaccine candidates under development for SARS-CoV-2 only eight candidates have already entered phase III efficacy trials [1]. Two of them are based on inactivated vaccines (SinoPharma and Sinovac). Four are based on non-replicating adenoviruses. Among them are one from chimpanzees (ChAdOx1 nCoV-19) developed by the University of Oxford and the pharmaceutical AstraZeneca [27], and three based on human adenoviral type 5 and 26 developed by CanSino Biological Inc. in collaboration with the Beijing Institute of Biotechnology (Ad5), Janssen Pharmaceutical Companies (Ad26COVS1) and the Gamaleya Research Institute from Russia (rAd26-S+rAd5-S). Finally, there are two other candidates based on the novel technology of encapsulated mRNA developed by Moderna/NIAID [28] and BioNTech/Fosun Pharma/Pfizer [29], respectively. Although, recently some concerns have been raised about the preclinical results of challenge experiments with rhesus macaques immunized with ChAdOx1 nCoV-19 which need further consideration [30], preliminary results in humans seems promising in terms of induction of NAb [31]. However, it is well known that induction of anti-vector (adenovirus) immunity will significantly decrease the potency of booster doses and additional shots might be needed to sustain a protective anti-SARS-CoV-2 NAb response. That might explain why the Gamaleya Research Institute developed a prime-boost schedule using two types of adenoviruses and some promising results with this approach have recently been showed in a non-randomized phase 1/2 [32]. Most importantly, considering a very recent report on the probable serious side effect observed in the efficacy trial of the ChAdOx1 nCoV-19 vaccine candidate a dark shadow cast over the future of this approach [33] and highlight the importance of having a cautious eye on those candidates based on adenovirus and viral vectors in general. On the other side, preliminary reports of phase I/II trials for novel vaccine candidates based on encapsulated mRNA also look promising [28,29] but this approach faces a major drawback of not having any previous licensed vaccine based on the same technology. Thus, taking into consideration all the accumulated evidence it seems that inactivated vaccines are well placed to be among the first candidates to get licensure at the international level as anti-coronavirus vaccine in the near future.

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