Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Perspective

Protection from infection or disease? Re-evaluating the broad immunogenicity of inactivated SARS-CoV-2 vaccines

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How do we measure vaccine efficacy? The strictest but also easiest parameter to determine vaccine efficacy is its ability to block infection. Indeed, if a vaccine is able to block infection, this necessarily follows that it will also prevent both disease development and viral transmission. As a consequence, antibodies, specifically neutralising antibodies, have been used as the "gold standard" correlate of protection to measure SARS-CoV-2 vaccine efficacy, given their ability to block infection. Since SARS-CoV-2 infects cells by the binding of its spike protein to the host ACE-2 receptor, a vaccine that is able to induce a large quantity of antibodies able to block the interaction between the ACE-2 receptor and spike protein should theoretically be highly efficacious. Given this "antibody-centric" method of evaluating of a vaccine, it is clear why spike mRNA vaccines have to date been regarded the most effective COVID-19 vaccine in the market.

The ability of spike mRNA vaccines to induce extremely high quantities of anti-spike antibodies is superior to all the other vaccine platforms (Zhang Z. et al., 2022), and is the reason why spike mRNA vaccines were initially highly efficacious in preventing symptomatic infection caused by the ancestral strain of SARS-CoV-2 (Thomas et al., 2021). On the contrary, inactivated SARS-CoV-2 vaccines (CoronaVac-Sinovac and BBIBP-CorV-Sinopharm), which were extensively distributed in 2021 and make up almost half of the 7.3 billion doses of SARS-CoV-2 vaccines used worldwide (Mallapaty, 2021), elicit a lower level of neutralising antibodies that wanes quickly (Lim et al., 2021; Peng et al., 2022; Zhang H. et al., 2022). This likely explains why inactivated SARS-CoV-2 vaccines are less effective in preventing symptomatic infection when compared to other vaccine platforms (Jara et al., 2021).

The epidemiological, virological, and immunological landscape of COVID-19 has evolved significantly since the onset of the pandemic in late 2019 (Van Dorp et al., 2021). In particular, the ancestral SARS-CoV-2 lineage has been substituted by diverging lineages with increasing number of mutations in the spike protein. These mutations render vaccine-induced antibodies less effective in neutralising emerging variants (Omicron lineages) (Liu L. et al., 2022). This has led to a shift in the perspective of what vaccination can and should protect a vaccinated individual from: rather than focusing solely on the ability of a vaccine to prevent infection, we should instead evaluate it based on its efficacy in preventing severe disease.

Here, T cells play a significant role (Bertoletti et al., 2022). Indeed, while the primary role of antibodies is to prevent the virus from infecting target cells, $CD4^+$ and $CD8^+$ T cells perform non-redundant immunological functions directed towards the reduction of viral load after infection. $CD8^+$ T cells detect virally infected host cells by recognizing short viral sequences (called epitopes) derived from the different viral proteins synthesized within the host cells and presented by the major histocompatibility complex (MHC)-class I molecules. $CD4^+$ T cells can also recognize and directly lyse virus-infected cells (Heller et al., 2006) but are mainly activated by epitopes derived from the processing of viral proteins internalized by uninfected professional antigen-presenting cells (mainly dendritic cells or other myeloid-lineage cells) and presented by the MHC-class II molecules. $CD4^+$ T cells are not only involved in the direct recognition of virus-infected cells, but also promote antibody maturation, sustain $CD8^+$ T cell function and release cytokines like IFN-γ that can have direct anti-viral effects.

It is important to highlight that the presentation of viral epitopes by MHC-class I or II molecules is not dependent on the location of the viral proteins within the virions, but by other parameters such as the kinetics and quantity of the viral protein synthesized within the infected cells. This means that spike-specific T cells are not more efficient than nucleoprotein-specific T cells in recognizing virus-infected cells or even in helping anti-spike specific antibody production. We have tried to explain such concepts in a recent review (Bertoletti et al., 2022) and these concepts are the basis of why we embarked on a detailed analysis of $CD4^+$ and $CD8^+$ T cell response elicited by inactivated vaccines in comparison to mRNA vaccines. This work has been recently published in *Cell Reports Medicine* (Lim et al., 2022).

Since T cells specific for different viral proteins can have excellent protective effects against disease, our focus was to understand whether inactivated virus vaccines, comprising all the structural viral proteins present in the virions, can elicit a T cell response not only against spike

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but also other distinct structural SARS-CoV-2 proteins (membrane and nucleoprotein). We were also interested in determining whether the combined magnitude of T cells specific for spike, membrane and nucleoprotein elicited by the inactivated vaccines were comparable to the sole spike-specific T cell response elicited by the spike mRNA vaccines.

Through a comparative analysis of SARS-CoV-2-specific T cells in healthy individuals vaccinated with inactivated SARS-CoV-2 or mRNA vaccines, we showed that in line with the lower humoral immunogenicity against spike, inactivated SARS-CoV-2 vaccines induced a lower magnitude of spike-specific T cell response compared to spike mRNA vaccination. However, the lower magnitude of spike-specific T cell response was compensated by the induction of T cell response specific for membrane and nucleoprotein, the other structural proteins present in inactivated SARS-CoV-2 vaccines. This has led to a comparable magnitude of the combined inactivated vaccine-induced T cell response (spike-, membrane- and nucleoprotein-specific) to that stimulated by spike mRNA vaccines (spike-specific only), with the added theoretical advantage that a T cell response against different epitopes located in multiple viral antigens could better tolerate the viral mutations present in the new SARS-CoV-2 lineages. It is important to note that although the impact of amino acid mutations in spike protein is often well tolerated by spike-specific T cells (Gao et al., 2022; Keeton et al., 2022; Liu J. et al., 2022; Tarke et al., 2022), ~10% of spike mRNA vaccines have spike-specific T cells that are profoundly inhibited by the spike mutations detected in Omicron lineages (Naranbhai et al., 2022).

In addition to these quantitative aspects, we also detected important qualitative differences in the T cell response induced by spike mRNA and inactivated SARS-CoV-2 vaccines. While spike mRNA vaccines stimulated a coordinated spike-specific $CD4^+$ and $CD8^+$ T cell response, the multi-antigenic T cell response induced by inactivated SARS-CoV-2 vaccines was mediated by the selective priming of $CD4^+$ T cells. The reduced ability of "protein-based" vaccines to elicit $CD8^+$ T cells has been also observed in a recent published work on the immunogenicity of the spike protein-based vaccine (NVX-CoV2373) (Rydyznski Moderbacher et al., 2022). Similar to our data, the authors showed that NVX-CoV2373 elicited robust Th1 and T follicular helper $CD4^+$ T cell responses but a very limited $CD8^+$ T cell response, even when quantified through the expression of activation markers by flow cytometry, a technique that we have shown in our work to overestimate the presence of vaccine-specific $CD8^+$ T cells.

It is challenging to define the impact of the lack of $CD8^+$ T cell induction by SARS-CoV-2 inactivated vaccine on its efficacy, but several lines of evidence in animals support the sufficiency of virus-specific $CD4^+$ T cells in controlling viral disease. The induction of $CD4^+$ T cells specific for the nucleoprotein of SARS-CoV in the nasal cavity of mice protected the animal from lethal disease after infection with different coronaviruses (Zhao et al., 2016). Similarly, memory $CD4^+$ T cells also directly and indirectly mediated protective effects in influenza A virus infected mice (Mckinstry et al., 2012). Furthermore, $CD4^+$ T cells were shown to be necessary for the effective induction of local immunity in the mucosal/olfactory tissues that prevents neuroinvasion following nasal vesicular stomatitis virus infection in mice (Wellford et al., 2022).

It is therefore possible that the multi-antigenic $CD4^+$ T cell response induced by inactivated virus vaccine (spike-, membrane- and nucleoprotein-specific) might be equally protective in ameliorating COVID-19 severity when compared to the mono-antigenic (spike) coordinated $CD8^+$ and $CD4^+$ T cell response induced by spike-based mRNA vaccination (Fig. 1). Recent clinical data from Hong Kong measuring the protective efficacy against mild and severe COVID-19 in healthy adults infected with Omicron showed similar efficacy between spike mRNA and inactivated SARS-CoV-2 virus vaccine preparations after three doses (Mcmenamin et al., 2022). This observation suggests that both the lack of the coordinated activation of $CD4^+$ and CD8 T^+ cells and lower ability to induce neutralising antibodies observed in inactivated virus vaccine recipients can be compensated by the multi-antigenic nature of the $CD4^+$ T cell response that could better tolerate the mutations present in Omicron.

Our study also showed that the level of multi-specific $CD4^+$ T cell responses remained stable six months after vaccination and did not wane significantly unlike antibody titers. On the other hand, a third dose of inactivated vaccine did increase antibody levels but had negligible effect on the frequency of $CD4^+$ T cells. Longitudinal studies will therefore be necessary to evaluate the persistence of such multi-specific $CD4^+$ T cell response after inactivated virus vaccination, even though cellular immunity has been shown to be much more stable overtime than antibody levels both after other vaccination and natural infection (Le Bert et al., 2020; Dan et al., 2021). Furthermore, our data also show that heterologous vaccination with mRNA vaccine and inactivated virus vaccines induce SARS-CoV-2 multi-antigenic specific $CD4^+$ and spike-specific $CD8^+$ T cells, reaffirming that heterologous vaccination with different preparations could be a feasible strategy for possible long term protection (Cerqueira-Silva et al., 2022; Khoo et al., 2022).

In our opinion, the changing landscape of the SARS-CoV-2 pandemic with the emergence of novel lineages that can easily escape the protective ability of antibodies have prompted a re-evaluation of what inactivated SARS-CoV-2 vaccines can do. We believe that vaccine efficacy should no longer be evaluated solely by its ability to prevent infection,

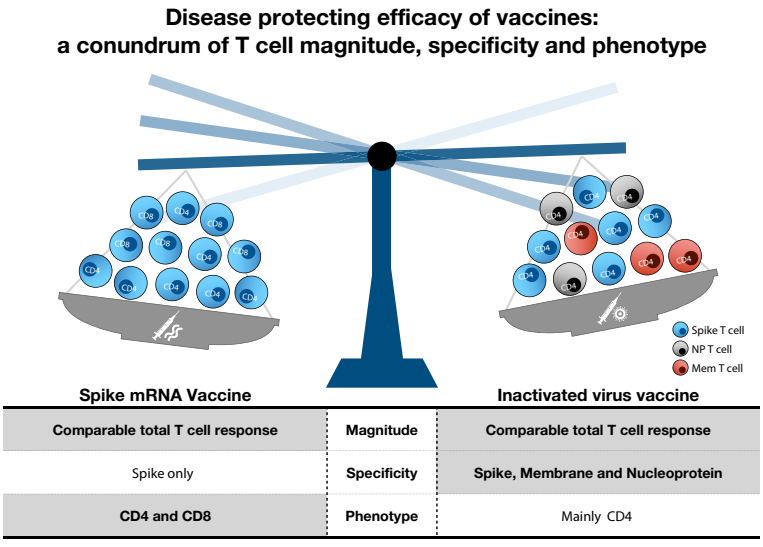

Fig. 1. Characteristics of spike mRNA and inactivated SARS-CoV-2 vaccine-induced T cell response. NP, nucleoprotein; Mem, membrane.

but also by its capability to protect from the development of severe disease. From this perspective, it does not seem wise to relegate inactivated virus vaccines to an inferior league based only on the assessment of humoral immunity response. It would be more prudent to understand how the superior breadth of the vaccine T cell response can represent a real long term protective asset and as such be utilised in this long drawn battle against COVID-19.

Footnotes

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